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RESEARCH ARTICLE

A Novel Automated System for the Design of Knuckle Joint

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ABSTRACT

The design of Knuckle joint assembly is very time consuming and complex activity. In this paper, a program has been developed for the design of Knuckle joint. The existing program uses knowledge database and reasoning. It tries to touch the unexplored field of design of joints by automated system. The use of AUTOKNUCK helps the designer to design the components of the assembly with high accuracy and dramatically reduced lead time especially for small scale industries having limited resources.

Keywords: Knuckle joint, Automation, lead time, small scale Industries

INTRODUCTION

Knuckle joint is a temporary joint that connects two rods which are subjected to tensile loading conditions. The typical applications of the Knuckle joint are bicycle chains, suspension bridges, roof trusses, Tractors, cranes and structural members [1]. The design process of knuckle joint in automated system is complex and requires iterations. To meet critical need of this kind, constraint based design helps to offer a unified network [3]. An expert system was developed to study the design for manufacturability of die Cast components using section thickness, rib based, boss based, Core based, Fillet based and Draft based guidelines as the parameters[2].Very little work has been done in the field of designing joints by automated systems. A method for automated gear box design using specificheuristics and stochastic search using simulated annealing was discussed [4]. There is requirement of flexi-dynamic and versatile techniques for sustaining in global market. Computer aided systems plays vital role in accomplishing this endeavor [5]. In this paper the authors have used the language C for designing an automated system **AUTOKNUCK**

Theoretical Design of knuckle joint

The diagrammatic representation of knuckle joint assembly is shown in the figure 1. In order to design the assembly the following assumptions [1] are made:

a The rods are subjected to tensile force only.
b Tensile stress is equal to compressive stress and permissible shear stress is half of that of permissible tensile stress.
c The effect of Stress concentration is neglected.

Empirical Relations used for the design [1]:

De = 1.1 ×D, h1 = 1.25× D, h2 = 0.75 × D, dp = 1.5 × dk, do = 2 ×dk
Taking 1st rod on the fork side and assuming that it is subjected to a force *F* to its left; to be in the state of equilibrium, equal and opposite force must be there so dividing the force in half and applying it to the fork ends to the right (fig.2).Taking 2nd rod on the eye end and assuming that it is subjected to a force *F* to its right; to be in the state of equilibrium, equal and opposite force must be there so applying force to the eye end to the left (fig. 3).The forces on the pin are opposite and equal as a reaction to the forces applied by both fork and pin (fig.4). The Table 1 given below show the different parts subjected to possible failure under loading conditions F

System Design

AUTOKNUCK requires the input from the user for selecting the type of material, factor of safety and the design load required for the knuckle joint design (fig. 5). If the user is not sure about the material, it automatically takes up the weakest material in its database for design purpose. In this database medium carbon steel is used for designing knuckle joint assembly ranging from 30C8 to 55C8 (fig. 5). The different parameters of the assembly are calculated by the autoknuck (fig. 6). The best possible design based on theoretical iteration is suggested on the screen to user. AUTOKNUCK automatically checks the design for failure of the knuckle joint and in case of failure redesigns the whole joint assembly until it is safe for the given load (fig. 7).

RESULTS AND DISCUSSIONS

AUTOKNUCK calculates all the proportions of knuckle joint assembly with high accuracy and reliability. Moreover the time taken to design knuckle joint is greatly reduced. The automated system is made plug and play. It requires very less system requirements to run as an executable file. The Table 2 below gives the values of different proportion for different loading conditions and materials assuming 5 as factor of safety.

CONCLUSION

Since finite element analysis softwares and skilled man power are costly, so AUTOKNUCK is fairly good alternative for micro and small enterprises as it will be very cost effective. It can be used even by a person having basic knowledge of computer. This automated system shows great potential in teaching as it is a handy tool for students and teachers to verify their design results by manual calculations. This software can also be used for the accurate design of various machine elements subjected to tensile loading like tractor trailer system and to reduce the accidents due to overloading failures.

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Nomenclature	
dk = diameter of knuckle pin	h2 = thickness of each eye of the fork
dp =diameter of pin head,	y =distance of center of fork radius r
do = outside diameter of eye and fork	σt = permissible/ design tensile stress
D = diameter of each rod,	σc = permissible /design compressive stress
De = enlarged diameter of each rod,	τ = permissible/design shear stress
h1 = thickness of eye end of the second rod,	F= axial load acting

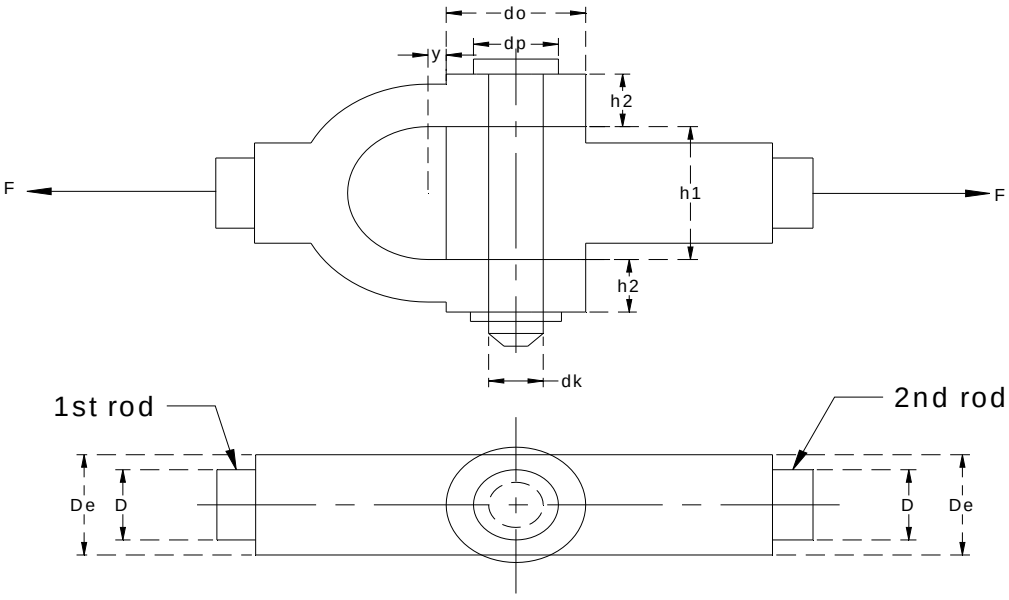


Figure 1. Knuckle joint assembly

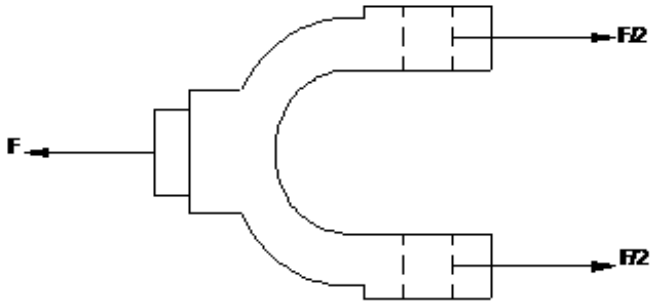


Figure 2 .Fork

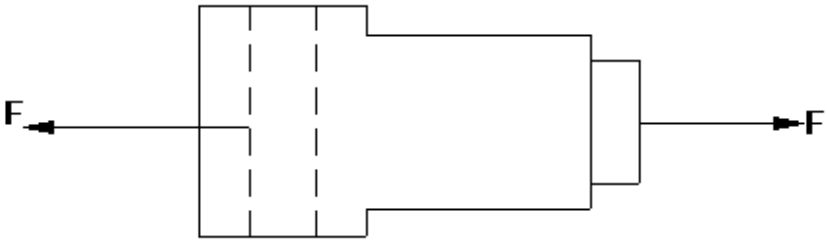


Figure 3. Eye

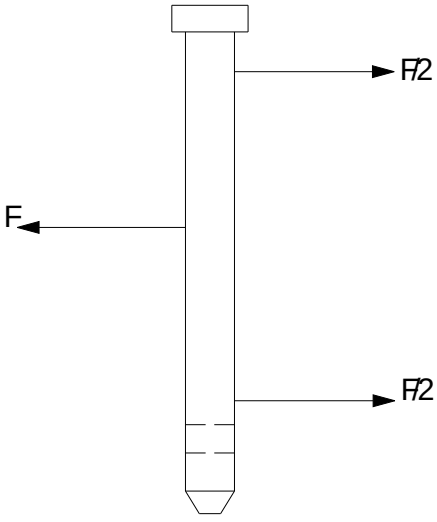


Figure 4.Pin

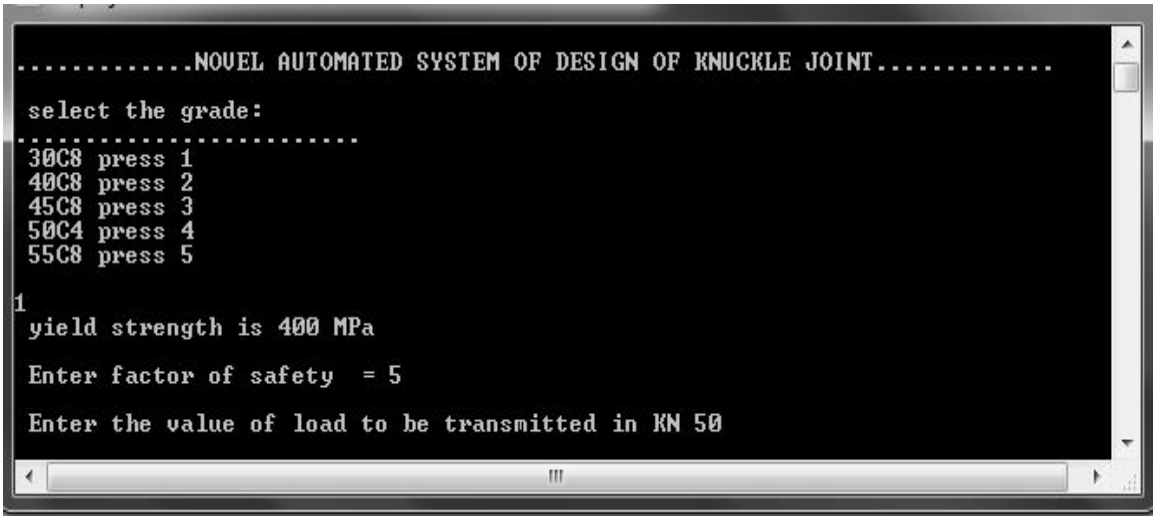


Figure 5: Screenshot of Auto knuck showing material type, Factor of Safety and Design load

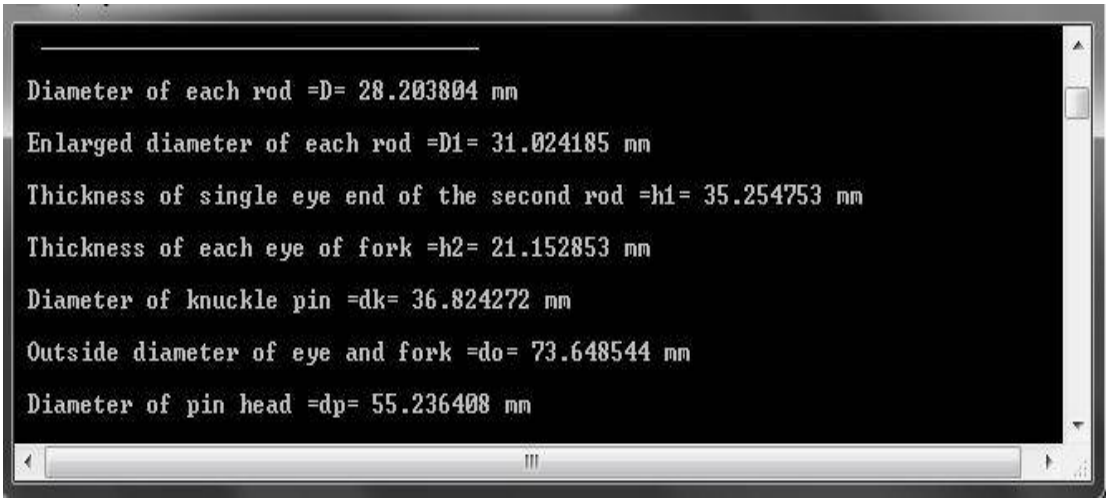
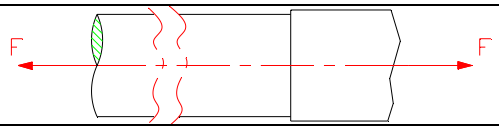
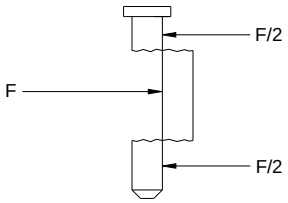
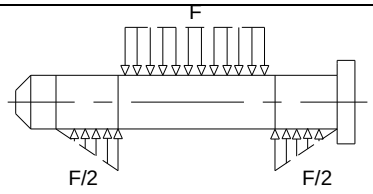
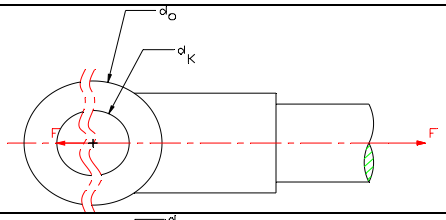
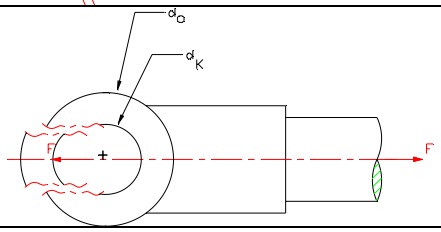
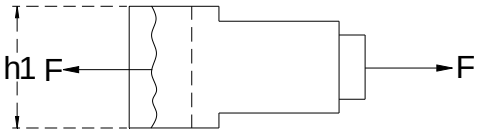


Figure 6: Screenshot of Auto knuck showing values of different parameters

Figure 7: Screenshot of Auto knuck showing values of failure stresses

Table1. Failure of Individual parts

PART	FAILURE	FORMULA	DIAGRAM
Rods	Tensile	$\sigma t = \frac{F}{\frac{\pi * D^2}{4}}$	
PIN	Shear	$\zeta = \frac{F}{2*\left(\frac{\pi * dk^2}{4}\right)}$	
	Bending	$\sigma b = Bending\ stress = (4 * F) * \left(\frac{3*h1+4*h2}{3*\pi * dk^3}\right)$	
EYE	Tensile	$\sigma t = \frac{F}{h1*(do-dk)}$	
	Shear	$\zeta = \frac{F}{h1*(do-dk)}$	
	Crushing	$\sigma c = \frac{F}{h1*dk}$	

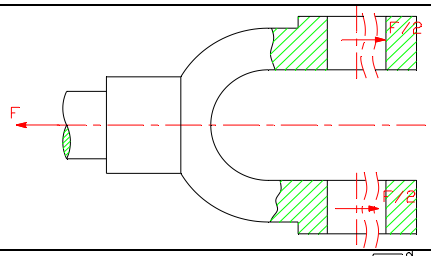
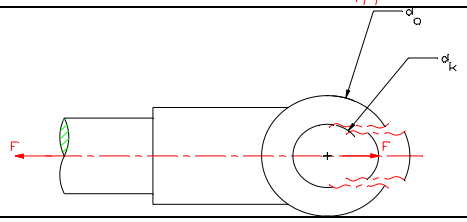
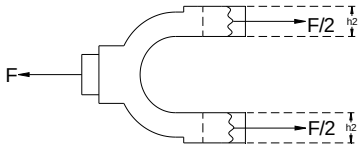
FORK	Tensile	$\sigma t = \frac{F}{2*h2*(do-dk)}$	
	Shear	$\zeta = \frac{F}{2*h2*(do-dk)}$	
	Crushing	$\sigma t = \frac{F}{2*h2*dk}$	

Table 2.Different Loading Conditions

For a loading of 50 KN

Proportions (in mm) ⇨ / Material ⇩	D	D1	h1	h2	dk	do	dp
30C8	28.20	31.02	35.25	21.15	36.82	73.64	55.236
45C8	28.93	31.83	36.17	21.70	37.77	75.55	56.66
55C8	26.93	28.93	32.87	19.73	34.34	68.68	51.51

For loading 75KN

Proportions (in mm) ⇨ / Material ⇩	D	D1	h1	h2	dk	do	dp
	34.54	37.99	43.17	25.90	45.90	90.18	67.63
45C8	35.43	38.98	44.29	26.57	46.26	92.52	69.39
55C8	32.21	35.43	40.26	24.15	42.05	84.10	63.07

For loading of 100KN

Proportions (in mm) ⇨ / **Material** ⇩	**D**	**D1**	**h1**	**h2**	**dk**	**do**	**dp**
45C8	40.92	45.01	51.15	30.69	53.41	106.82	80.11
55C8	37.19	40.91	46.49	27.89	48.54	97.09	72.82

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RESEARCH ARTICLE

Selective Oxidation of Benzyl Alcohol to Benzaldehyde by using Nickel Oxide Nanoparticles

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ABSTRACT

NiO nanoparticles were prepared by the direct precipitation method. The as-prepared nanoparticles were characterized by X-ray diffraction (XRD), FTIR and UV-Visible spectrometer analysis techniques. The average particle size of nanoparticles was calculated from the XRD study was 18 nm. According to the UV-Visible spectrum, the band gap value of 5.41 eV was obtained for the NiO nanoparticles. The catalytic activity of NiO nanoparticles has been studied for the selective oxidation of benzyl alcohol to the benzaldehyde using air as the oxidizing agent. The NiO oxide nanoparticles showed excellent catalytic activity for the oxidation of benzyl alcohol to the benzaldehyde. The complete conversion of all the benzylic alcohols to the corresponding benzaldehyde is achieved within a 3 hour reaction period at 80 ^{0}C. The obtained product of the reaction is identified by TLC, IR and Tollun's test which was shown benzaldehyde.

Keywords: Nickel oxide nanoparticles, Catalyst, selective oxidation, Benzyl alcohol

INTRODUCTION

Recently, the metal oxide nanoparticles have been interested by the researchers. The structure of nickel oxide is a premium example, which has a large exaction binding energy and a wide band gap ranging from 3.6 to 4.0eV (Purushothaman and Muralidharan, 2008; Deraz, et al., 2008). Previously, Nickel oxide becomes considerable

attention by researcher because of their catalytic, electrical and magnetic properties (Gaecia, et al., 2003). It has several applications in the different fields such as magnetic materials (Wang, et al., 2005; Xiang, et al., 2002), photovoltaic devices (Wang and Ke, 1996), electro chromic films (Li and Xu, 2005), Gas sensor (Zhang, et al., 2004) and fabrication of catalysis (Li, et al., 2000; Panocove, 1971). The NiO nanoparticles have large surface area to volume ratio and small particle size by comparing to bulk-sized NiO particles. The nano-sized of NiO particles have a large magnetic behavior such as super paramagnetic and catalytic properties (March, 1985).

In the last decades, the high catalytic efficiency of nanoparticles in organic synthesis has attracted the interest of organic chemists to apply in performing many organic reactions, and some of them were successful like oxidation, reduction, disulfide formation. The oxidation of alcohols to aldehydes are one of the most used reactions in organic synthesis, as the produced aldehyde could be involved in many organic reactions such as chain extension reactions to produce alkenes, hemiacetal, and acetal formation, imine (Schiff base) formation …etc. The classical oxidation of alcohols to aldehydes includes the use of acidified solution of ordinary oxidizing agents such as Jones reagent ($K_2Cr_2O_7$), but the direction of primary alcohol oxidation usually further oxidized to the carboxylic acid, and the reaction is not clean with several safety issues (Omura and Swern, 1978) Swern oxidation is also another classical method, whereby a primary or secondary alcohol could oxidize to an aldehyde or ketone using oxalyl chloride, dimethyl sulfoxide (DMSO) and an organic base, but this reaction is messy and not very common nowadays. Today's the mildest method for selective oxidation of primary alcohols to aldehydes without further oxidation to carboxylic acids is the use of Chromium-based reagents, such as Collin reagent and pyridinium chlorochromate (PCC). Unlike chromic acid, PCC will not oxidize aldehydes to carboxylic acids, if the right mole equivalents are used, and the use of water is avoided, but again this method is not favorable as the chromium is reported as carcinogen compound, and the byproducts (featured in Gary) are Cr(IV) as well as pyridinium hydrochloride is very difficult to remove and affects the overall yield of the reaction (Collins, et al., 1968). In this work, NiO nanoparticles synthesized via a direct precipitation process in the presence of sodium hydroxide. The purpose of this work is to oxidize benzyl alcohol to benzaldehyde selectively by using prepared NiO nanoparticles.

MATERIALS AND METHODS

Synthesis of nickel oxide nanoparticles

All the chemicals were used as received without further purification. The precursor was synthesized by direct precipitation method. In the direct precipitation method, 0.5M of $NiSO_4$ nickel sulphate prepared in 250 ml of distilled water at room temperature leading to a clear green color solution. In another beaker 0.5M NaOH, sodium hydroxide prepared in 250 ml of distilled water. The sodium hydroxide solution was added to the nickel sulphate solution dropwise. The mixture was stirred for 5 hours at room temperature until form the light green precipitate. The precipitate was filtrated, washed three times with distilled water to remove impurities. Finally, the precipitate was dried at room temperature and for forming NiO nanoparticles the precipitate was calcined for two hours at 300°C in the furnace. Until the color of precipitate change from light green to black, which is indicated the NiO nanoparticles.

$$2NaOH_{(s)} \longrightarrow 2Na^{+}_{(aq)} + 2OH^{-}_{(aq)}$$

$$NiSO_{4\,(s)} \longrightarrow Ni^{+2}_{(aq)} + SO_4^{-2}{}_{(aq)}$$

$$Ni^{+2}_{(aq)} + 2OH^{-}_{(aq)} + xH_2O_{(aq)} \longrightarrow Ni(OH)_2.xH_2O_{(s)}$$

$$Ni(OH)_2.xH_2O_{(s)} \xrightarrow{50\text{-}100^oC} Ni(OH)_{2\,(s)} + xH_2O_{(g)}$$

$$Ni(OH)_{2\,(s)} \xrightarrow{300^oC} NiO_{(s)} + H_2O_{(g)}$$

Scheme 1: Precipitate reactions of NiO nanparticles

Catalytic Oxidation reaction

The oxidation of benzyl alcohol over the NiO nanoparticles catalyst was carried out in a magnetically stirred reactor, provided with a mercury thermometer and reflux condenser. By dissolving 1 mmol of benzyl alcohol in 3ml of THF and 3 mmol of catalyst was added to the reaction mixture and heated at 80 ^{o}C. The completion of the reaction was followed by monitoring TLC. After completion of the reaction mixture was diluted with water, acidified with HCl and then extracted with ether, dried over magnesium sulphate, filtered and the solvent was evaporated to give the required aldehyde.

RESULTS AND DISCUSSION

FT-IR Analysis of Nickel Oxide Nanoparticles

An FTIR spectrum of NiO nanoparticles shows several significant absorption peaks. The broad absorption band in the region of 600–700 cm^{-1} is assigned to Ni–O stretching, vibration mode; the broadness of the absorption band indicates that the NiO powders are nanocrystals. The size of samples used in this study was much less than the bulk forms NiO so that NiO nanoparticles had its FTIR peak of Ni–O stretching vibration and shifted to blue direction. Due to their quantum size effect and spherical nanostructures, the FTIR absorption of NiO nanoparticles is blue-shifted compared to that of the bulk form. Besides the Ni–O vibration, it could be seen from Figure 1 that the broad absorption band centered at 3440 cm^{-1} is attributable to the band O–H stretching vibrations and the weak band near 1635 cm^{-1} is assigned to H–O–H bending vibration mode were also presented due to the adsorption of water in the air when FTIR sample disks were prepared in an open air. These observations provided the evidence to the effect of hydration on the structure. Meanwhile, it implied the presence of hydroxyl in the precursor.

UV-Visible Analysis of Nickel Oxide Nanoparticles

The UV–visible spectroscopy measurement was carried out by using a double-beam spectrophotometer Cary 500 scans and operated in the range of 200–500 nm at a resolution of 2.0 nm. The photo-absorption ability of the NiO nanoparticle was detected by the UV–Visible spectrum as shown in figure 2. The NiO nanoparticle showed strong absorption at the wavelength 229 nm. The band gap energy (Eg) of the NiO nanoparticle calculated by the formula: $Eg = 1{,}240/\lambda g$, where λg is the wavelength. The wavelength of the absorption edge of the prepared NiO nanoparticle sample was 229 nm. Thus, the band gap energy estimated from the absorption edge was about 5.41 eV. This result indicates that the NiO nanoparticle suspension has a high ability to absorb ultra-violet light.

XRD Analysis of NiO nanoparticles

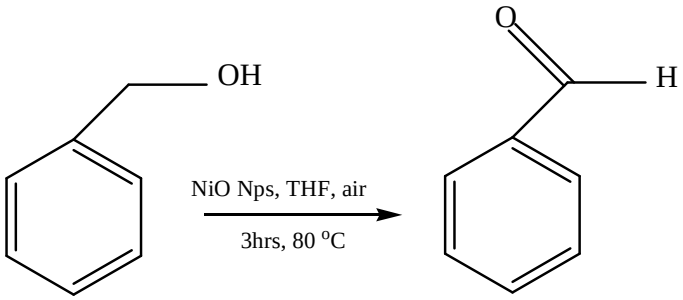
The Structure of the NiO nanoparticles was characterized by using X-ray diffraction (XRD). The XRD was collected by using a Rigaku Mini with Cu Kα radiation (λ = 0.1541 nm). The diffractograms were recorded in a range of 10-90°. Figure 3 shows an X-ray diffraction study of Nickel oxide nanoparticles synthesized by direct precipitation method. From the XRD pattern, it is clear that nanoparticles synthesized purely crystalline in nature. All the peaks found to be the broadened and indicating the formation of small crystallites. The average NiO nanoparticles size was calculated by using the Debye-Scherer formula.

$$D = \frac{K\lambda}{\beta cos\theta}$$

Where 'D' is the particle diameter size , 'K' is the shape factor, 'λ' the X-ray wavelength (0.1541nm), 'θ' the Bragg's angle in radians and 'β' the full width at half maximum in radians. The average crystalline size is calculated by using the above formula is 18nm.

Oxidation of benzyl alcohol into benzaldehyde

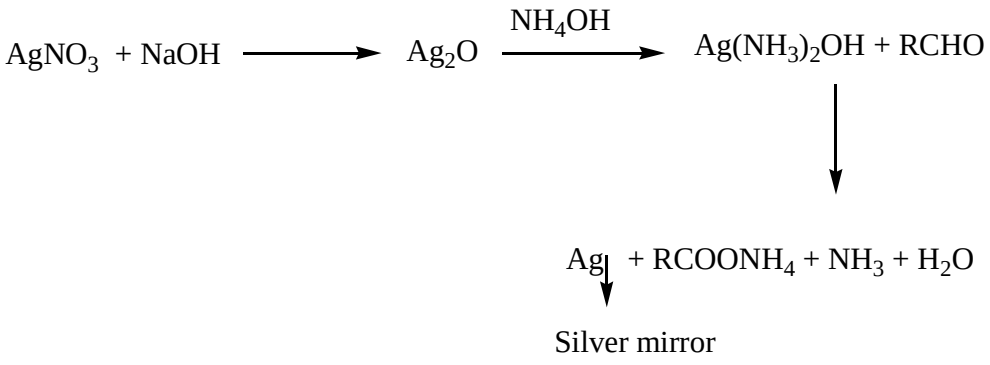
The NiO nanoparticles were used to oxidize benzyl alcohol to benzaldehyde selectively, without further oxidation to carboxylic acid. The oxidation reaction occurred at 80^{0}C and 3hour reaction period in the presence NiO nanoparticle catalyst.



Scheme 2: Oxidation reaction of benzyl alcohol

Substituted benzyl alcohol (p-hydroxy benzaldehyde) was also oxidized successfully using the above condition to check the versatility of the reaction**.** The reaction completion was monitored by the Thin Layer chromatography (TLC). The completion of the reaction was confirmed by TLC, in comparison to both starting material ($PhCH_2OH$) and product (PhCHO) as shown from the TLC figure 4a, (5:1 petroleum ether/Ethyl acetate as eluting solvent) after 30 minutes indicated the top spot for the produced aldehyde and the bottom spot is for the benzyl alcohol as a starting material; Further TLC was checked to follow the reaction process, and it was confirmed that within 3 hours almost all of the benzyl alcohol was oxidized to the corresponding benzaldehyde as shown in figure 4b, there is no or faint amount of benzyl alcohol is left with no furthur oxidation to benzoic acid. The same TLC results were obtained for the oxidation of p-hydroxy benzyl alcohol

Further analysis of the product was conducted by using Tollun's test as a qualitative specific test for aldehydes only. The product showed positive test to the reagent by forming a clear silver mirror.



Scheme 3: Tollun's test for benzaldehyde

In addition to the TLC monitoring of the reaction and Tollun's test as a qualitative specific test for aldehydes, IR spectroscopy was also used as another analysis method for further confirmation of the success of the reaction. Figure 5 shows spectrum for the p-hydroxy benzaldehyde, and it's clearly confirmed the success of the reaction, as shown in the C=O stretching band at 1637 cm^{-1}, and two bands as doublet at 1905 and 2016 cm^{-1}, the first band for the C-H aldehyde functional group and the latter is the overtone of C-H bending of the aldehyde functional group. The other

characteristic bands are also present such as C=C stretching band at 1596 cm^{-1}, phenolic OH at 3156 cm^{-1} and C-O is stretching band at 1159 cm^{-1}.

CONCLUSION

Nickel Oxide nanoparticles catalyst has been successfully synthesized by direct precipitation method. The synthesized catalyst characterized by employing Fourier transform infrared spectroscopy (FTIR) , UV-visible spectrophotometer and X-ray diffraction (XRD) which confirmed the formation of NiO nanoparticles in the range 20-90 nm . The catalytic oxidation of benzyl alcohol to benzaldehyde in presence NiO nanoparticles catalyst carried out at 80^0C and 3 hours reaction time in the reflux condenser. The conversion reaction of benzyl alcohol to benzaldehyde monitored with the help of TLC and the product of the reaction identified by TLC, IR and Tollun's test which was benzaldehyde. For this reason, the present work provides an inexpensive and efficient way for oxidation alcohols.

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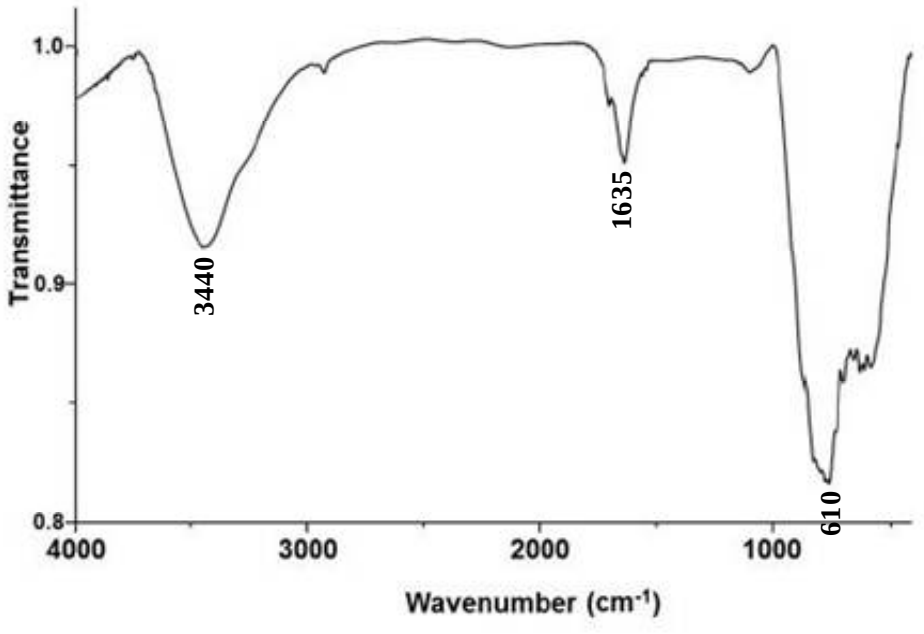

Fig. 1: FT-IR Spectrum for prepared NiO nanoparticle

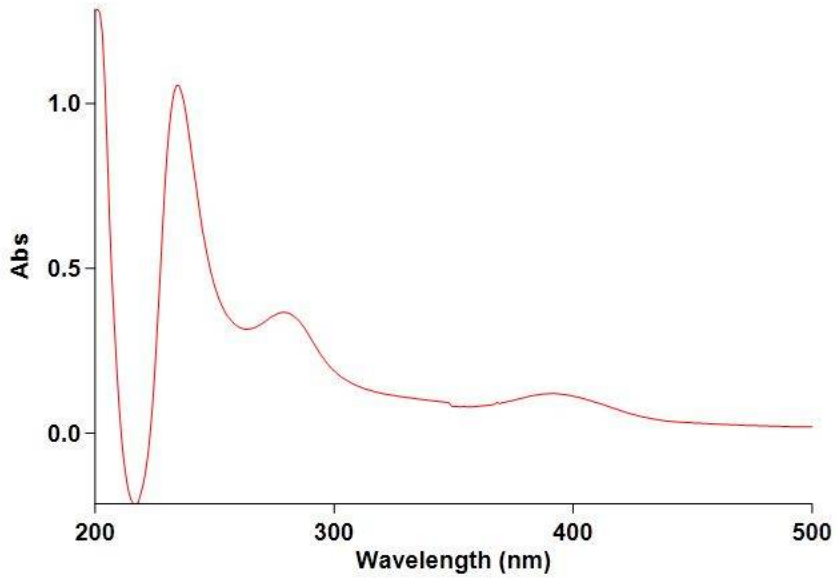

Fig. 2: UV-Visible spectra of NiO nanoparticles

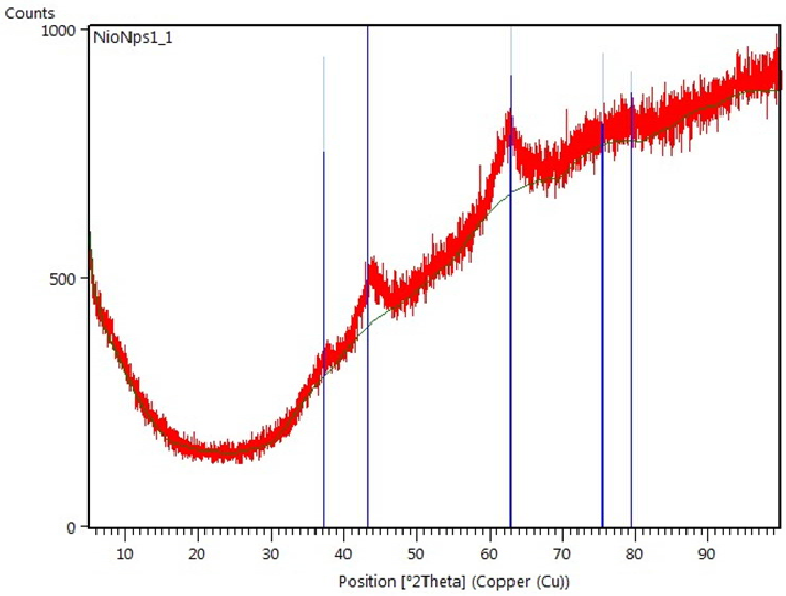

Fig. 3: XRD pattern of NiO nanoparticle

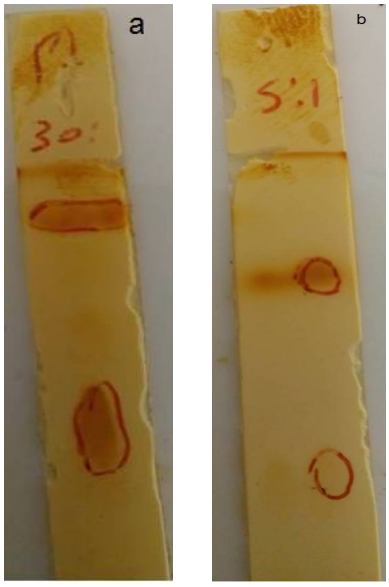

Fig. 4: TLC monitoring for oxidation reaction completion

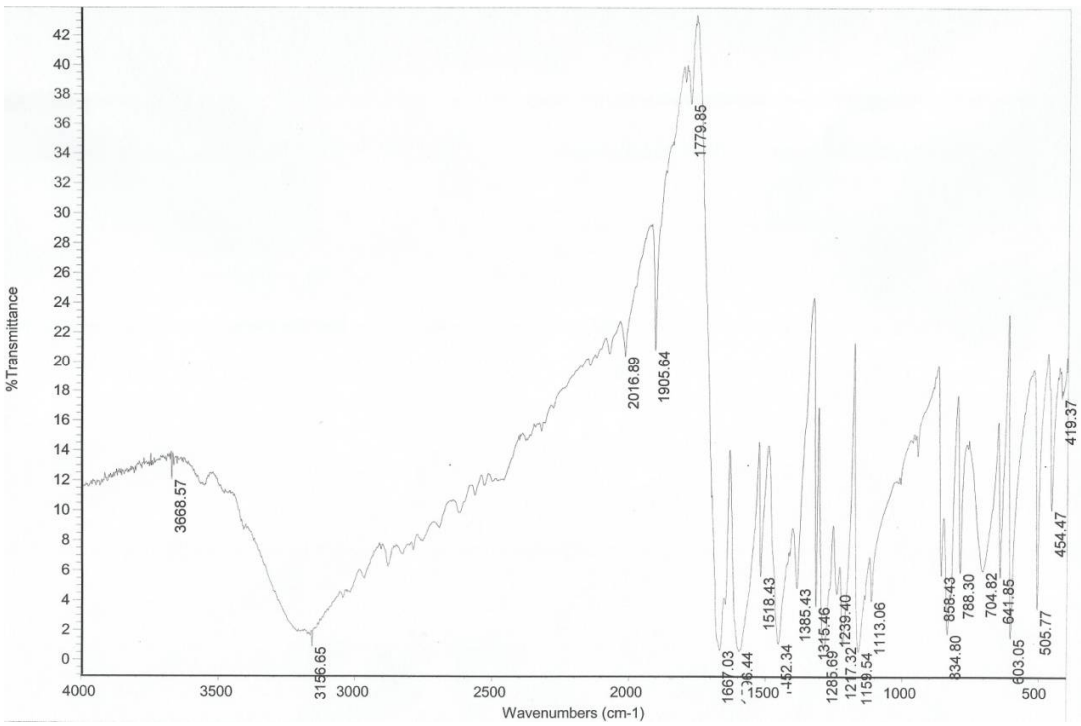

Fig. 5: IR Spectrum for p-hydroxy benzaldehyde

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RESEARCH ARTICLE

Phytochemical Screening and Anti-Bacterial Activity of Silver Nanoparticle Synthesis from *Psidium gujava* L. Leaves Extract

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ABSTRACT

Silver is considered to have an inhibitory effect on microbes and the pure silver can be isolated as nanoparticles using nanotechnology. The silver nanoparticles when reduced by plant sources can yield the green synthesis of silver nanoparticles. The present study was carried out to analyse the phytochemical constituents and antimicrobial activity of silvernanoparticles containing *Psidium guajava* leaves extract. The green synthesis of silver nanoparticles from aqueous silver nitrate was prepared by treating them with*Psidium guajava* leaves extract which used as reductant. The synthesized silver nanoparticles *Psidium guajava* leaves extract were screened for phytochemical studies which revealed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins in the extracts. Antibacterial activities were further done by disc diffusion method against *Escherichia Coli*, *Salmonella typhi* and *Staphylococcus aureus*. Inhibition zone on the plates indicate the anti-bacterial activity of synthesised silver nanoparticles leaves extract of *Psidium guajava*.The results suggest that the silver nanoparticles green synthesis from *Psidium guajava* leaves extractcan be potent natural antioxidants and can be used in drug therapy for those bacterial infection.

Keywords: Anti-bacterial action, Disc diffusion, Guava, Phytochemicals and Silver nanoparticle synthesis.

INTRODUCTION

Use of nanotechnology in drug therapy and drug delivery is easier for the human body to metabolize, utilize in natural mechanism and readily available for uptake by the diseased cells. Silver nanoparticles of biological synthesis such as plant sources are non-toxic and eco-friendly (Aguilar et al., 2011). The use of plant compounds such as phytochemicals are can be of interest in therapeutic and are used in traditional medicinal system in the treatment of infectious disease. Previous studies on various plant compounds were conducted all around the world and hence proved their potential activity against microbial pathogens (Nascimento et al., 2000). Analysis is based on the extraction from plants by any of the methods which includes maceration, infusion, percolation, digestion, decoction, Soxhlet extraction, aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultra sound extraction, supercritical fluid extraction, and phytonic extraction (Green et al., 2004). Amongst all the variety of medicinal species studied, we can highlight *Psidium guajava* L. that are highly implicated in therapeutic medicine against cancer, bacterial infections, inflammation and pain (Mahfuzul et al., 2007). *Psidium guajava* L. (guava) belongs to Myrtaceae family is a shrub from Central and South American origin commonly naturalized in tropical and subtropical parts of the world. Guava leaves are reported for anti-plasmodial, anti-inflammatory, hepatoprotective, anticancer and antioxidant activity. Leaves of *P.guajava* has been previously analysed to contain flavonoids, triterpenoids, tannins, carotenoids and other biologically active secondary compounds which responsive for mentioned useful pharmacological activities (Flores et al., 2015). The present study was to synthesize the silver nanoparticles of *P.guajava* leaves extract and assess them for phytochemical analysis and anti-bacterial activities against *Escherichia Coli*, *Salmonella typhi* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Preparation of plant extract

The leaf samples of guava were washed in tap water, dried, and placed into a blender to be grounded into powder. For aqueous extract, 100 g of powdered leaves was taken in a beaker and suspended in 600 ml of water and mixed. For the methanolic extract, 100 g of powder was added to 500 ml of methanol solvent and mix thoroughly. The filtrates of both extracts were collected through Whatmann filter paper No. 4. This extract was used for preliminary tests and anti-microbial activity. The extract was dried through vacuum rotary evaporator at 20 rpm in a temperature below 40°C, under reduced pressure. The condensed form of extract was collected, transferred to an air-tight container and stored in a freezer at −20°C till subsequent use (Kanojiya et al., 2015).

Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, 0.1M silver nitrate aqueous solution (90ml) was prepared and to this, 10ml of methanolic *Psidium guajava* leaves extract was added. The mixture was vigorously stirred and kept at room temperature. Reduction takes place and a change in colour from light yellow to yellowish brown appeared that indicated the synthesis of nanoparticles (Kheybariet al.,2010).

Phytochemical analysis

Preliminary phytochemical analytical tests such as test for saponins, phenols, tannins, terpenoids and flavonoidsfor screening the bioactive chemical compounds in the guava were carried out with the silver nanoparticles extract using the standard procedure as described by Tanaka et al. (1992).

Antibacterial activity

The bacterial cultures such as *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus* were obtained. Each of the bacterial strains was maintained by cultured on the nutrient agar plates. The colonies formed after overnight incubation were selected and transferred to a glass tube of sterile physiological saline. Antimicrobial activity test was done using the well-diffusion method (Reller et al., 2009) using the silver nanoparticles of *P.guajava* leaves extract. Control for each strain was used. After 24 hours of incubation at 37°C, the plates were observed for inhibition zones which were measured in millimetres using a ruler. Each experiment was checked for their reproducibility using triplicates.

RESULTS AND DISCUSSION

Silver nanoparticle synthesis

The green synthesis of silver nanoparticles using *Psidium guajava* leaves extract was confirmed by change in colour as shown in (Figure 1) which may be due to excitation of surface plasmon vibrations of silver nanoparticles.

Phytochemical analysis

The green synthesis of silver nanoparticles using *Psidium guajava* leaves extract have been analysed for their phytochemical constituents. The test results confirm that the leaves extract contains secondary metabolites like alkaloids, flavonoids, phenolic compounds, saponins and sugars. The results were qualitatively expressed by positive sign and show the observed colour changes in various phytochemical tests as in Figure 2.

Anti-bacterial activity

The green synthesis of silver nanoparticles using *Psidium guajava* leaves extract showed strongest inhibition zone (Figure 3) against three microbes such as *Escherichia Coli*, *Salmonella typhi* and *Staphylococcusaureus* by 12mm, 10mm and 10mm respectively. The results are in line with the study observed by Cho etal. (2005) where anti-microbial activity was observed against *Staphylococcus aureus* and *Escherichia coli* by silver and platinum nanoparticles.

ACKNOWLEDGEMENTS

None declared

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Figure 1: Flasks containing the *Psidium guajava* leaves extract before (a) and after (b) incubation in an aqueous solution of AgNO3 solution.

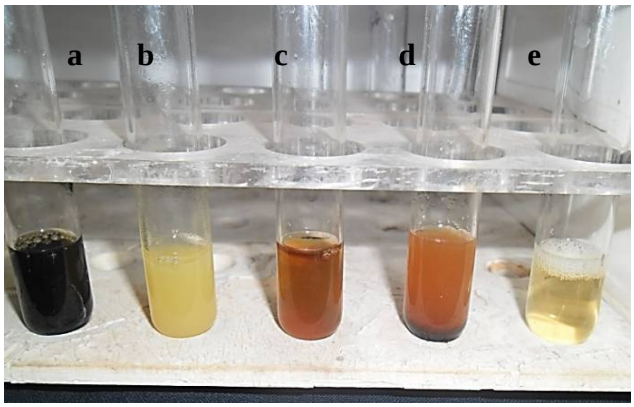

Figure 2: Phytochemical analysis of *Psidium guajava* leaves extract silver nanoparticles showing (a) phenol, (b) Flaavonoids, (c) Sugar, (d) Alkaloids and (e) Saponins.

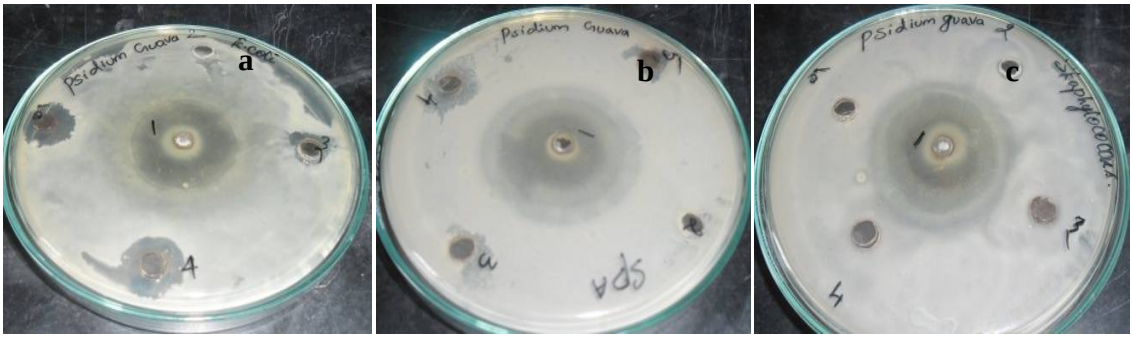

Figure 3: Antibacterial activity of silver nanoparticles (*Psidium guajava)* leaves extract against (a) *Escherichia coli,* (b)*Salmonella typhi* and (c) *Staphylococcus aureus.*

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REVIEW ARTICLE

Transient Heat Transfer Analysis in Laminar Flow Condition: A Review

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ABSTRACT

The flow may be a laminar or turbulent in different types of flow fields. In the laminar flow, the fluid flow is highly ordered and it is possible to identify streamlines along which fluid particles move. The term transient designates a phenomenon which is time dependent. The transient measurement of temperatures is usually performed by mounting sensors embedded inside the heated material surface. The surface heat fluxes are then estimated from the measured temperature history through one-dimensional analytical heat transfer modeling. Transient heat transfer can also be found by using computational fluid dynamics by using following method finite integral transform technique, finite element method, finite volume method ,finite difference method, backward and forward differencing scheme. Conduction of heat in unsteady state refers to the transient conditions wherein the heat flow and the temperature distribution at any point of the system vary continuously with timeMeasurement of transient surface temperature and heat flux is the major requirement in many scientific and engineering applications such as design of combustion chamber in internal combustion engines, design of systems/sub-systems like heat exchanger, steam/gas turbines and thermal protection systems for high speed Aerodynamic vehicles. Therefore the purpose of this work is to review the progress, challenges and opportunities in transient heat transfer research as applied to laminar flow.

Keywords: Transient heat transfer, laminar flow, finite volume method, central differencing scheme, boundary layer, velocity profile, temperature profile

INTRODUCTION

Heat transfer is defined as energy-in-transit due to temperature difference. Heat transfer takes place whenever there is a temperature gradient within a system or whenever two systems at different temperatures are brought into thermal contact. Heat, which is energy-in-transit, cannot be measured or observed directly, but the effects produced by it can be observed and measured. If the temperature of a body does not vary with time, it is said to be in a steady state but if there is an abrupt change in its surface temperature, it attains an equilibrium temperature or a steady state after some period. During this period the temperature varies with time and the body is said to be transient state. The term transient designates a phenomenon which is time dependent. The steady state is thus the limit of transient temperature distribution for large values of time. During an unsteady state the change in temperature may follow a periodic or non-periodic variation. Non-periodic transient state, the temperature at any point within the system varies non-linearly with time. While periodic transient state, temperatures undergo periodic changes which are either regular or irregular but definitely cycle. Transient heat transfer can be calculated when the flat plate is subjected to constant heat flux or constant wall temperature as shown in figure below.

Heat transfer coefficients and the free convection thermal boundary layer about a uniformly heated vertical plate is calculated from Zehnder-Mach interferometer . Plate is immersed in water and the steady state boundary layer ,as well as its transient development from an initial state at rest and with uniform temperature to steady state condition, is investigated when a step function in the power input to the plate is applied .Results for the steady state runs agree very well with the results of an analysis by Sparrow and Gregg. Two experiments are performed. In the first one, the boundary layer characteristics and heat transfer are investigated for steady state condition. The second group dealt with the transient development of the free convection boundary layer and result are excellent agreement with the analysis of sparrow and Gregg and Siegel literature [1].An Analytical solution of transient heat transfer for unsteady incompressible laminar flow between parallel plates is discussed. The solution is first obtained for the case where the inside surfaces of the channel walls undergo a specified step in temperature, that is, the heat transfer resistance of the wall is neglected. Results have been presented for transient heat transfer arising from simultaneous changes with time of the channel wall temperature and fluid pumping pressure in the two-dimensional velocity distribution [2].Forced, convection heat transfer is considered for laminar flow across a flat plate whose surface temperature varies with time. The case analyzed first is that of a step change in surface temperature, and series solutions are obtained which apply for both small and large time. These series results are used to construct an approximate solution which describes the entire time-history of the unsteady heat-transfer process. The following general equation are used in this paper.

$$\theta = \frac{T - T_\infty}{T_w - T_\infty}, \qquad (1)$$

Energy Equation

$$\frac{\partial \theta}{\partial t} + u\frac{\partial \theta}{\partial x} + v\frac{\partial \theta}{\partial y} = \alpha\frac{\partial^2 \theta}{\partial y^2} \qquad (2)$$

The analytical result as described above has excellent agreement with the previous published literature [3]. The general approach to the problem of heat transfer to laminar or turbulent flow in annular passages developed in this paper will be applied and compared with experimental results. In general the superposition concept is found to be supported by the data. The effects of asymmetry in the heat flux of wall temperatures are usually significant, while the influence of axial variations on the local convection heat-transfer processes is less pronounced [4]. Hydrodynamically fully developed flow is discussed in this paper. This includes evaluation of the four fundamental solutions in the thermal energy regions by solution of the eigenvalue problem, including development of asymptotic expressions for the higher Eigen values. The analytical predictions are excellent agreement with experimental

measurements data [5]. Analysis of the laminar flow heat transfer in an annulus with simultaneously developing velocity and temperature distributions and constant wall heat flux is discussed. A solution is obtained first for the hydrodynamic problem, and then for the combined hydrodynamic and thermal problem by an integral method. Results are tabulated for several inner to outer tube radius ratios and Prandtl numbers. Experimental measurements made for Prandtl number = 0.7 showed excellent agreement with the theoretical analysis. The general formula used in this analysis is [6]

$$\frac{\partial u}{\partial x}+\frac{v}{r}+\frac{\partial v}{\partial r}=0 \qquad (3)$$

$$u\frac{\partial u}{\partial x}+v\frac{\partial u}{\partial r}=-\frac{1}{\rho}\frac{\partial p}{\partial x}+\upsilon\left(\frac{\partial^2 u}{\partial r^2}+\frac{1}{r}\frac{\partial u}{\partial r}\right) \qquad (4)$$

$$u\frac{\partial T}{\partial x}+v\frac{\partial T}{\partial r}=\alpha\left(\frac{\partial^2 T}{\partial r^2}+\frac{1}{r}\frac{\partial T}{\partial r}\right) \qquad (5)$$

The present analysis and the subsequent numerical results have clearly demonstrated the usefulness of the extended Leveque solution for computing heat-transfer rates or wall temperatures in the thermal entrance region with fully developed laminar flow. With a maximum of six terms in the fundamental solutions this method takes over at the point where the eigenvalue solutions become cumbersome due to the large number of terms required. The fact that with this type of solution one cannot discriminate between different kinds of boundary conditions at the opposite wall is of no consequence if the solutions are used only for $X < 10^{-2}$; up to this point the differences between solutions of the first and the third kind and between solutions of the second and the fourth kind are quite negligible [7].

Unsteady laminar heat transfer in a duct with periodically varying inlet temperature and time- and space-dependent wall temperature is determined. The wall temperature variation is dynamically determined by a balance of the heat-transfer rate and the energy storage. The series expansion properties of the corresponding complex Eigen functions, which are essential to the solution, are verified. Numerical results are obtained for the time and space dependence of the wall and bulk temperatures and of the Nusselt number.. Wide range of operating conditions, the overall performance can be described by a single curve. For comparison purposes, results for the overall performance are also derived using the quasi-steady model. Quasi-steady results are evaluated for both spatially uniform and spatially varying heat-transfer coefficients. The general equation used in this analysis is [8]

$$\rho c_p\left(\frac{\partial T}{\partial t}+u\frac{\partial T}{\partial x}\right)=k\frac{\partial^2 T}{\partial y^2} \qquad (6)$$

A new analytical method of finding solutions valid for all times is presented. Results for wall flux or wall temperature transients are displayed graphically for Prandtl numbers ranging from 0.01 to 100. For Prandtl number (0.72 to l00), the time required for the thermal layer to achieve steadiness varies inversely with the free stream velocity and directly with 1/3 power of the Prandtl number.

The present analytical procedure provides a relatively simple and rapid means of calculating the instantaneous surface heat flux and temperature characteristics, it does not lend itself conveniently to the determination of the transient temperature field although, in principle, this can be done. The general equations are used in this case are as follow [9].

$$\frac{\partial u}{\partial x}+\frac{\partial v}{\partial y}=0 \qquad (7)$$

$$\frac{\partial T}{\partial t}+u\frac{\partial T}{\partial x}+v\frac{\partial T}{\partial y}=k\frac{\partial^2 T}{\partial y^2}+\frac{\nu}{c_p}\left(\frac{\partial u}{\partial y}\right)^2 \quad (8)$$

$$u\frac{\partial u}{\partial x}+v\frac{\partial u}{\partial y}=\nu\frac{\partial^2 u}{\partial y^2} \quad (9)$$

Incompressible, steady-state laminar flow heat transfer in the combined entrance region of a circular tube for a constant wall heat flux and constant wall temperature is analyzed numerically. The development of velocity profile is obtained from Sparrow's entrance region solution. This velocity distribution is used in solving the energy equation numerically to obtained temperature profiles.varition of heat transfer coefficient for these two different boundary conditions for the early stages of boundary layer formation on the pipe wall is obtained .Local Nusselt numbers are calculated and the results are compared with whose given by Ulrichson and Schmitz.. Nusselt number between present analysis and analysis made by Ulrichson and Schmitz is mainly due to the use of different expressions for the velocity development along the pipe. The general equation used in this paper is [10]

$$k\frac{\partial^2 T}{\partial y^2}=\rho c_p u\frac{\partial T}{\partial x} \quad (10)$$

An analytical solution of transient laminar natural convection in a rectangular cavity containing either one fluid or two immiscible liquids is discussed. The resultant differential equations were integrated numerically and computed results are presented for the transient streamline patterns and for the isotherms, for a variety of conditions including high, low and intermediate values of the Prandtl number. The computed results agree with experimental data, both obtained in this study, and reported by other investigators [11].

Exact solution of transient heat or mass transfer in fully developed laminar tube flows is obtained. The solutions for the impulse and step function response are considered separately for the boundary conditions for a constant wall temperature or concentration and for an adiabatic wall. The solution permits calculation of the local temperatures or concentrations as well as heat or mass fluxes. Typical results are presented for downstream conditions of local temperature and Nusselt number .The general equation is used in this case are as follow [12]

$$\rho C_v\left[\frac{\partial T}{\partial t}+u\frac{\partial T}{\partial x}\right]=k\left[\frac{\partial^2 T}{\partial r^2}+\frac{1}{r}\frac{\partial T}{\partial r}+\frac{\partial^2 T}{\partial x^2}\right] \quad (11)$$

An exact solution of the equation of transient forced convection for time varying inlet temperature with a general, space dependent boundary condition of an incompressible laminar forced convection heat transfer with fully developed flow between two parallel plates is obtained. The finite integral transform technique has been used as the method of analysis. Analytical results for laminar and turbulent flow are presented. The results are confirmed experimentally by the frequency response method [13]

$$\frac{\partial \theta}{\partial t}+u\frac{\partial \theta}{\partial x}=\alpha\frac{\partial^2 \theta}{\partial y^2} \quad (12).$$

An exact analytical solution is obtained for the transient surface heat flux and temperature distribution in the fluid, moving over a plate which is cooled from below, caused by a step change in the fluid temperatureat the plate leading edge. The result has been generalized to handle arbitrary fluid temperature variation with time.The solution indicates, for the step change in fluid temperature at x = 0 and t = 0, the lack of the infinite and very large flux associated with step changes in the wall temperature [14]

$$\frac{\partial\theta}{\partial t}+U_{\infty}\frac{\partial\theta}{\partial x}=\alpha\frac{\partial^{2}\theta}{\partial y^{2}} \qquad (13)$$

Unsteady compressible laminar boundary layer flow on an arbitrary cylinder due to an incident stream whose velocity varies arbitrarily with time is considered. The method presented is based on the separation between the convective and diffusive quantities. By defining some new variables, the splitting appears rather naturally, and the initialization problem can be solved without difficulty. The transformed equations are solved with the help of a semi-implicit finite difference scheme which is unconditionally linearly stable. The computations have been applied to flows past a cylinder with constant and fluctuating free-stream velocities [15].Transient thermal response of a power-law type non-Newtonian, laminar boundary layer flow over a wedge is investigated. Consideration is given to the case of a step change in surface temperature. Details of the transient heat flux and the temperature field are obtained and have been presented graphically. The range of Prandtl numbers investigated is from 5 to 1000 while the viscosity index was allowed to vary from 0.1 to 5.0 [16].The unsteady heat transfer from a solid spherical particle in the Stokes flow is investigated by the finite difference method in the case of intermediate Peclet numbers and high thermal conductivity of the sphere. It is found that when the parameter $(pc)_{21}$, is varied in the range 0.2-22, the relaxation time of the thermal field in the vicinity of the sphere's surface, and the characteristic time of cooling (heating) of the particle are of the same order of magnitude. Under these conditions, the asymptotic regime of heat transfer (at $\tau\rightarrow\infty$) may be considerably different from the steady-state heat transfer. The asymptotic Nusselt number is significantly lower than its corresponding steady-state value. In some cases, the local Nusselt numbers at the rear part of the sphere become negative and an 'attached thermal wake' appears behind the sphere. The asymptotic Nusselt number may be satisfactorily estimated by the use of a simple 'film' model [17].

Transient natural convection heat transfer problem between two horizontal isothermal cylinders is formed within the Boussinesq approximation and solved numerically through the vorticity-stream function approach. Discretizing of vorticity and energy equations is done by alternating direction implicit (ADI) method and the stream function equation by the successive over relaxation (SOR) method. In this case the transient time from the transient state to the steady state is found to be very small in comparison with a typical operation time. Numerical results are summarized by three Nusselt number vs Grashof number curves with the diameter ratio as a parameter, which serve as a guide to natural convective heat transfer calculations for an annulus [18].Unsteady heat transfer in laminar flow of a non-Newtonian fluid flowing over a flat plate is investigated .Effect of Prandtl number and the viscosity index on the transient is also studied. The steady state temperature profile obtained from the model agrees with the published results. The following equation is used in this paper [19].

$$\left(\frac{\partial v_{x}}{\partial x}\right)+\left(\frac{\partial v_{y}}{\partial y}\right)=0 \qquad (14)$$

$$\left(v_{x}\frac{\partial v_{x}}{\partial x}\right)+vy\left(\frac{\partial v_{y}}{\partial y}\right)=\frac{1}{\rho}\left(\partial\tau_{yx}/\partial y\right) \qquad (15)$$

$$\left(\partial T/\partial t\right)+v_{x}\left(\partial T/\partial x\right)+v_{y}\left(\partial T/\partial y\right)=\left(k/\rho c_{p}\right)\left(\partial^{2}T/\partial y^{2}\right) \qquad (16)$$

Method for solving transient problems of a laminar channel or tube flow is presented and the results are given for a step change of inlet fluid temperature for the cases where wall and fluid temperatures are constant initially. This paper is an extension of the method of Sucec for a channel flow and results are compared with those of Sucec. Calculated results are given in the case of a tube flow and these are compared with measured results [20].Effects of conduction heat transfer in the pipe wall on the unsteady forced convective heat transfer in the flow in a long circular pipe resulting from a step change in uniform wall heat tlux over a finite length of the pipe. Substantial influences are

observed for the variations of Peclet number, radius ratio, conductivity ratio, and diffusivity ratio on the transient heat transfer characteristics. In particular, the wall-to-fluid heat capacity ratio is found to have a decisive impact on the unsteady heat transfer in the flow [21].

$$\frac{\partial\theta_f}{\partial c}+P_e\left(1-\eta^2\right)\frac{\partial\theta_f}{\partial\xi}=\frac{1}{\eta}\frac{\partial}{\partial\eta}\left(\eta\frac{\partial\theta_r}{\partial\eta}\right)+\frac{\partial^2\theta_f}{\partial\xi^2} \quad (17)$$

$$\frac{\partial\theta_w}{\partial\tau}=A\left[\frac{1}{\eta}\frac{\partial}{\partial\eta}\left(\eta\frac{\partial\theta_w}{\partial\eta}\right)+\frac{\partial^2\theta_w}{\partial\xi^2}\right] \quad (18)$$

Unsteady entrance heat transfer in the combined entrance heat transfer region of laminar pipe flows resulting from time-varying inlet temperature are numerically investigated .Three non-dimensional parameters,Nu_0 a*,and f are identified in the study.Also,their effects on the non-dimensional duct wall heat flux are discussed in great detail.Comparisions are made with the zero thermal capacity wall solution [22]. The problem of unsteady laminar flow and heat transfer in an incompressible micro polar fluid past an infinite porous flat plate is investigated numerically .The flat plate is subjected initially to constant suction velocity followed by a step function of time t > 0.An explicit finite difference scheme has been used to solve the governing equation of motion and energy .The velocity ,micro rotation and temperature distribution have been displayed through graphs for various values micro polar parameters R at different time levels [23].Unsteady free convection from the outer surface of a vertical circular cylinder is analyised numerically. The ordinary differential equation are solved numerically using a fourth order Runge-Kutta scheme and the gradient method .Numerical results are obtained for the study of boundary layer characteristics. The general analysis developed in this study corresponds to the case of surface temperature that varies exponentially with time and uniform with respect to the axial coordinate.

The effect of Prandtl number on the boundary layer characteristics and the maximum value of the vertical component of the velocity are studies [24].The axial distribution of the air-side heat and mass transfer coefficients occurring in regenerative dehumidifiers is evaluated from experimental breakthrough curves obtained from single-blow experiments. It is shown that the heat and mass transfer coefficients vary little with position in the flow direction for the slowly moving mass transfer wave. This result validates the assumption of constant transfer coefficients in the modelling of rotary dehumidifiers. The technique which is used to compute the distributions was introduced by Ghezelayagh and Gidaspow for isothermal mass transfer. This study extends the use of this technique to simultaneous heat and mass transfer occurring in desiccant regenerators. It is shown that this technique can provide a powerful procedure for analyzing experimental breakthrough curves.

The variation of the transfer coefficients due to axially varying wall fluxes can also be examined by Graber's analysis. His correlation shows that the variation of the local Nusselt number is proportional with the first derivative of the logarithm of the wall heat flux with respect to position in the flow direction [25].Theoretical study of laminar forced convection in the thermal entrance region of a rectangularduct, subjected to a sinusoidally varying inlet temperature, is presented. Several boundary conditions that account for uniform wall heat flux and/or external convections with or without wall thermal capacitance effects are considered. Analytical expressions for these problems are obtained through extending thegeneralized integral transform technique. The centerline temperature amplitudes are determined as a function of Biot number, fluid-to-wall thermal capacitance ratio and dimensionless inlet frequency of inlet heat input oscillations. The effects of these variables on the solution are discussed. The eigen values and corresponding coefficients are given in tabular forms [26].The following general equation are used in this paper

$$\frac{\partial T}{\partial t}+u(y)\frac{\partial T}{\partial x}=\alpha\frac{\partial^2 T}{\partial y^2} \quad (19)$$

Approximate analytical method based on a linearization of the energy equation is developed for the area mean Nusselt number for free convection heat transfer from isothermal spheres for the range of Rayleigh number $0 \leq Ra \leq 10^8$ and all Prandtl numbers. In the process of linearization, the energy equation is reduced to the form of the transient heat conduction equation for which the solution exists. Comparison of the final correlation of Nu against Ra (which is an explicit form of linear superposition of the diffusive limit and boundary layer solution) with other correlation and experimental air data reveals very good agreement with a maximum difference of less than 5% [27].Unsteady non-similar forced convection flow over a longitudinal cylinder, which is moving in the same or in the opposite direction to the free stream, has been investigated. The unsteadinessis due to the free stream velocity, cylinder velocity, surface temperature of the cylinder and the mass transfer, and non-similarity is due to the transverse curvature. The partial differential equations, governing the flow, have been solved numerically, using an implicit finite-difference scheme in combination with a quasi-linearization technique. The results show that both, skin friction and heat transfer, are appreciably affected by the free stream velocity distributions and by the cylinder velocity. Also, skin friction as well as heat-transfer is found to increase as the transverse curvature or the suction increases, but the effect of injection is just the opposite, The heat transfer is significantly affected by the viscous dissipation and variation of surface temperature with time [28].

$$(ru)_x + (rv)_r = 0 \qquad (20)$$

$$u_t + uu_x + vu_r = \left(v/r\right)(ru_r)_r + (u_e)_r \qquad (21)$$

$$T_t + uT_x + vT_r = Pr^{-1}\left(v/r\right)(rT_r)_r + (\mu/\rho c_p)(u_r)^2 \qquad (22)$$

Numerical solution for the transient natural convection over heat generating vertical cylinders of various thermal capacities and radii is presented. A fully implicit finite difference technique is used to solve the non-linear set of equations. The rate of propagation of the leading edge effect is given special consideration. It is found that this rate, predicted by the one-dimensional conduction solution, is slower than that resulting from the boundary layer solution. Also, it increases as the radius and thermal capacity of the cylinder decrease, and as the surface heat flux increases. The transient boundary layer thickness is found to exceed its steady-state value while the transient average heat transfer coefficient is found to reach a minimum, as low as 53% of its steady-state value for the highest value of the modified Grashof number studied. Excellent agreement with previous experimental steady-state data as well as with one-dimensional theoretical results is obtained [29].The transient heat transfer in forced convection for simultaneously developing laminar flowinside a parallel-plate channel is studied by solving the steady momentum equation with the generalized integral transform technique and the transient energy equation through a hybrid approach that combines the integral transform method with a second-order accurate finite-differences scheme. Semi-analyticalresults are then presented for the fluid bulk temperature and local Nusselt number along the channel as a function of position and time [30].An inverse problem utilizing the conjugate gradient method of minimization with adjoint problems used to estimate the timewise variation of the inlet temperature of a thermally developing hydrodynamically developed laminar flow between parallel plates by utilizing transient temperature measurements from a single thermocouple located downstream of the entrance. The effects of functional form of inlet temperature, sensor position magnitude of measurement error and data sampling rate on the accuracy of estimates are examined. The inverse analysis considered here could predict the time wise variation of inlet temperature even under such strict conditions The estimates are notably more accurate when the thermocouple is placed near the entrance [31].

Steady-state natural convection over a sphere has been studied numerically. Heat transfer and drag coefficient for a wide range of Grashof number ($10^1 \leq Gr \leq 10^8$) and prandtl numbers 0.72 and 7.0 have been obtained. A plume with a mushroom-shaped front forms above the sphere whose length and thickness decrease with increasing Gr.At high Gr ($Gr \geq 10^7$ and Pr=0.72),flow separation and an associated recirculation vortex exist in the wake of the sphere. The vortex size increases with Gr.The local Nusselt number along the sphere surface first decreases, reaches a minimum, and then increases steeply at the rear of the sphere [32].

$$\nabla.V = 0 \quad (23)$$

$$\rho\frac{\partial v}{\partial t} + \rho(V.\nabla)V = -\nabla p + F + \mu\nabla^2 \quad (24)$$

$$\rho c_p\frac{\partial T}{\partial t} + \rho c_p(V.\nabla)T = k\nabla^2 T \quad (25)$$

Numerical study for the periodically fully-developed flow in two-dimensional channels with streamwise-periodic round disturbances on its two walls is obtained. To accurately describe the round disturbance boundary condition, a body fitted grid was used. The flow and heat transfer have been studied in the range of Reynolds number and Prandtl number Pr=0.71. The influences of disturbance parameters and Reynolds number on heat transfer and friction have been investigated in detail. Some of the solutions have been examined using both steady and unsteady finite difference schemes; and the same results have been obtained. The results show that different flow patterns can occur with different deployments of the disturbances. With appropriate configuration of the disturbances, the Nusselt number can reach a value four times greater than in a smooth channel at the same condition, with the penalty of a much greater pressure drop [33].Heat transfer characteristics and the flow behavior of cross flow over a transversely oscillating cylinder are investigated. The lock-on phenomenon has been predicted numerically and its influence on the heat transfer performance of the cylinder is evaluated. The SOLA method is employed to solve the unsteady velocity field in a non-inertial reference frame and the energy equation is solved by a finite volume method. Transient variations of the Nusselt number and the drag and lift coefficients are calculated for various oscillation calculations. The range of the dominant parameters considered in this study are $0 \le Re \le 300$, $0 \le S \le 0.3$ and $0 \le A/D \le 0.7$.The prandtl number is considered to be 0.71 or 7.0.In the lock-on regime, an appreciable heat transfer increase caused by the oscillation is observed ;however ,outside this regime, the heat transfer is almost unaffected by the oscillation. A correlation formula expressing the dependence of heat transfer on these dominant parameters in this lock-on regime is presented.

The numerical predictions have been compared with the existing information, and good agreement has been found [34]. Numerical investigation of the two-dimensional laminar flow of air around a heated circular cylinder has been carried out. A very efficient finite volume code employing multi-grid and local grid refinement techniques led to highly accurate results for the drag coefficient and Strouhal and Nusselt numbers. In the Reynolds number range 10^{-4} – 200 the characteristics of the different flow regimes are described. The behavior of the relevant parameters C_D, St and Nu with the Re is investigated in detail first for the case of a small temperature loading (τ=1.003), when the fluid properties may be treated as constant. Good agreement is found in comparison with available analytical solutions and also with experimental data in the range of their validity. The critical Reynolds number where vortex shedding starts is determined to be Re_c = 45.9. The temperature loading is varied between 1.003 and 1.5 and the resultant effect on the C_D, St and Nu is analyzed [35]. Unsteady laminar flow and heat transfer of a particulate suspension in an electrically conducting fluid through channels and circular pipes in the presence of a uniform transverse magnetic field is formulated using a two-phase continuum model. Two different applied pressure gradient (oscillating and ramp) cases are considered. The general governing equations of motions (which include such effects as particulate phase stresses, magnetic force, and finite particle-phase volume fraction) are non-dimensional zed and solved in closed form in terms of Fourier cosine and Bessel functions and the energy equations for both phases are solved numerically. Numerical solutions based on the finite difference methodology are obtained and graphical results for the fluid-phase volumetric flow rate, the particle-phase volumetric flow rate, the fluid-phase skin-friction coefficient and the particle-phase skin-friction coefficient as well as the wall heat transfer for plane and axisymmetric flows are presented and discussed [36]

$$\frac{\partial F}{\partial \tau} = \frac{\partial^2 F}{\partial \eta^2} + \frac{j}{\eta}\frac{\partial F}{\partial \eta} + k\alpha(F_p - F) - M^2 F + G(\tau) \quad (26)$$

$$\frac{\partial F_p}{\partial \tau} = \beta \frac{\partial^2 F_p}{\partial \eta^2} + \frac{j}{\eta}\frac{\partial F_p}{\partial \eta} + \alpha\left(F - F_p\right) \quad (27)$$

$$\frac{\partial \theta_p}{\partial \tau} = \frac{1}{Pr}\left(\frac{\partial^2 \theta}{\partial \eta^2} + \frac{j}{\eta}\frac{\partial \theta}{\partial \eta}\right) + Ec\left(\frac{\partial F}{\partial \eta}\right)^2 + Eck\alpha\left(F_p - F\right)^2 + EcM^2F^2 + k\gamma\varepsilon\left(\theta_p - \theta\right) \quad (28)$$

Where

$\beta = \mu_p / \mu$

$\alpha = a^2\rho/(\mu\tau_v)$

$M = \sqrt{\sigma/\mu}\, B_0 a$

$Pr = \mu c / k$

$\gamma = c_p / c$

$Ec = \left(G_0 a^2/\mu\right)^2/\left(cT_1\right)$

$\varepsilon = a^2\rho/\left(\mu\tau_T\right)$

are the viscosity ratio, momentum inverse Stroke number, the Hartmann number, the Prandtl number, the specific heat ratio, the Eckert number, and the temperature inverse Stokes number.Transient forced convection heat transfer from a fixed, semi-infinite, flat plate situated in a fluid which, at large distances, is, moving with a constant velocity parallel to the plate. Both the fluid and the plate are initially at a constant temperature and the transients are initiated when the zero heat flux at the plate is suddenly changed to a constant value. The thermal boundary-layer equations are solved using numerical techniques to extend a series which is valid for small times and describe fully the development from the initial unsteady state solution (small times) to the ultimate steady state solution (large time) [37].Transient heat transfer in a steady and two-dimensional (2D) laminar boundary layer flow on a wedge with sudden change of thermal boundary conditions of uniform wall temperature (UWT) and heat flux (UHF) is discussed. Additionally, a correlation of unsteady forced convection was also formulated through an exact solution of transient heat conduction (ξ=0) and the similarity solutions of a steady forced convection on a wedge (ξ= 1) in this study. Particularly, for the wedge with -0.198838 $\leq \xi \leq$1, the deviation of the wall temperatures estimated by correlation is less than 7.5% within full time of $0 \leq \xi \leq 1$ comparing with numerical results in the case of UHF ranging from Pr= 10^{-4} to10^4 [38].Pulsation effect on heat transfer in laminar incompressible flow, which led to contradictory results in previous studies, is theoretically investigated in this work starting from basic principles in an attempt to eliminate existing confusion at various levels. First, the analytical solution of the fully developed thermal and hydraulic profiles under constant wall heat flux is obtained. It eliminates the confusion resulting from a previously published erroneous solution.

The physical implications of the solution are discussed. Also, a new time average heat transfer coefficient for pulsating flow is carefully defined such as to produce results that are both useful from the engineering point of view, and compliant with the energy balance. This rationally derived average is compared with intuitive averages used in the literature. New results are numerically obtained for the thermally developing region with a fully developed velocity profile. Different types of thermal boundary conditions are considered, including the effect of wall thermal inertia. The effects of Reynold and Prandtl numbers, as well as pulsation amplitude and frequency on heat transfer are investigated. The mechanism by which pulsation affects the developing region, by creating damped oscillations along the tube length of the time average Nusselt number, is explained [39]. Numerical simulation is performed to study the flow structures and heat transfer characteristics of a heated transversely oscillating cylinder in a cross flow. The variations of flow and thermal fields are classified into a class of moving boundary problems. The moving interfaces between the fluid and cylinder have been considered. An arbitrary Lagrangian–Eulerian kinematic

description method is adopted to describe the flow and thermal fields. A penalty consistent finite element formulation is applied to solve the governing equations. The subsequent developments of the vortex shedding and heat transfer characteristics around the heated cylinder are presented in detail. The effects of Reynolds number, oscillating amplitude, oscillating speed on the flow structures and heat transfer characteristics are examined. The results show that the interaction between the oscillating cylinder and vortex shedding from the cylinder dominates the state of the wake. The flow and thermal fields may approach a periodic state in lock-in regime. The heat transfer of the cylinder in the lock-in regime is enhanced remarkably [40].

Numerical study of the transient forced laminar convection flow over a flat plate, when thermal conditions are due to arbitrary wall heat flux variations in space. The energy governing equation is modelled using the Karman–Pohlhausen integral approach in the wide range of Prandtl numbers. The influence of both the thermal problem nature (transient heating and/or cooling processes) and the wallflux function on the resulting mathematical expressions is evidenced and the thermal boundary layer thickness behavior is discussed. Detailed thermal responses and convective heat coefficient evolutions due to the change of wall conditions are presented. Finally, a simple relationship has been proposed to obtain a first evaluation of the transient duration suitable whatever the thermal problem nature, namely heating or cooling one [41].

Unsteady laminar flow and heat transfer in a sharp 180° bend is studied numerically to investigate a convective heat transfer regime of special relevance to electronic systems. Unsteady heat transfer simulations are performed for $50 \leq Re \leq 1000$. Results show that the flow remains steady until $Re \approx 600$. In this steady regime, the re-attachment length increases gradually with the Reynolds number. For $Re > 600$, the flow becomes unsteady with large-scale vortices emanating from the sharp edge dominating the flow field. Flow oscillation causes a substantial reduction in the re-attachment length and a dramatic heat transfer enhancement. As the vortices move downstream, the Nusselt number along the wall oscillates significantly. The correlation between the flow structure and the heat transfer is found to be strong [42].

The finite difference method was applied for the numerical simulation of unsteady laminar flow and forced convection from a fixed cylinder placed in a uniform flow. Primitive variable formulation is used for the fluid flow, and the fluid is assumed to be incompressible and of constant property. The viscous energy dissipation term is neglected in the energy equation since its value is small at low Reynolds numbers. By using boundary-fitted coordinates, interpolation of the boundary conditions becomes unnecessary. An orthogonal transformation provides a fine grid scale in the vicinity of the cylinder and a coarse grid in the far field. Time derivatives are approximated by forward differences, space derivatives by fourth order central differences, except for convective terms which were approximated by a third order modified upwind scheme. The code developed is applied to the investigation of both flow around a circular cylinder and forced convection from the cylinder. The non-dimensional vortex shedding frequency (Strouhal number), time-mean values of drag and of base pressure coefficients, further the root-mean-square values of lift, drag, base pressure, and Nusselt number are determined for Reynolds numbers from 50 to 180. Where possible, results are compared with experimental data and excellent agreement was obtained. The distribution of the local Nusselt number over the cylinder surface was also investigated over a complete cycle. It was found that the curves belonging to different phases are similar in shape and magnitude, but shift slightly over the whole periphery of the cylinder, and the shift is largest on the downstream side of the cylinder. This shift increases with increasing Reynolds number.

The good agreement found between experimental and computational values encourages the author to extend the investigation in the future to the cases of forced convection from an oscillating cylinder, and to the three-dimensional case [43].Transient laminar heat transfer of a rotating disk heated to a constant initial temperature and sudden subjection to unsteady cooling by still air. Both numerical simulations of conjugate heat transfer inside the disk and convection between the disk and air, as well as a self-similar solution show that the heat transfer coefficient becomes time independent very quickly and equal to its value at steady-state conditions. It appeared that the widely

employed solution of unsteady one-dimensional heat conduction in a semi-infinite plate over estimates significantly the disk temperature and consequently the heat transfer coefficient calculated from this solution using known experimental instant disk surface temperature [44].The transient natural convection has been analyzed for the temperature dependent viscosity of fluids in spherical annulus and between two vertically eccentric spheres. Using the modified Sorenson's method to generate the grid line can get the grid system with orthogonality along all boundaries. The grid system goes along with weighting function scheme to discretize the general governing equation. Numerical solutions are obtained for Rayleigh numbers (5.0×10^3–6.5×10^4) at a radius ratio of 2.0 with the dimensionless vertical eccentricity of the outer sphere for variable viscosity fluids at different Prandtl numbers (158, 405 and 720). The results of this analysis show that heat and flow patterns vary with the Rayleigh number and the eccentricity; besides, the effect of variable viscosity is investigated. The present calculations applied to constant viscosity are compared with the results of other papers, and these comparison results are agreeable [45].

Conjugate heat transfer by forced convection through an externally heated pipe has many important engineering applications. In the present work, the radial and axial heat conductions and thermal stresses in a pipe with uniform or non-uniform wall heat flux of fully developed laminar forced convective conjugate heat transfer are considered for analysis. The analysis is based on the two-dimensional steady-state heat conduction equation and laminar boundary layer equation for the flowing fluid by using a finite difference scheme. Water has been used as a fluid. Numerical calculations have been performed by using the FLUENT 4.5. The temperature and stress ratio distributions inside the pipe wall, heated from the outer surface by applying uniform and non-uniform heat fluxes, are presented for two different mean flow velocities. The temperature distributions of the flowing fluid inside the pipe have also been presented for all investigated cases [46].Two-dimensional Naiver -Stokes and energy equations are solved numerically for unsteady laminar flow in periodic wavy (sinusoidal and triangular) channels. The flow in the channels has been observed to be steady up to a critical Reynolds number. Beyond the critical Reynolds number the flow becomes self-sustained quasi periodic oscillatory. This transition of flow occursat lower Reynolds number for triangular channel relative to sinusoidal channel. The frequencies of oscillations, the friction factors and Nusselt numbers are reported [47].

The steady and incompressible flow of power-law type non-Newtonian fluids across an unconfined, heated circular cylinder is investigated numerically to determine the dependence of the individual drag components and of the heat transfer characteristics on power-law index ($0.5 \le n \le 1.4$), Prandtl number ($1 \le Pr \le 100$), and Reynolds number ($5 \le Re \le 40$). The momentum and energy equations are expressed in the stream function/vorticity formulation and are solved using a second-order accurate finite difference method to determine the pressure drag and frictional drag as well as the local and surface-averaged Nusselt numbers and to map the temperature field near the cylinder. The accuracy of the numerical procedure is established using previously available numerical and analytical results for momentum and heat transfer in Newtonian and power-law fluids. The results reported herein provide fundamental knowledge of the flow and heat transfer behavior for the flow of non-Newtonian fluids over a circular cylinder; these results further show that the effect of the power-law index on such behavior is strongly conditioned by the kinematic conditions and less so by the type of thermal boundary condition prescribed atthe cylinder surface [48].

The effect of Peclet number ($0.7 \le Pe \le 4000$) and blockage ratio (β = 1/8, 1/6 and 1/4) on the flow and heat transfer characteristics of a square cylinder confined in a planar channel has been investigated in the Reynolds number range $1 \le Re \le 45$ in the 2D steady flow regime. The effect of the type of thermal boundary condition at the cylinder surface, i.e., constant cylinder temperature and constant heat flux, on the rate of heat transfer has also been studied. The use of the constant heat flux boundary condition yields slightly higher values of the Nusselt number than those for the constant temperature case under otherwise identical conditions of β, Re and Pr. The difference in the computed values of the average Nusselt number for the two types of thermal boundary conditions increases as the Prandtl number is increased for fixed values of the Reynolds number for all blockage ratios. Also, this difference increases with the increasing Reynolds number for fixed value of Prandtl number for all blockage ratios. The local Nusselt number variation on each face of the cylinder has been determined. Further insights into the role of blockage ratio,

Reynolds and Prandtl number on the detailed flow and temperature fields have been provided by including streamline, vorticity and constant temperature contour plots. The average Nusselt number increases monotonically with an increase in the Reynolds number and/or Prandtl number. Finally, heat transfer correlations have been obtained for the constant temperature and constant heat flux cases on the solid square cylinder in crossflow over the range of physical parameters considered in this study [49].Convective heat transfer from a rectangular cylinder placed in the middle of a channel was investigated numerically. The unsteady laminar flow equations were discretised using finite volume method for the range of Reynolds numbers between 50 and 200. Computations were performed for cylinder aspect ratios of 0.5, 1 and 2. Results of flow and thermal fields areobtained for both the instantaneous and mean flow. Nusselt number distribution along each side of the cylinder showed that front side has maximum heat transfer rate compared with other sides. Results of mean total Nusselt number variation with Reynolds number for different aspect ratios showed that increasing aspect ratio decreases total Nusselt number for the Reynolds numbers considered in this study. Heat transfer from a rectangular cylinder in unsteady laminar flow showed that increasing aspect ratio in the range of 0.25 to 4 decreases mean total Nusselt number, while increasing Reynolds number in the range of 100 to 200 increases Nusselt number [50].

The effects of Reynolds number (Re), Prandtl number (Pr), and power law index (n) on the heat-transfer characteristics of an unconfined sphere submerged in an isothermal and incompressible power law fluid, for different thermal boundary conditions (isothermal and isoflux) on the sphere surface, are investigated numerically for the 2-D axisymmetric and steady flow by using a finite volume method over the ranges of conditions as $5 \leq Re \leq 200$, $1 \leq Pr \leq 400$ (the maximum value of the Peclet number is 2000), and $0.5 \leq n \leq 2$. Based on the numerical results obtained herein, simple heat-transfer correlations are developed for the constant-temperature and the constant heat-flux boundaryconditions to estimate the value of the Nusselt (Nu) number in a new application. Furthermore, the variation of the local Nu over the surface of the sphere has been studied to delineate the effects of Re, Pr, and n on heat transfer from a sphere, thereby showing the extent of heat transfer from the front and the rear parts of the sphere [51].An inverse thermal problem is considered for two-phase laminar flow in a parallel plate duct. The inlet temperature, which varies temporally as well as spatially, is estimated when measured temperatures are available at downstream of the duct. In the present study, the problem is solved through a minimization of an objective function by using two regularization methods, i.e., the iterative conjugategradient method (CGM) and the Tikhonov regularization method (TRM). The effects of the functional form of inlet temperature profile, the number of the measurement points and the measurement errors are investigated and discussed. The computational accuracy and efficiency of these two regularization method are compared and discussed [52]

.In the present study, the effects of Prandtl number($0.7 \leq Pr \leq 400$) and of the two commonly used thermal boundary conditions on the forced convection heat transfer from an unconfined sphere have been investigated in the Reynolds number range of $5 \leq Re \leq 200$ in the steady symmetric regime. For $Re > 20$, the local Nusselt number on the sphere surface decreases from its maximum at the front stagnation point up to the point of separation and then again increases from the point of separation to the rearstagnation point. The average Nusselt number increases monotonically with Reynolds number and/or Prandtl number and it are always higher for the UHF boundary condition than that for the CWT condition. While the present results for the CWT condition are in good agreement with the previous results, no prior results are available for the UHF condition. Finally, Eqs.(14) and (15)capture well the dependence of Nusselt number on the Reynolds and Peclet numbers. In addition, the dependence of the mean Nusselt number on the Reynolds and Prandtl numbers has been linked to the distribution of the local Nusselt number on the surface of the sphere [53].An investigation of 2D heat conduction effects on the transient heat transfer of a rotating disk heated up to a non-uniform initial temperature and suddenly subjected to unsteady cooling by still air. A self-similar solution of the transient laminar convective heat transfer confirmed that the heat transfer coefficient rapidly becomes time-independent and equal to its value at steady-state conditions. An analytical solution of the unsteady two-dimensional heat conduction inside a disk made of Plexiglas confirmed that the known infinite-slab approach can still be used as a transient technique for determining heat transfer coefficients. Use of the regular heat transfer regime theory for the same purpose can be recommended only for the cases with the moderate initial

temperature non-uniformity [54]. Flow in a laminar boundary layer is modeled using a slip boundary condition. The slip condition changes the boundary layer structure from a self-similar profile to a two-dimensional structure. Although the slip condition generally leads to decreased overall drag, two-dimensional effects cause local increases in skin friction. Other effects include thinner boundary layers, delayed transition to turbulence, and changes in the heat transfer at the wall. Without a thermal jump condition, slip will lead to increased heat transfer. When a thermal jump boundary condition is added to simulate real gases, the heat transfer decreases to below the no-slip values .The equations for a laminar boundary layer can be solved non-dimensionally in the presence of slip. The boundary layer loses the self-similarity of the no-slip Blasius solution. The loss of self-similarity leads to an increase in skin friction under some conditions. Other effects of slip included a thinner boundary layer and a more stable velocity profile. The heat transfer in the boundary layer is also affected by the presence of slip. In liquid flows, where there is no temperature jump, the heat transfer increases as the slip velocity increases. In gas flows, a temperature jump condition is added and shown to scale with the velocity slip. The presence of the thermal jump condition decreases the heat transfer in the system. For slightly rarefied flows, increased fluid velocity near the wall more than offsets effects of the temperature jump, and the heat transfer will still be greater than for the no-slip case. For more rarefied flows, the heat transfer will decrease to values less than those of the no-slip case. These results can be applied to analyze a variety of systems, including potential micro device designs and flight in low-density Atmospheres [55].

The full Naiver Stokes equations and the energy equation for laminar natural convection heat transfer over an isothermal sphere have been discretized using the finite control volume formulation and solved by employing the SIMPLEC method. Transient and ‘‘steady-state’’ results have been obtained for a wide range of high Grashof numbers ($10^5 \leq Gr \leq 10^9$) and a wide range of Prandtl numbers (Pr = 0.02, 0.7, 7 and 100).A plume with a mushroom-shaped cap forms above the sphere and drifts upward continuously with time. The upward movement of the plume cap is slowed as the Prandtl number increases. The size and the level of temperature of the transient cap and plume stem decrease with increasing Gr and Pr. The time at which the ‘‘steady-state’’ is reached, increases with the Prandtl number. The presence of a vortex in the wake of the sphere has been predicted and has been clearly delineated as a function of both Grashof and Prandtl numbers. The overall Nusselt numbers and total drag coefficients for the range of Grashof and Prandtl numbers investigated are presented and they are in very good agreement with studies available in the literature [56].

A coupled solver for the solution of the transient conjugate heat transfer problem is developed and validated against analytical results. The time accurate solution is obtained in a multi physics and multi domain configuration through an iterative boundary condition exchange. Several transient problems are investigated transient heat transfer in composite solids; the effect of oscillating inlet temperature in laminar forced convection and the transient heat transfer problem in developing and fully developed laminar pipe flow. The results confirm the accuracy of the new solution method in time and its suitability for the coupling of different specific numerical solver in a multi physics environment. For the solution of thermal coupled problem, the boundary coupling presented is an effective alternative to the solution of a fully coupled monolithic system. The advantages reside in the possibility of utilizing existing dedicated solvers for the coupled solution of specific interacting thermo-physical problems. The validating cases showed demonstrate a high accuracy in time of the method in simple test cases that are representative of Industrial configurations [57].An analytical and experimental method to find the transient temperature distribution in a flat plate subjected to a convective heat transfer on a face and to a heat flux on the other face. The heating flux is maintained during a t_1 time. The heating phase is followed by a relaxation one. The theoretical method is uses the Green’s functions method to determine the analytical solutions of the heat propagation equation in the plate during the heating and relaxation phases. These analytical solutions find the temperature distribution as well as wall heat flux versus time. Results are presented in this article as evolutions of dimensionless temperatures with the Biot and Fourier numbers. These models as well as the theoretical method can be useful to evaluate influence of parameters such as the conductivity or the diffusivity of a plate, during the identification of the boundary conditions like the heat flux or the convection coefficient, in industrial processes confronted with this kind of systems or else to teach

transient conduction using an innovative approach, not much used in thermal science and based on the use of the Green's function. The basic equation and boundary condition are [58].

With H=$\frac{h}{k}$ and assumed to be independent from both x and t

The following equation are obtained in this article

$$\theta^*(0,F_0)=\left\{1-2Bi\sum_{n=1}^{\infty}\frac{\mu_n^2+Bi^2}{\mu_n^2+Bi^2+Bi}\frac{\cos\mu_n}{\mu_n^2}e^{-\mu_n^2F_O}\right\} \quad (30)$$

$$\theta^*(0,F_0\to\infty)\cong\left\{1-2Bi\frac{\mu_1^2+Bi^2}{\mu_1^2+Bi^2+Bi}\frac{\cos\mu_1}{\mu_1^2}e^{-\mu_1^2F_O}\right\} \quad (31)$$

$$\theta^*(0,F_0\approx 0)\cong 2Bi\left\{2\sqrt{\frac{F_O}{\pi}}e^{-1/4Fo}-erfc\left(\frac{1}{2\sqrt{F_0}}\right)\right\} \quad (32)$$

Forced convection heat transfer across a circular cylinder rotating with a constant non-dimensional rotation rate (α) varying from 0 to 6 are investigated for Reynolds numbers of 20–160 and a Prandtl number of 0.7. Flow transitions are reported here for a wider range of Reynolds number and rotation rates. Heat transfer visualization technique using heat lines is implemented here, probably for the first time, in finite volume framework for the unsteady heat transfer problem in complex domain and used for heat flow analysis. Rotation can be used as a drag reduction and heat transfer suppression technique [59]The aim of this work is to model the unsteady thermal boundary layer developing along a finite thickness plate under a ramp type variation of temperature on the bottom plate surface. The hydrodynamic boundary layer was considered laminar and at steady state. To model the transient heat transfer, two mathematical approaches were used: the integral method based on Karman-Polhausen methodology and the full Naiver-Stokes system of equations, numerically solved with the commercial solver FLUENT. As a case study, a laminar water flow over a steel plate was considered, but the models remain valid for all combinations of incompressible fluids with Pr> 0.7 and solid materials. The results were expressed in terms of a deviation factor, defined as the ratio between the instantaneous heat flux associated with a finite thickness plate and the instantaneous heat flux associated with a zero thickness plate, both computed at the same space coordinate. Both methods were validated for steady state regime and zero plate thickness, by comparison with solutions commonly reported in the literature. The numerical results revealed that the two methods agree within 5% for the steady state and 2.6% for transient conditions [60].

Non-Fourier hyperbolic heat conduction analysis for heterogeneous hollow cylinders and spheres made of functionally graded material (FGM). All the material properties vary exponentially across the thickness, except for the thermal relaxation parameter which is taken to be constant. The cylinder and sphere are considered to be cylindrically and spherically symmetric, respectively, leading to one-dimensional heat conduction problems. The problems are solved analytically in the Laplace domain, and the results obtained are transformed to the real-time space using the modified Durbin's numerical inversion method. The transient responses of temperature and heat flux are investigated for different inhomogeneity parameters and relative temperature change values. Thecomparisons of temperature distribution and heat flux between various time and material properties are presented in the form of graphs [61].The effects of periodically moving heat source on a circular steel pipe heated partlyfrom its outer surface under stagnant ambient conditions. While the pipe is heated with this heat source applied on a certain section having a thickness of heat flux, the water flows through it to transfer heat. It is assumed that the flow is a fully-developed laminar flow. The heat source moves along from one end ofthe outer to the other end with a constant speed and then returns to the first end with the same speed. It is assumed that the heat transfer rate has a constant value, and that the

thermo-physical properties of the steel do not change with temperature (elastic analysis). The numerical calculations have been performedindividually for a wide range of thermal conductivity of steel and for different thicknesses of heat flux. The moving heat source produces the non-uniform temperature gradient and the non-uniform effective thermal stress, and when it arrives at the ends of the pipe, the temperature and effective thermal stress ratio profiles rise more excessively. The tangential component is more dominant in the effective thermal stress than the radial component [62].Theoretical estimations of boundary layer thickness and heat transfer coefficient is examined using Computational Fluid Dynamics (CFD) for laminar air flow. The feasibility and accuracy of using CFD to calculate convective heat transfer coefficients is examined. A grid sensitivity analysis is performed for the CFDsolutions, and it is used to determine the convective heat transfer coefficients. The coefficients are validated using analytical solution. In addition the local Nusselt number are obtained, which can be used in estimation of flow and heat transfer performance over a flat plate. The results tell us that for the laminar forced convection simulations the convective heat transfer coefficients differed from analytical values by 5%. The result also tells us that the boundary-layer thickness for laminar flow decreases with distance from the leading edge of the flat plate and increases with Reynolds number. The effect of Reynolds number, Prandtl number on flow is also investigated. These estimations can quickly give us the conclusion of dependences between the variables of interest [63].

A computational fluid dynamics methodology is developed to study transient, laminar flow and heat transfer in a periodic zigzag channel with a semi-circular cross-section. The computational domain consists of seven repeating zigzag units with smoothly joined inlet and outlet sections. Reynolds numbers ranging from 400 to 800 and Prandtl numbers ranging from 0.7 to 20 are examined for constant wall heat flux and constant temperature thermal boundary conditions. Simulation results show that the flow reaches a ‘‘developed’’ state after around three units, where the local velocities fluctuate with time but give well defined average heat transfer rates and pressure loss. The power spectra of the velocity at monitor points located periodically along the channel also become very similar. Significant heat transfer enhancement isobserved in the transient regime studied, which is accompanied by a modest pressure-drop penalty, both of which increase with increasing Reynolds number. Vortex structures are visualized at different simulation times and Reynolds numbers and it is found that with increasing Reynolds number, vortices with smaller length-scale are generated, which contribute largely to the enhancement of heat transfer. The results for different Prandtl numbers show that the heat transfer enhancement is proportional to $Pr^{1/3}$. The Nusselt number for the T boundary condition is found to be always higher than that for the H2boundary condition [64].

A new all-time model is developed to predict transient laminar forced convection heat transfer inside a circular tube under arbitrary time-dependent heat flux. Slug flow (SF) condition is assumed for the velocity profile inside the tube. The solution to the time-dependent energy equation for a step heat flux boundary condition is generalized for arbitrary time variations in surface heat flux using a Duhamel’s integral technique. A cyclic time-dependent heat flux is considered and new compact closed-form relationships are proposed to predict (i) fluid temperature distribution inside the tube, (ii) fluid bulk temperature and (iii) the Nusselt number. A new definition, cyclic fully developed Nusselt number, is introduced and it is shown that in the thermally fully developed region the Nusselt number is not a function of axial location, but it varies with time and the angular frequency of the imposed heat flux. Optimum conditions are found which maximize the heat transfer rate of the unsteady laminar forced-convective tube flow. We also performed an independent numerical simulation using ANSYS FLUENT to validate the present analytical model. The comparison between the numerical and the present analytical model shows great agreement; a maximum relative difference less than 5.3% [65].

CONCLUSION

Extensive research work has been carried out for experimental, theoretical and numerical approach on transient heat transfer analysis in laminar flow. Various researchers have developed different experimental models to conduct the experiments; different theoretical models have also been developed. Numerical modeling has also been done using

different schemes like, finite difference method, finite volume technique, finite element scheme. Extensive computational work has been done by using ANSYS FLUENT. Different analytical model by using green's function theorem has also been discussed [58]. The analysis and measurement becomes even more vital and crucial for industries dealing with heat exchanger, automobiles, boilers, space mechanisms, electronic equipment's and almost all the daily life applications. It can be also used in a education purpose for teaching transient heat transfer.

Nomenclature

T_∞ = Ambient temperature , K

c_p = Specific heat at constant pressure , J/kg K

T_w = Wall temperature , K

t = Time , s

x = Axial coordinate , m

θ = T-T_0 , temperature difference

α = Thermal diffusivity , m^2/s

y = Transverse coordinate ,m

u = Axial velocity , m^2s

k = Thermal conductivity , W / m K

r = Radial distance , m

U_∞ = Free stream velocity , m^2s

v = Transverse velocity , m^2s

T_∞ = Free stream temperature , K

ρ = Density , kg/m^3

T = Temperature , K

υ = Kinematic viscosity , m^2/s

C_v = specific heat at constant volume , J/kg K

τ_{yx} = shear stress , N/m^2

v_x , v_y = velocity component in x and y direction

η = dimensionless radial coordinate

ξ = dimensionless axial coordinate

τ = dimensionless time.

P_e = Peclet number

$u(y)$ = velocity profile along test section

A = wall-to-fluid thermal diffusivity ratio

r , t=derivative with respect to r , t , u

μ = coefficient of viscosity , Ns/m^2

Pr = Prandtl number

V=velocity vector

p = pressure , N/m^2

F=body force , N

Bi = Biot number

F_0=Fourier number

h=convective heat transfer coefficient ,W/m^2K

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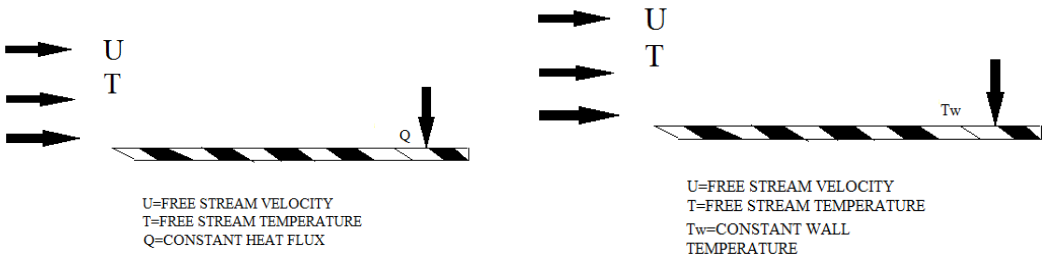

Figure 1.Flat plate subjected to constant heat flux or wall temperature.

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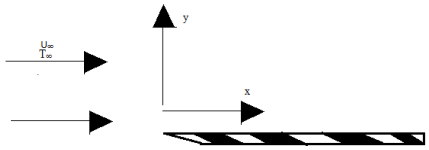

Figure.2 Flow over a flat plate

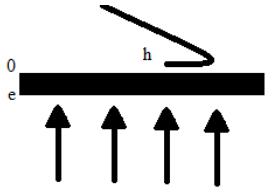

Figure.3 Flat plate with constant heat flux from bottom

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RESEARCH ARTICLE

Effect of Gamma Radiation on the A.C Electrical and Dielectric Properties of Prepared Pure and Doped Polyaniline Salt

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ABSTRACT

Purepolyaniline and doped with hydrochloric acid was prepared in different molarities at room temperature. The a.c electrical properties were stadied.AC conductivityσ_{ac} (ω), is found to vary as ω^s in the frequency range (100Hz-10MH), S< 1and decreases indicating a dominate hopping process. Thedielectric constant $\varepsilon_1$and dielectric loss ε_2 have been determined for bulk polyaniline. ε_1 decrease with the increase frequency. Electrical conductivity measurements increase with the increases both of the amount of HCl and the dose of radiation. The dielectric investigations show decrease with dose radiation.

Keywords: polyaniline, gamma radiation, electrical conductivity, dielectric constant

INTRODUCTION

Conducting polymers have received much attention due to their potential usage in several applications such as biosensor [1] electrochemical display [2] corrosion protection [3] or even rechargeable batteries [4]. Polyaniline is a type of conductingpolymer which received the most attention due to the discovery of its high electrical conductivity [5] reversible acid-base chemistry in aqueous solution, thermal and environmental stabilities and easiness of synthesis [6].Since the discovery of electrically conducting polymer by Alan MacDiarmid, Alan J. Heeger, and Hideki Shirakawa in 1976, intensive investigations have been carried out on the new generation of "synthetic metals" due to their unique combination of electronic and optical properties and processing advantages [7]. The electrical conductivity is achieved in the conjugated polymers by means of delocalized of the π-electrons that allow charge mobility along the backbone of the polymer chain. The synthesis of conducting polymers has been accomplished by oxidizing or reducing process either through chemical doping [8] or electro-chemical doping [9]. The aniline polymers have the general formula $[(- B- NH-B-NH)_y(-B-N =Q=N-)_{1-y}]_x$ in which B andQ denote the C_6H_4 rings in the

benzenoid and quinonoid forms, respectively. Thus, the aniline polymers are basically poly (p-phenyleneamine)s, in which the intrinsic oxidation states can vary from that of fully reduced leucoemeraldine (LM y=1), through that of the 50%intrinsically oxidized prenigraniline (PNAy=0).The polymer canachieveits highly conductive state either through protonation (doping) of the aminenitrogens(=N-) in its EM oxidation state or through the oxidation of the amine nitrogens(-NH-) [10]. A numberof studies have been reported on the electrical and dielectric properties of polymericnanocomposite of PANI, as wellas,polypyrrolecomposites.The properties of these systems aresensitive to particle, inter-particle interaction and temperature.Synthesisof materials with a large dielectric is very important for the development of a new generation dynamic RAM and microelectro-mechanicalsystem. High dielectric behavior is possible for application in conductive paints, rechargeable batteries, sensors and actuator [11].The aim of this work is to study the effect of gamma rays on the electrical properties of prepared polyaniline.

Experimental work

The preparation of (PANI) is based on the oxidation of (0.2M) aniline hydrochloride with (0.25M) ammonium peroxydisulfate in aqueous medium. The pure sample was prepared in distilled water and the doped sample with different molarities of HClaquous solution (0.5M, 1M and2M). To prepared sample doped with 2M aniline hydrochlorid was dissolved in (1M) HCl in a volumetric flask to 50ml of solution, ammonium pexroywas similarly dissolved in (1M) HCl also to 50ml of solution both solutions are mixed at room temperature in a rounder, and gentlestirring to polymerize the mixture is left to rest to the next day. The (PANI) precipitate is collected on a filter and washed with three 100ml of (0.2M) HCl, and 150ml of acetone. Polyaniline (emeralidin) hydrochloride powder is dried in air for about one hour then in vacuum oven about (80 ºC) for 6 hours the average yield was (1.85)gm. The A.C electrical measurements are used to investigate polyaniline samples doped during polymerization with various molarities of HCl. The polyaniline powder was thoroughly grounded in a morter to obtain very fine particles, and then it was compressed under a pressure 10 tone in the form of a pellet. The resulting pellet has a diameter of 1.3cm and thickness of (1.88-1.79mm). To improve theelectrical contact the faces of the pellet were coated with aluminum by thermal evaporation. The LCR meter models (HP-4274A and HP-4275A) were used for the ac measurements. The sample was placed in a holder specially designed to minimize stray capacitance. The range of frequency was 100Hz - 10MHz. For the sample under investigation, the specimen capacitance C, dissipation factor D and resistance R were measured. The total conductivity was calculated from the following equation: $\sigma_t(\omega) = d/RA$, where d is the thickness of the sample and A is the cross-section area.

The ac conductivity $\sigma_{ac}(\omega)$ was calculated by using the relation: $\sigma_{ac}(\omega) = A\omega^S$where$\omega$ isangular frequency, A is a constant;S (≤ 1.0) is frequencyexponent. The dielectric constant ε_1 wascalculated from the equation: $\varepsilon_1 = Cd/A\varepsilon_o$,where $\varepsilon_o$is the permittivity of free space =8.854x10^{-14}(F/cm). The dielectric loss$\varepsilon_2$was calculated from the equation: $\varepsilon_2 = \varepsilon_1 \tan\delta$, where tan$\delta$ is the dielectric tangent loss ($\delta = 90 - \phi$).

RESULTS AND DISCUSSION

Gamma rays (γ- radiation) imparted it is energy in the medium through various processes such as ionization and excitation of atoms, chemical bound scission, grafting, cross-linking and disintegration of molecules. Figure 2 shows the variation of the total conductivity as a function of frequency for polyaniline pure and doped withHCl (0.5, 1 and 2M) at various doses. The total conductivity can be expressed as in equation (1).

$\sigma_T(\omega) = \sigma_{dc} + \sigma_{ac}(\omega)$ ----------(1)

σ_{dc} is the dc conductivity.

At frequency independent, the conductivity is served by weakly disassociated ions by irradiation such as Cl^-, H^+ and OH^- while at frequency dependant the conductivity is served by relaxed and phonon assisted process [12].$\sigma_{ac}$is obtained by subtracting the dc conductivity from the measured total conductivity according to Eq.(1). Figure (3) shows the dependence of a.c. conductivity on frequency atvarious doses at room temperature. It is clear from the

figure that σ_{ac} increases with the increase in frequency. The frequency exponent s can be calculated from the slope of the straight lines in figure 3, the exponent s is less than unity. The general values of s appear to be consistent with a hopping process of charge carriers (protons) between polymer chains. Figure 4 shows the variation of S values as a function of HCl M concentration and listed in Table (1).

Polyaniline can be made more conducting by protonation with an acid such as hydrochloric acid (HCl) [13]. The presence of the acid result is the protonation (increased proton concentration) of nitrogen atoms; the degree of protonation depends on the PH of the acid solution [14]. Fig.5 show the effect of HCl content on the ac conductivity at room temperature, the time of radiation at 1.20 hour in this plot indicates that the low frequency behavior is less than 10^4Hz of all the sample looks like a straight line dc conductivity dominated and then the absolute conductivity for individual sample increases as a function of frequency and the conductivity increases with increased in HCl concentration (0.5M, 1M and 2M).Figure 6 shows the variation of electrical conductivity at 100 KHz as a function of HCl concentration(protonconcentration) at various doses. Themeasurement of radiation-induced conductivity inpolymers has been developed as a technique to study theinfluence of radiation on the electrical behavior of polymeric layers used in radiation environments. Electrical conductivity of organic polymers can be significantly increased during the time that the material is exposed to a radiation flux due to the formation of transient conductive species (electrons, holes). Thespecies also known as charge carriers rapidly recombine once the irradiation is stopped with the result that the conductivity quickly decreases to near the initial value. Theabsorption of relatively high doses, however, may cause permanent changes in the conductivity [15, 16].

The dielectric constant was calculated from the measured value of capacitance C_p in the range of frequency 100Hz-10MHz. The frequency dependence of ε_1 at different doses is shown in Fig. 7. It is clear from the figure that ε_1 decreases with the increase in frequency. The variation is small at high frequencies. The decrease of ε_1 with frequency can be explained as follows: at low frequencies ε_1 for polar materials is due to the contribution of multi-component of polarizability, deformational polarization (electronic, ionic, orientation, and interfacial).When the frequency is increased, the dipole will no longer be able to rotate sufficiently rapidly. So their oscillations are lagging behind those of the field. As the frequency is further increased, the dipole will be completely unable to follow the field and the orientation stopped, so $\varepsilon_1$decreases at a higher frequency approaching a constant value due to the interfacial polarization [17].

CONCLUSION

A.C conductivity and dielectric behavior of polyaniline /HCl have been presented in this work. These are synthesized by the 'in-situ polymerization. The AC conductivity of these composites will obey the power law well above the critical frequency .The dielectric behavior of PANI shows nearly a Debye-type relaxation, because of this, there is a decrease in the dielectric constant with increase in frequency. Numbers of blends which are different in composition were exposed to gamma radiation to various doses and the effect of irradiation time and composition of polymers used in the blends on the conductivity of films were investigated by using conductivity measurements, PANI- has also been found to be very efficient in inducing conductivity in gamma-irradiated PANI. The results clearly showed that ionizing radiation is an effective tool to induce conductivity in the blends of PANI. The main mechanism behind this radiation-induced conductivity is insitu doping of PANI-base with HCl released from partner polymers and compounds by the effect of radiation.

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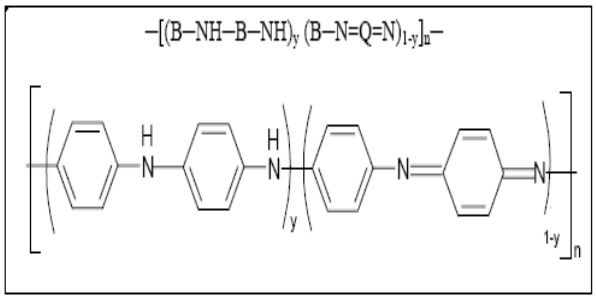
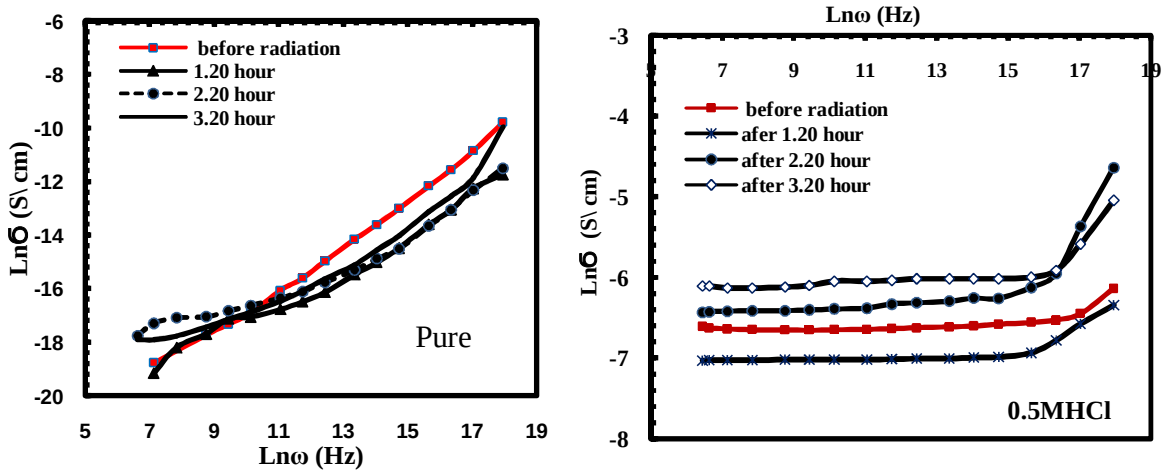


Figure 1: The structure of the polyaniline chain [10].



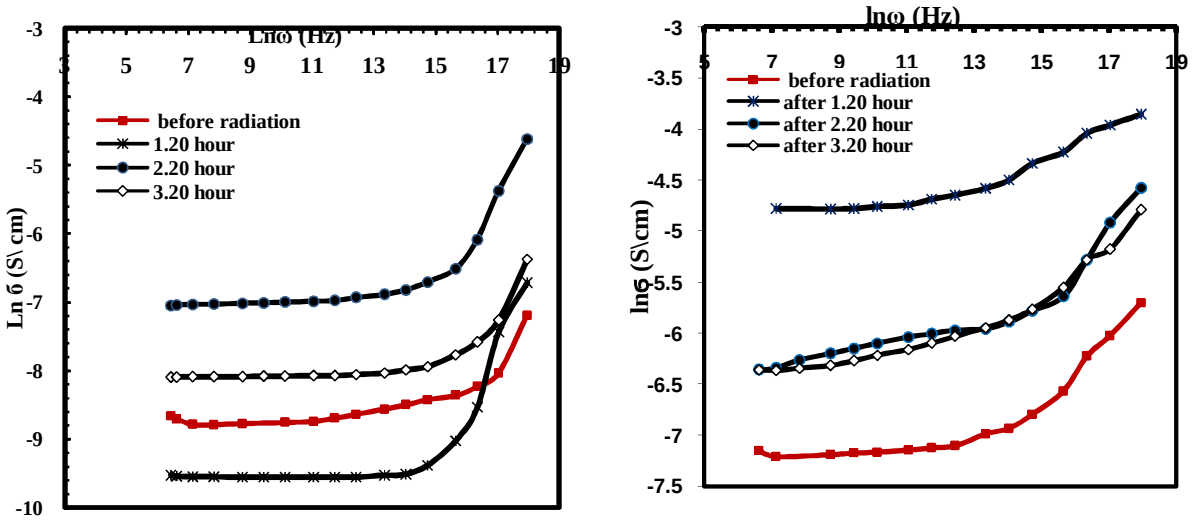
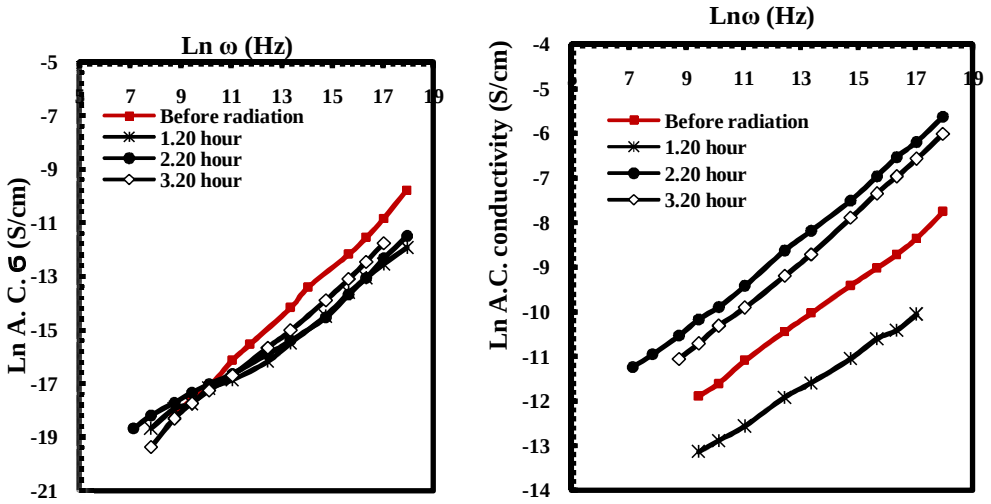


Figure2:Frequency dependence of σ_t for polyaniline pure, 0.5 M, 1M, and 2M HCl before and after radiation.



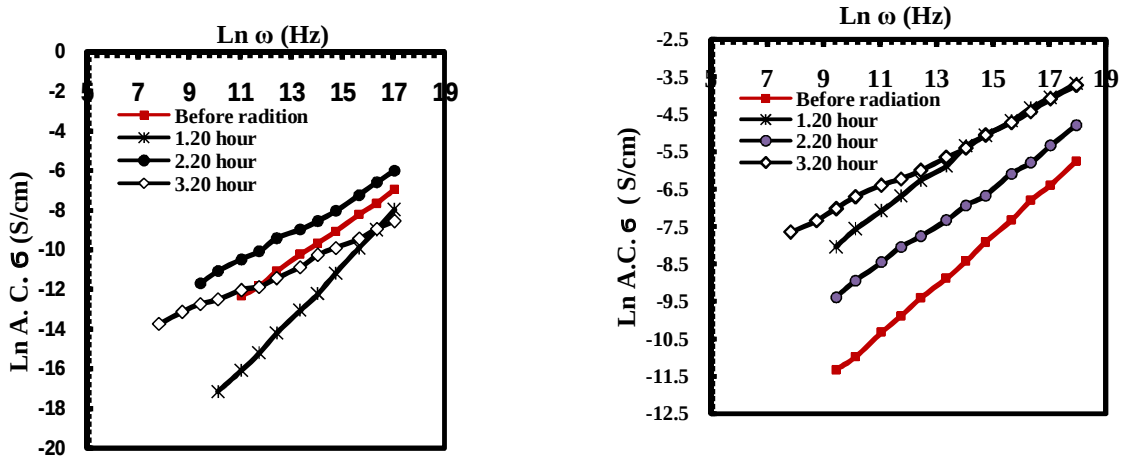


Figure 3: Variationofa.c conductivity as a function of frequency at various doses of radiation

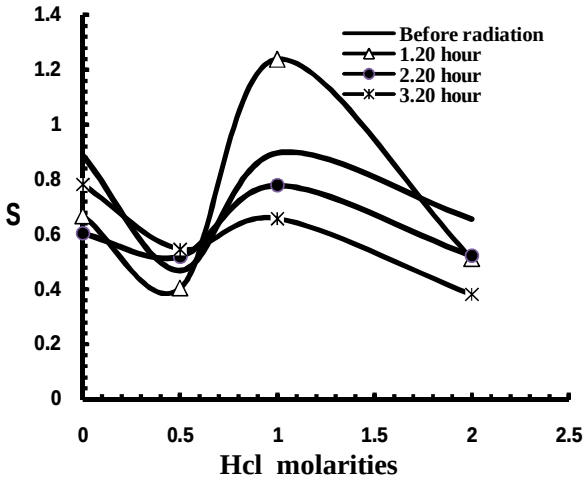


Figure 4: Variation of S with HCl Mconcentration

Table 1: The values of S

concentration HCl Mol.	Before radiation	1.20 hour	2.20 hour	3.20 hour
0	0.888	0.668	0.604	0.782
0.5	0.468	0.405	0.519	0.546
1	0.897	1.238	0.779	0.658
2	0.656	0.512	0.522	0.381

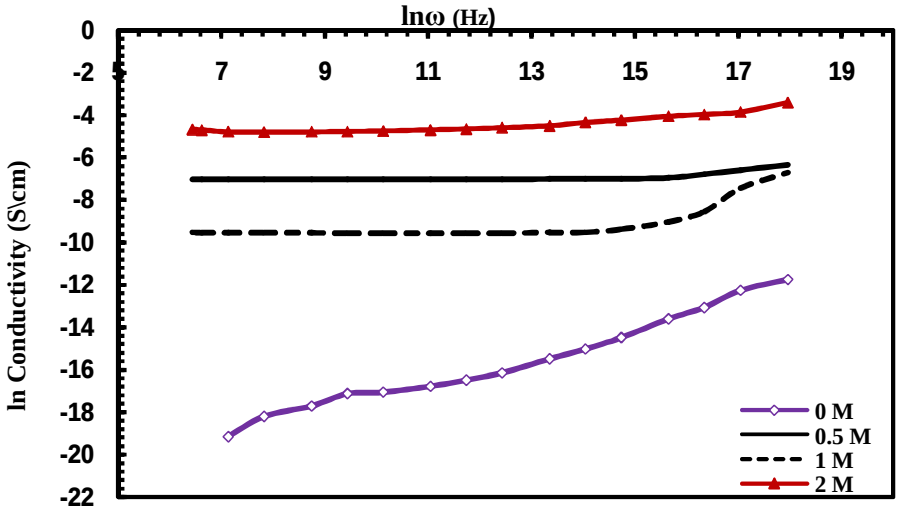


Figure 5: Variation of electrical conductivity as a function of frequency for different concentration of HCl M at time 1.20 hour.

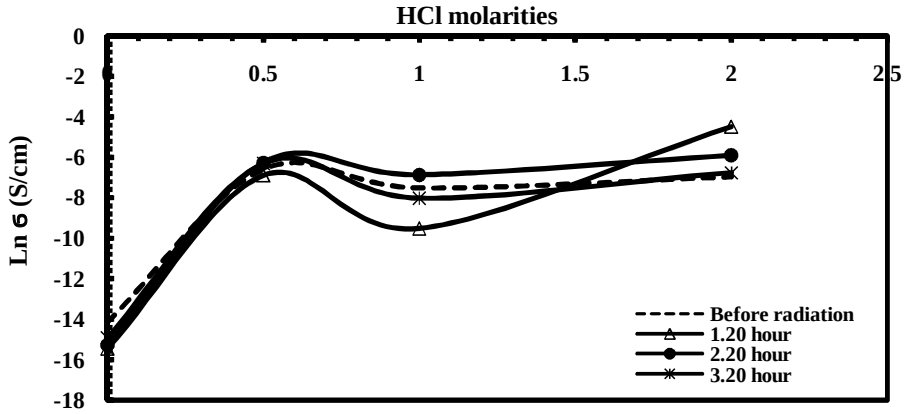


Figure 6: Variation of conductivity at100KHz as a function of HCl molarities in different doses.

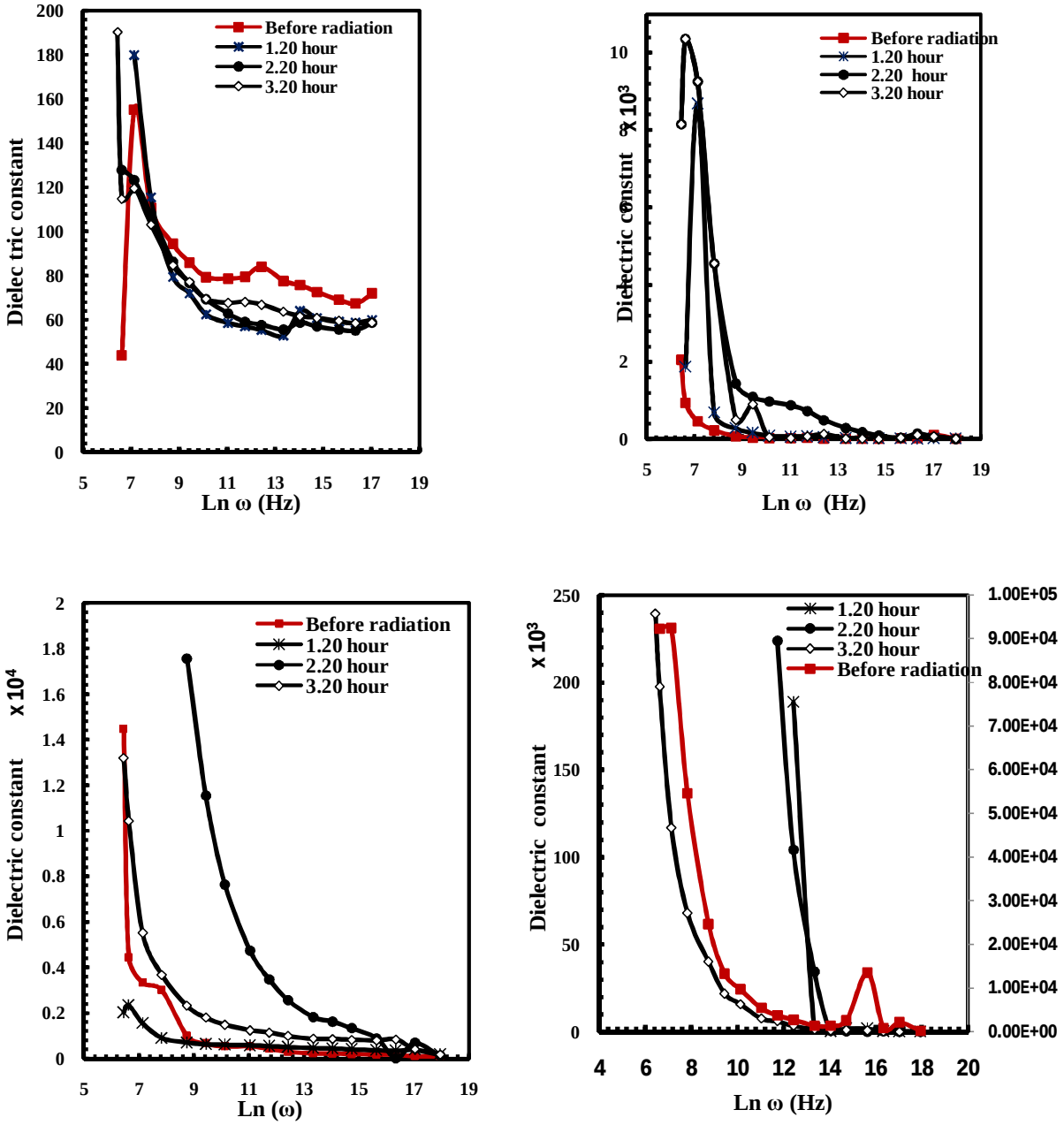


Figure 7: The variation of dielectric constant as a function of frequency.

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RESEARCH ARTICLE

Anti Dyslipidemic Effect of Extracts of *Phyllanthus emblica* Leaves against Triton Model in Rats.

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ABSTRACT

The present study was conducted to evaluate the antidyslipidemic effect of petroleum ether, aqueous, hydroalcoholic and ethanolic extract of *Phyllanthus emblica* against triton WR-1339 (200mg/kg) induced hyperlipidemic model in albino wistar rats. The serum was collected at different interval time (0, 18, 24, 40 and 48h) and estimated for total cholesterol and triglyceride levels. Triton induced hyperlipidemic control group showed a significant (P<0.05) increase in the total cholesterol and triglyceride level compared to normal control group. Among the various extracts, ethanolic extract showed significant (P<0.05) decrease in the serum lipids compared to hyperlipidemic control group at the dose rate of 200mg/kg b.w. compared to other extracts like petroleum ether, hydroalcoholic and aqueous. Further, the phytochemical screening of the ethanolic extract revealed the presence of phytoconstituents such as alkaloids, flavonoids, sterols and phenolic compounds etc. which may be responsible for the anti-dyslipidemic activity. It can be concluded from these study that, ethanolic extract of *Phyllanthus emblica* leaves have significant antidyslipidemic activity.

Key words: *Phyllanthus emblica*, anti-dyslipidemic, Triton WR-1339, albino Wistar rats, ethanolic extract

INTRODUCTION

Cardiovascular disease (CVD) is one of the major contributing factors to the total global mortality. Atherosclerosis is one of the major risk involved in cardio vascular disease, where oxidative stress plays a major important role in developing this condition (Mishra *et al.* 2011). According to global health observatory data the increase in cholesterol level causes 2.6 million deaths in a year (WHO, 2014). Hence it is necessary to develop an alternative therapeutic agent for treatment and management of dyslipidemia. Hyperlipidemia/dyslipidemia leads to various cerebrovascular disease, peripheral vascular disease and coronary heart disease. It is a metabolic syndrome with diverse lipid profiles such as increase in low density lipoprotein cholesterol and also increase in the level of total cholesterol and triglyceride levels. Herbal drugs are widely used and accepted by large population due to its lesser side effects compared to synthetic drugs even when used for long term (Pandit *et al*., 2011)

Phyllanthus emblica, commonly known as Indian gooseberry or Amla, belongs to the Phyllanthacae family and occupies a hallowed position in the Indian indigenous system of medicine. It is the most mentioned herb in the "Charaka Samhitha", the ayurvedic medicine literature (500 BC). According to the ancient Indian mythology, it is believed that this is the first tree to be created on the earth and a gift of nature to mankind which is used as a rejuvenator in ayurveda. In Hindu religious mythology this tree is worshipped as the "Earth Mother" as its fruit nourishes the mankind. In Sanskrit it is called as amlaki which means the fruit where the goddess of prosperity resides (Onions, 1994). Each part of this plant is considered to have the medicinal importance and it is also used for the treatment of several diseases from the ancient times and reported for free radical scavenging, anti-inflammatory, hepatoprotective, anti-mutagenic and immunomodulatory activities It has shown efficacy in prevention and therapeutic approach towards diseases like cancer, atherosclerosis, diabetes, hepatic ailments, cardiovascular disease and also various other numerous diseases (Dasaroju and Gottumukkala, 2014). Fruits of *Phyllanthus emblica* were used traditionally as a hypolipidemic agent and have been scientifically validated through different studies, both clinically and experimentally (Antony *et al.,* 2008).

The systemic administration of non-ionic detergent surfactant triton to mice or rats results in a biphasic elevation in the concentration of serum cholesterol and triglycerides level (Frantz and Hinkelman, 1995). Triton WR-1339 causes a high increase in hepatic cholesterol synthesis and also increase in HMG Co-A reductase activity in rats and mice within the twenty four hour of intravenous or intraperitoneal administration. Triton WR-1339 stimulates the hepatic cholesterogenesis by decreasing the hepatic cholesterol and trapping them in the blood compartment (Stanley Goldfarb 1978).

MATERIALS AND METHODS

Collection and identification of plant material

Phyllanthus emblica leaves were collected from the Wayanad district of Kerala in the month of May 2015. The plants was identified and authenticated in the Department of botany, University of Calicut and voucher specimen was deposited with accession number.

Preparation of plant extracts

Leaves of experimental herb collected were cleaned and shade dried under room temperature. They were pulverized to coarse powder using electrically operated plant sample grinder and kept in air tight containers till used for extraction purpose. Weighed quantities of dried leaf powder of *P. emblica* were extracted in soxhlet apparatus separately with the solvents such as 95% ethanol, distilled water and ethanol at 1: 1 ratio and petroleum ether to get the respective extracts. For the preparation of aqueous extract decoction method was used in which weighed quantity

of dried *P. emblica* leaf powder was mixed with distilled water in the ratio of 1:4 and reduced the quantity to 1/4th of initial volume by boiling. The extracts so obtained from soxhlet method/decoction method were evaporated and concentrated using Rotary vacuum evaporator (m/s Buchi, Switzerland) under reduced pressure of 96 mbar at a temperature range of 40-50°c. The concentrated extract was air dried at room temperature and stored in refrigerator until use. The percentage yield of each extract was calculated separately using the formula below:

$$\text{Extractive yield (\%)} = \frac{\text{Weight of the extract} \times 100}{\text{Weight of the sample taken}}$$

Experimental animals

Forty eight male and six female albino Wistar albino rats of 6 to 8 weeks age were procured from small animal breeding station, Mannuthy, Kerala. All the animals were kept in well ventilated cages under standard laboratory conditions and acclimatized for two weeks prior to experimentation. They were given standard diet and clean drinking water ad-libitum.

Chemicals

All the chemicals used were of analytical grade and procured from Merck or Himedia, India unless otherwise specified. The diagnostic kits for the study were procured from span diagnostics ltd., India.

Serum lipids estimation

Triglycerides (TG)

Serum TG was estimated by glycerol-3-phosphatse oxidase- peroxidase method using commercial kit (*source*: M/s Span Diagnostics Ltd., Mumbai).

$$\text{TG concentration of sample (mg/dl)} = \frac{\text{Absorbance of sample} \times 200}{\text{Absorbance of standard}}$$

Total Cholesterol (TC)

Serum TC was estimated by cholesterol oxidase-phenol amino antipyrene method using commercial kit (source: M/s Span Diagnostics Ltd., Mumbai) and calculated as below

$$\text{TC conc. of sample (mg/ dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 200$$

Experimental design

The present study was conducted in three phases as mentioned below:
Phase I: Screening of different extracts of *P. emblica* leaves in triton induced acute hyperlipidemic/ dyslipidemic model for selection of the most potent extract.
Phase II: Phytochemical screening of the selected extract.
Phase III: Acute oral toxicity evaluation of the selected extract

Phase I: Screening of different extracts of *P. emblica* leaves in triton induced acute hyperlipidemic/ dyslipidemic model for selection of the most potent extract.

Forty eight albino Wistar rats were randomly divided into eight groups of six animals each. Group I served as a normal control, in which all the animals were administered with distilled water. Group II and III that served as triton /hyperlipidemic control were administered orally with vehicles distilled water (Group II) and coconut oil (Group III) along with the triton treatment, whereas group IV to VIII were treated orally with atorvastatin at 7.2 mg/kg, ethanolic, hydroalcoholic, aqueous and petroleum ether extracts respectively at 200mg/kg b.wt, single dose, along with triton treatment.

Distilled water was used as vehicle for all the drug/extracts administered except for petroleum ether extract in which, coconut oil served as vehicle. Triton treatment indicated single intraperitoneal (i/p) administration of triton WR-1339 (Tyloxypol) at 200mg/kg body weight dose level after vehicle/atorvastatin/extracts administration.The animals were fasted overnight; blood was collected from retro orbital plexus before the triton WR-1339 and drug/vehicle treatment (zero hour) administered and at time intervals of 18, 24, 40 and 48 h post treatment for the estimation of serum total cholesterol (TC) and triglyceride (TG). The absolute values in respective hyperlipidemic control groups were considered 100% and percent reduction in other treatment groups at different intervals were calculated.

$$\text{Per cent reduction (Specified time)} = \frac{\text{Serum conc. (hyperlipidemic control – treated group)} \times 100}{\text{Serum conc. of hyperlipidemic control}}$$

Subsequently the extract showing the maximum antihyperlipidemic activity among the tested extracts was selected for further evaluation in cholesterol and cholic acid induced dyslipidemia.

Phase II: Phytochemical screening of the selected extract

The extract selected from the tested extracts owing to the maximum antidyslipidemic potential was ethanolic extract of *P. emblica* and it was further subjected to phytochemical screening by various qualitative tests for detecting the presence of active phytochemical constituents namely alkaloids, tannins, glycosides, phenolic compounds, steroids and saponins as per the procedure quoted by Harborne (1991) and Raaman (2006) as enlisted below.

Phase III: Acute oral toxicity evaluation of the selected extract

Acute oral toxicity test was performed as per the Organization for Economic Co-operation Development (OECD, 2000) test guidelines No.423. The ethanolic extract of *P. emblica* was solubilized in distilled water and administered orally at dose level of 2000 mg/kg b. wt. in six animals, in a two-step manner with three animals per step. On the day of dosing, all the animals were observed for mortality and clinical signs for the first 30 min, 1 h, 2 h and 4 h after dosing and thereafter daily for 14 days. During the first 1 hour after the drug administration, the rats were observed for any gross behavioural changes like hyperactivity, grooming, convulsions, sedation, and loss of righting reflex, changes in respiration, salivation, urination and defecation. After the observation period of 14 days, all surviving animals were sacrificed and subjected to detailed necropsy.

Statistical analysis of data

All the results were expressed in Mean ± SEM. The statistical tests were performed by Statistical Package for Social Sciences (SPSS) version 21. The data were analyzed using one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1994) with Bonferroni or Dunnett T_3 method as post hoc test. Statistical significance was set at $p < 0.05$.

RESULTS

Plant identification

The plants were identified and authenticated in the Department of Botany, University of Calicut, Kerala, India and the voucher specimens was deposited with the accession number, *Phyllanthus emblica* L., Accession number: CALI 6812.

Preparation of different extractsand phytochemical screening

The extractive yield of 100g powdered leaves of *Phyllanthus emblica* in different solvents such as ethanolic, petroleum ether, hydroalcoholic (1:1) and aqueous.Phytochemical screening of ethanolic extracts of *Phyllanthus emblica* revealed the presence of various active chemical constituents such as alkaloids, tannins, flavonoids, glycoside, phenolic compounds, steroids, saponins, carbohydrates.

Screening of the potent extract in triton WR-1339 (Tyloxypol) induced hyperlipidemic model

All the experimental animals used for this model was kept for fasting overnight. Tyloxypol was once administered to all the animals except the normal control group at the dose rate of 200 mg/kg b.wt. Intraperitoneally. The serum triglycerides and cholesterol was estimated at different time interval 0, 18, 24, 40 and 48th h**.**

Serum triglyceride

The ethanolic extract of *Phyllanthus emblica* showed a significant reduction in the serum triglyceride level at different interval period of time (0, 18, 24, 40 and 48th h) when compared to triton WR-1339 control group.

Serum total cholesterol

The ethanolic extract of *P. emblica* showed a significant reduction in the serum total cholesterol level at different interval period of time (0, 18, 24 and 48th h) when compared to triton WR-1339 control group.

Acute oral toxicity of ethanolic extract of *P. emblica*

No mortality was recorded among the groups of rats administered with ethanolic extract of *P. emblica* single oral dose of 2000mg/kg body weight. The treated groups of rats exhibited normal behaviour and there were no apparent clinical signs of toxicity.

DISCUSSION

The present study revealed significant increase in the serum cholesterol and triglyceride level in triton induced hyperlipidemic model where in significant ($P<0.05$) decrease in the serum cholesterol and triglyceride level were observed in ethanolic extract treated group compared to other extract treated groups. The Hypolipidemic agents which interfere in both the phases of triton administration is due to the inhibition of the hepatic cholesterol biosynthesis in the synthetic phase and increased excretion of bile acid synthesis (Chitra and Leelamma, 1997). The interference of the ethanolic extract of *P. emblica* in both the phase of the triton administration revealed that the extract is active in both the synthetic phase and the excretory phase and this resulted in decreased serum cholesterol and triglycerides level and the percentage of reduction were observed high compared to other extract treated group. The reference drug atorvastatin also showed a significant ($P<0.05$) decrease in the serum cholesterol and triglycerides level compared to triton induced model the mechanism is through inhibiting cholesterol biosynthesis by inhibition of HMG CoA reductase activity which in turn reduces the triglyceride and cholesterol levels (Gayathri *et al.,* 2013).

Hence the possible mechanism attributed in the ethanolic extract of *Phyllanthus emblica* is due to the interference of the extract in inhibiting the hepatic cholesterol biosynthesis and might be due to the increased excretion of bile acid synthesis.Phytosterols present in the plants are responsible for lipid lowering activity by increasing the cholesterol and bile acid metabolism (Sharma *et al*., 2013). The presence of alkaloid ingredient in the plant inhibits the cholesterol biosynthesis which in turn decreased the total plasma lipids and cholesterol level in rats (Koriem, 2014). The presences of glycoside and poly phenolic compounds are reported to inhibit HMG CoA reductase activity (Gayatri *et al.,* 2013). Flavonoids present in the plants are responsible for reducing the risk of CVD by facilitating the removal of cholesterol from the peripheral tissues.

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Conflict of Interests

None declared

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Table 1: Experimental protocol for Anti-dyslipidemic activity of different extracts of *Phyllanthus emblica* leaves.

Group	Treatment
I	Normal/vehicle control group :Animals receiving distilled water @ 10 ml/kg orally
II	Hyperlipidemic control I: Animals receiving distilled water and triton WR-1339 (200 mg/kg; i.p.) treatment
III	Hyperlipidemic control II: Animals receiving coconut oil (10 ml/kg orally) and triton WR-1339 (200 mg/kg; i.p.) treatment
IV	Animals receiving atorvastatin @ 7.2 mg/kg b.w. and triton treatment
V	Animals receiving ethanolic extract@ 200 mg/kg b.w. and triton treatment
VI	Animals receiving hydroalcoholic extract @200 mg/kg b.w. and triton treatment
VII	Animals receiving aqueous extract @ 200 mg/kg b.w. And triton treatment
VIII	Animals receiving petroleum ether extract @ 200 mg/kg b.w. and triton treatment

Table 2: Phytochemical analysis of Methanolic extract of *Phyllanthus emblica* leaves:

Sl. No.	Phytochemical constituents	Reagents	Inference
1	Alkaloids	Mayer's reagent	Creamy white precipitate
2	Tannins	1% Ferric chloride solution	Blue / green / brownish green colour
3	Flavonoids	Neutral ferric chloride	Green colour
4	Glycosides	20% Sodium hydroxide solution	Yellow colour
5	Phenolic compounds	10% Ferric chloride solution	Dark brown colour
6	Steroids	Chloroform, concentrated sulphuric acid	Red colour
7	Saponins	Foam test	Persisting foam for 10 minutes
8	Carbohydrates	Molisch test	Violet ring at junction

Table 3. Extractive yield of dried leaf powder of *Phyllanthus emblica*

Sl. No.	Solvent	Yield (%)
1	Ethanolic	32.18
2	Petroleum ether	2
3	Hydroalcoholic	14.82
4	Aqueous	11

Table 4: Effect of different extracts of *E. officinalis* leaves on triglyceride levels in Triton WR-1339 treated Wistar rats

Treatment groups	Serum triglycerides (mg/dl)				
	Hour 0	**Hour 18**	**Hour 24**	**Hour 40**	**Hour 48**
I Normal control	56.33±2.54	57.83±2.89	58±2.5	56.67±2.29	53.67±1.76
II (Hyperlipidemic control I)	57.83±1.99	1264.17±39.81[a]	701.33±25.23[a]	384.67±43.13[a]	112±3.83[a]
III (Hyperlipidemic control II)	58	1189	674	356	110
III Atorvastatin (7.2 mg/kg)	58±2.07	439.83±22.47[b] (65.21)	222.33±15.08[b] (68.29)	123.67±12.77[b] (67.85)	70.33±1.99[b] (37.21)
IV ALEO (200 mg/kg)	58.33±1.31	625.17±29.12[b] (50.55)	334.67±19.61[b] (52.28)	171.33±11.8[b] (55.46)	80.17±2.21[b] (28.42)
V HAEO (200 mg/kg)	57.67±2.19	851.33±26.16[b] (32.66)	584.67±7.86 (16.63)	205±12.99 (46.71)	90±3.34[b] (19.64)
VI AQEO (200 mg/kg)	57±1.71	722.17±37.47[b] (42.87)	435.33±23.57[b] (37.93)	190.33±4.37 (50.52)	85.33±3.29[b] (232.81)
VII PEEO (200 mg/kg)	57.83±1.64	971.83±39.84[b] (23.13)	666.5±24.21 (4.97)	296.67±19.7 (22.88)	90.83±3.09[b] (18.90)
VIII EEEO (200 mg/kg)	57±1.81	899.67±45.48[b] (28.83)	588±28.31 (16.16)	263.5±13.42 (31.51)	110.17±7.81 (1.79)

Values are expressed as mean ± SEM; n=6, [a]p< 0.05, Vehicle control VsDyslipidemiccontrol; [b]p< 0.05, Dyslipidemic control Vs Treated groups, Values in parentheses are % reduction as compared to Dyslipidemic control
ALEO: Alcoholic extract of *PhyllanthusEmblica;* HAEO: Hydroalcoholic extract of *PhyllanthusEmblica*
AQEO: Aqueous extract of *PhyllanthusEmblica;* PEEO: Petroleum ether extract of *PhyllanthusEmblica*
EEEO: Ethylacetate extract of *PhyllanthusEmblica*

Table 5: Effect of different extracts of *E. officinalis* leaves on total cholesterol levels in Triton WR-1339 treated Wistar rats

Treatment groups	Serum total cholesterol (mg/dl)				
	Hour 0	Hour 18	Hour 24	Hour 40	Hour 48
I Normal control	56.67±2.17	57.33±2.16	57.67±2.09	57±1.93	56.5±2.01
II (Hyperlipidemic Control I)	53.17±3.13	208±10.6[a]	197±8.21[a]	106.33±3.88[a]	93.67±2.09[a]
III (Hyperlipidemic Control II)	56.00±1.81	208±10.82[a]	186.67±9.70[a]	104.67±3.17[a]	89.67±2.60[a]
IV Atorvastatin (7.2 mg/kg)	57.17±2.06	113±8.37[b] (45.67)	95.5±4.43[b] (51.52)	88.17±6.97[b] (17.08)	65.5±2.69[b] (30.07)
V ALEO (200 mg/kg)	51±2.74	156.17±3.58[b] (24.92)	146.67±2.86[b] (25.55)	112.67±3.57 (-5.96)	71.83±2.01[b] (23.32)
VI HAEO (200 mg/kg)	58±2.58	173.17±5.08 (16.75)	161.67±3.03 (17.93)	153.17±2.24[b] (-44.05)	111.17±4.12[b] (-18.68)
VII AQEO (200 mg/kg)	57.67±1.89	161.67±2.29 (22.27)	153.5±2.51[b] (22.08)	118.33±3.11 (-11.29)	92.17±1.9 (1.60)
VIII PEEO (200 mg/kg)	60.67±2.04	193±6.09 (7.21)	176±3.59 (10.66)	160.5±2.74[b] (-50.95)	119.17±4.5[b] (-27.22)

Values are expressed as mean ± SEM; n=6, [a]p< 0.05, Vehicle control VsDyslipidemiccontrol; [b]p< 0.05, Dyslipidemic control Vs Treated groups, Values in parentheses are % reduction as compared to Dyslipidemic control.
ALEO: Alcoholic extract of *PhyllanthusEmblica,* HAEO: Hydroalcoholic extract of *PhyllanthusEmblica*
AQEO: Aqueous extract of *PhyllanthusEmblica;* PEEO: Petroleum ether extract of *PhyllanthusEmblica*
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RESEARCH ARTICLE

Development and Quality Evaluation of Novel Chicken Cutlet Incorporated with *Amorphophallus paeoniifolius* (Elephant Foot Yam)

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ABSTRACT

Chicken cutlets were formulatedwithextenders at the rate of 30%;control cutlets, P with 30 % potato as extender and treatments Y with 30 % yam as extender and PY with 15 % potato and 15 % yam as extender. Physico-chemical, microbiological and sensory quality characteristics of cutlets incorporated with elephant foot yam at different levels were analysed and compared with potato incorporated control chicken cutlets. pH of Y was found to be lower (p□0.01) than P and PY , TBARS and TV did not differ significantly between control and treatments. Phenolic antioxidants were found to be higher (p□0.01) in Y than PY and P. DPPHradical scavenging assay showed higher free radical scavenging activity in yam than potato. Y had higher (p□0.05) protein, carbohydrate, ash and dietary fibre levels,similar energy

values and lower fat content when compared to P. Instrumental texture analysis showed significantly higher (p□0.01) hardness for yam cutlet and no significant difference in cohesiveness, springiness and adhesiveness between control and treatments. Hunter L, a, b colour estimation showed no difference in 'L' and 'a'values but potato cutlet showed higher (p□0.01)b value. No significant difference was observed in colour, flavour, texture, juiciness and overall acceptability scores in sensory evaluation between treatments and control.Cost of production was same for Y, PY and P. Yam can successfully replace potato as extender in cutlet with improved nutritive value and health benefits.

Key words: Chicken cutlets, yam, potato, quality characteristics

INTRODUCTION

The strong link between diet and health is a topic of serious discussion and people are well aware of a healthy diet with less fat and salt and high levels of dietary fibre, omega 3 fatty acid and antioxidants. Meat is a rich source of protein of high biological value and is a food with high net protein utilisation. It is also rich in iron of high bioavailability and vitamins, especially B complex vitamins. Recent trend in meat industry is development of value added comminuted or minced meat products avoiding intense processing methods like curing and grilling of meat. Development of extended meat products with non-meat ingredients rich in dietary fibre, anti-oxidants etc. is a promising way to functional meat products with low cost of production and added health benefits. They are therefore a convenient meat based value added snack which can be stored in a ready-to-fry form. Meat cutlet is a comminuted meat product very popular in Kerala, prepared from beef or chicken and used as a snack. Traditionally potato is used as an extender in meat cutlets. Most of the tuber crops including potato are contraindicated for diabetic patients due to their high glycaemic index, but an exception for this is elephant foot yam which has a low glycaemic index (Li *et al.*, 2004). Elephant foot yam is a widely cultivated and conventionally used tuber in Kerala with great importance in Ayurveda. Modern researches have proved its hepatoprotective, immunomodulatory, antitumor, anthelminthic, antifungal and antibacterial activity for certain components of its extract (Singh and Wadhwa, 2014).The present study of incorporation of *Amorphophallus paeoniifolius* (elephant foot yam) in cutlet as extender would open up a way for value addition for meat as well as for the conventionally available tuber.Furthermore, the better health benefits of yam could be utilized in a well-accepted common snack.

MATERIALS AND METHODS

Broiler chicken of the same age group was procured from the local markets in Vythiri, Wayanad district and was brought to the Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Pookode. The birds were provided *adlibitum* water and proper rest. They were slaughtered, dressed under hygienic conditions and the carcasses were washed and chilled overnight (4±1^{0}C). Carcasses were cooked, deboned and meat was finely cut. Yam (*Amorphophallus paeoniifolius*) and potato were procured from local market, washed, peeled, cooked and mashed.

Preparation of cutlets

The cutlets prepared for the present study constituted 50% chicken, 20% spices and condiments and 30 % extender. Control cutlets, P contained 30 % potato as extender andtreatment cutlets, Y and PY respectively had 30 % yam and 15 % each of potato and yam as extenders.Spices and condiments were sauted in oil, mixed with finely chopped meat and extender to make the cutlet mix. The mix was mouldedinto flat, oval cutlets of around 35 g weight, dipped in duck egg and enrobed with rusk powder. The ready to fry cutlets were analysed for physico-chemical, microbiological, and sensory characteristics.

Physico-chemical characteristics

pH of the samples was measured using a digital pH meter as described by AOAC (1990). Ten grams of sample was blended with 50 ml distilled water for one min using a tissue homogenizer (Kinematica, Switzerland) at the speed of 4000 rpm. The pH of the homogenate was recorded by immersing the combined glass electrode of digital pH meter (EUTECH instruments pH 510, Singapore).

TBARS numbersof cutlet samples were determined as per Witte *et al*. (1970) with modifications. The absorbance wasexpressed as TBARS number of sample.Tyrosine values of the cutlet samples were estimated as per the method described by Pearson (1968). The absorbance was measured at 660 nm in a spectrophotometer. By referring to the standard graph of tyrosine, tyrosine values of samples were calculated and were expressed as mg of tyrosine/100g of sample.

Phenolics are antioxidants present in foods especially plant foods and estimation of total phenolics helps to assess the antioxidant level of food products. Total phenolics of yam and potato were estimated as per the procedure ofEscarpa and Gonzalez (2001).Yam and potato were peeled, cut into small pieces and dried in a hot air oven at 60^0C for 24 h. The dried pieces were powdered and extracted in Soxhlet solvent extraction system (SocsPlusSCS 06E, Pelican Equipment, Chennai) for 60 min in petroleum ether for fat extraction and then the fat free powder was subjected to extraction in ethanol for five hours. The extract was dried by putting in a rotaevaporator (BUCHI rotavapour, R-215, Switzerland). The dried extract was scraped and reconstituted in two ml ethanol and the ethanolic extracts were used for estimation of total phenolics. For cutlet samples, acetone extracts were taken and total phenolics were estimated as per the procedure ofDevatkal*et al.* (2010).

The standard graph was prepared by plotting optical density against micrograms of tannic acid using standard tannic acid solution (0.1 mg/ml) and absorbance of the sample extract was taken at 725nm and the corresponding tannic acid equivalent of phenolics per gram of sample was found out from the standard graph. 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was done to evaluate the antioxidant activity or free radical scavenging ability of the phenolic extract. This was done by the modified method of Singh *et al*.(2002). Ethanolic extracts of fresh as well as cooked yam and potato containing 50 and 100 µg each of phenolics were taken and DPPH assay was carried out.

The textural properties of the cutlets were evaluated as per Bourne (1978) using a Universal Testing Machine (TRAPEZIUM EZ-SX, Shimadzu, Japan) Texture profile analysis was performed using central portions of fried cutlets of size 1.5x1.5x1.5 cm which were compressed twice to 50% of the original height by using 50 mm diameter compression plate probe. A crosshead speed of 10mm/min was used, applying 500 N load cell and the texture parameters like hardness, cohesiveness, springiness and adhesiveness were estimated.Colour of the cutlet samples were determined objectively as per Kumar*et al*. (2010) using Hunter Lab Mini Scan ZX Plus Spectrophotometer (Hunter Lab, Virginia, USA) with diffuse illumination.The colour coordinates L (lightness), a(redness) and b(yellowness) of the samples were measured thrice and mean values were taken. Cutlets were analysed for proximate principles like moisture, fat, protein, carbohydrate and ash according to method of AOAC (1990). Samples were also analysed for omega 3 fatty acid and dietary fibre contents.

Microbiological parameters

Aerobic plate count (APC) was evaluated as per the procedure of Morton (2001).Psychrotrophic count was expressed as per the procedure of Beuchat and Cousin(2001). Yeast and mould count was expressed as per the procedure of Cousin *et al.* (2001).

Sensory Attributes

Sensory evaluation of chicken cutlets was conducted by a semi-trained panel consisting of seven panellists from the Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Pookode using a nine-point Hedonic score card according to (Badr*et al*., 2004).Cost of production was calculated and statistical analysis was done for the parameters observed.

RESULTS

Physico-chemical characteristics

The values of pH, TBARS numbers and tyrosine value of control as well as treatment cutlets are shown in Table 1. The pH of cutlets differ significantly (p□0.01)withP having the highest value of 5.79 ± 0.005 and the lowest pH (5.4 ± 0.004) was observed for Y and PY had a value in between. TBARS numbers and tyrosine values did not show any significant difference between treatments and control.Total phenolics was observed to be significantly higher (p□0.01)inY(11.75 ± 0.456µg tannic acid equivalents per g) when compared to the values of P (8.75 ± 0.332) and PY (10.37 ± 0.412) respectively.

DPPH radical scavenging assay was done to estimate the antioxidant activity of fresh and cooked yam and potato. DPPH radical scavenging activities of fresh and cooked yam (73.89 ±1.300and 83.30±0.637 %, respectively) were significantly (p<0.01) higher than those of fresh and cooked potato (59.27±0.904and75.06±0.963, respectively).On instrumental texture profile analysis, it was noted that there was significant (p□0.01) difference in hardness values between treatments and control and Y had the highest hardness value of 204.39 ± 2.25 N/cm^2 and P had the lowest value of 115.04 ± 3.108 N/cm^2 andPY showed an intermediate value of 145.75 ± 2.186 N/cm^2. There observed no significant difference in cohesiveness, springiness and adhesiveness between control and treatment cutlets.

There was no significant difference in Land a values between treatments and control, but b value wassignificantly (p<0.01)lower for P when compared to Y and PY. The texture parameter values and hunter values are given in Table 2Moisture content of cutlets did not show a significant difference between the cutlets and ranged between 60-62% in all. Protein content was found to be significantly lower (p□0.001) in P (16.25±0.184 %) when compared to Y (17.33±0.116 %) and PY (17.00±0.051 %).Fat content was significantly (p□0.001) lower in Y (6.16±0.045 %) when compared to P(7.05±0.103 %) and PY (6.68±0.103 %). Carbohydrate content of Y (14.32±0.08 %) was significantly (p<0.001) higher than P(13.86±0.26 %) and PY (13.27±0.25 %). Energy value of cutlets showed no significant difference between P, Y and PY, with values of 183.92±0.588, 181.96±0.48 and 182.83±0.910 kcal/100g, respectively.Y and PY had significantly (p<0.01)higher ash content(2.84±0.029 and2.84±0.036%, respectively) when compared to P(2.56±0.027 %). The values of proximate analysis are depicted in Table 3.Dietary fibre content of P was found to be 3.64% and those of Y and PY were 3.86% and 3.70% respectively and there was no significant difference. Omega 3 fatty acid content of cutlets dipped in backyard reared duck eggs was found to be 0.13g/100g in both potato and yam added cutlets.

Microbiological parameters

Aerobic plate count did not differ significantly between treatments and control which was found to be in the range of 4.54 ± 0.03log$_{10}$ CFU/g sample ofY, 4.47 ± 0.11 log$_{10}$ CFU/g sample ofPY and 4.37 ± 0.034log$_{10}$ CFU/g sample of P.Psychrotrophic growth was not observed in any of the cutlet sample. Yeast and mould counts also did not differ significantly between the cutlets. The microbiological counts are showed in Table 4.

Sensory Attributes

Sensory evaluation of cutlets were done using the nine-point hedonic scale and was evaluated for the attributes like colour, flavour, juiciness, texture and overall acceptability. There was no significant difference between P, Y and PY in any of these attributes. All the cutlets, P, Y and PY has sensory scores in the level of 'more acceptable'.Cost of production was calculated to be Rs. 265/- per kilogram of P, Y and PYcutlet mixes.

DISCUSSION

With increase in the level of potato in cutlet there observed an increase in pH and similar observation was made byChetana (2014), who observed a significant and gradual increase in pH when the level of potato incorporated in to chicken cutlet was increased. In the present study the TBARS number did not vary significantly between P, Y and PY and the numbers were low since the cutlets were fresh and were on the first day of preparation. Lee *et al*. (2005) described a delay in the lipid oxidation of burgers with added plant extract of 500 ppm of rosemary and had lipid oxidation lower than that of the control burgers (without antioxidant) and the oxidation was equivalent to burgers with 200 ppm of butylated hydroxyanisole(BHA). Balakrishnan and Kalirajan (2015) found out the presence of tyrosinase enzyme in yam tuber which could lead to lysis of free tyrosine amino acid formed due to proteolysis. However, no significant difference in tyrosine values was observed between cutlets in the present study and might be due to the analysis of samples on day of preparation.

Since the total phenolics were higher in yam the antioxidant content would be higher in yam and also the antioxidant activity was retained even after cooking of yam since we got a significantly higher value for DPPH scavenging activity for cooked yam than cooked potato.Angayarkanni (2010) observed a maximum of 68.6 % radical scavenging activity in *Amorphophalluspaeoniifolius* extract having 50μg phenolics as gallic acid equivalent.Kaur and Kapoor (2002) had estimated the DPPH activity of ethanolic extract of potato as 62.5%. Antioxidants could decrease or prevent the formation of free radicals which might otherwise cause oxidation of different cell components and would lead to disorders or diseases (Manach*et al*., 2004).

The hardness value observed was significantly higher for yam cutlet in instrumental texture profile analysis but it was not correlated to the sensory evaluation scoring in which the texture showed no appreciable difference. Caine *et al*. (2003) observed the regression analysis of texture profile analysis in beef steaks and reported that hardness and adhesiveness would be helpful in explaining the significant variations between the tenderness of the steaks, but juiciness and flavour intensity analysis during sensory testing were not well correlated with texture profile analysis results. According to Biswas (2011) texture profile analysis using texture analyser/Warner Bratzler shear press/Kramer shear apparatus showed a significant increase in hardness when there was an increase in fibre content however springiness, adhesiveness and cohesiveness showed irregular behaviour. In the present study there observed no difference in lightness and redness values between cutlets.Carpenter *et al*. (2007) observed no effect on the lightness value of cooked pork pattiesdue to addition of natural antioxidant sources like grape seed extract or bearberry.In contrast to this, Muthukumar*et al*. (2012) reported lowering of Lvalues of ground pork patties incorporated with*muringa* leaf extract and butylated hydroxyl toluene.Mbouguenge*t al.* (2015) observed significant correlation between different starch types like potato starch and cassava starch as well as different starch incorporation levels on the moisture contents of both raw and cooked patties and they opined that it might be due to the physico-chemical characteristics and inherent moisture content of the starch used. In the present study, no significant difference in moisture content was noticedbetween the cutlets. Mbouguenge*t al.* (2015) reported significant difference (p□0.05)in protein, fat and carbohydrate contents of beef patties due to incorporation of different starch types (cassava and potato). Similarly, significant differences in fat and protein contents were observed between P and Y cutlets. The mineral content in yam was observed to be higher, 5.743g% (Vora*et al*., 2015) than that of potato, 1.51 g% (Abbas *et al*., 2011). This difference due to incorporation of yam and potato was appreciable in the ash percentage of cutlets. Dietary fibre intake would increase the absorption of essential nutrients (Hayashi *et al.*

2001)and they could lead to production of short chain fatty acid and gut hormones which helped in decreasing different metabolic diseases (Lattimer and Haub, 2010). However, no significant difference was observed in dietary fibre levels of yam and potato incorporated cutlets.Increased microbiological counts were usually observed in extended meat products during storage and the low fat percentage in extended products were considered as the reason for higher count (Bhat *et al*., 2013). But the present study revealed lower microbiological count and it might be due to lower contamination during processing and due to thermal processing of ingredients like pressure cooking. Indumathi and Reddy (2015) observed no yeast and mould count in functional chicken nuggets incorporated with antioxidants like green tea extracts, guava leaf extract and curry leaf extracts and it was attributed to the antibacterial effect of spices added in the nuggets. Similarly the lower aerobic and yeast and mould count could be attributed to the higher proportion of spices and condiments (20%) added to all groups of cutlets. Sensory attributes like colour, flavour, texture, juiciness and overall acceptability showed higher scores for all cutlets and all cutlets scored 'well acceptable'in the sensory study. Chetana*et al.* (2014) also reported increased overall acceptability when they increased the level of incorporation of potato to 25%.

Complete or partial replacement of potato in chicken cutlets with elephant foot yam resulted in an organoleptically 'well acceptable' product with higher antioxidant activity, higher protein and ash contents and lower fat content. To conclude, the present study helped to developa novel meat productwith good organoleptic acceptability, higher nutritional and health benefits at the same cost as that of the traditional product.

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Table 1. Physico- Chemical Caharacteristics of Control and Treatment Cutlets

Cutlets	PH	Tbars Number	Tyrosine Value (Mg Of Tyrosine/100g Of Sample.	Total Phenolics Mg Tannic Acid Equivalents Per G
P	5.79 ± 0.005^{a}	0.06 ± 0.007	0.73 ± 0.021	8.75 ± 0.332^{c}
Y	5.4 ± 0.004^{c}	0.05 ± 0.004	0.73 ± 0.021	11.75 ± 0.456^{a}
PY	5.76 ± 0.003^{b}	0.06 ± 0.004	0.76 ± 0.03	10.37 ± 0.412^{b}
F-value	2271.455**	1.211^{ns}	0.489^{ns}	11.085**
P-value	0.001	0.328	0.782	0.001

**- significant at 1% level, ^{ns}- nonsignificant, means having same alphabets are homogenous

Table 2. Texture Profile and Hunter L, A, B Of Control And Treatment Cutlets

Cutlets	Hardness	Cohesiveness	Springiness	Adhesiveness	L	a value	b value
P	115.04 ± 3.108^{c}	0.21 ± 0.026	0.25 ± 0.003	-0.01 ± 0.002	43.19 ± 0.39	4.4 ± 0.12	14.22 ± 0.18^{b}
Y	204.39 ± 2.25^{a}	0.22 ± 0.004	0.28 ± 0.012	-0.02 ± 0.006	43.39 ± 0.60	4.72 ± 0.30	15.66 ± 0.26^{a}
PY	145.75 ± 2.186^{b}	0.21 ± 0.019	0.27 ± 0.011	-0.02 ± 0.005	43.91 ± 0.26	4.98 ± 0.27	14.98 ± 0.23^{a}
F-value	253.643**	0.178^{ns}	1.816^{ns}	0.875^{ns}	0.560^{ns}	1.078^{ns}	7.856**
P-value	0.001	0.969	0.140	0.507	0.729	0.392	0.001

**- significant at 1% level, ^{ns}- nonsignificant, means having same alphabets are homogenous

Table 3 Proximate Analysis of Control and Treatment Cutlets

Group	Fat (%)	Protein (%)	Carbohydrate (%)	Ash (%)	Energy Kcal/100g
P	7.05±0.103^{a}	16.25±0.184^{b}	13.86±0.26^{ab}	2.56±0.027^{b}	183.92±0.588
Y	6.16±0.045^{b}	17.33±0.116^{a}	14.32±0.08^{a}	2.84±0.029^{a}	181.96±0.48
PY	6.68±0.103^{a}	17.003±0.051^{a}	13.27±0.25^{b}	2.84±0.036^{a}	182.83±0.91
F value	31.898**	18.584**	6.002*	27.668**	2.103ns
P value	0.001	0.001	0.012	0.001	0.157

**- significant at 1% level,*- significant at 5% level, ^{ns}- nonsignificant, means having same alphabets are homogenous

Table 4. Microbiological Parameters of Control and Treatment Cutlets

Cutlets	Aerobic Plate Count	Yeast And Mould Count
P	4.37 ± 0.034	3.67 ± 0.048
Y	4.54 ± 0.03	3.66 ± 0.03
PY	4.47 ± 0.11	3.58 ± 0.061
F-value	1.302ns	0.802^{ns}
P-value	0.290	0.557

**- significant at 1% level, ns- nonsignificant, means having same alphabets are homogenous

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RESEARCH ARTICLE

Clinical Profile and Laboratory Analysis of Enteric Fever in Gulbarga Region

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ABSTRACT

A total of 89 patients with clinical and /or laboratory diagnosis of enteric fever admitted to the various private and government general hospitals of this region were studied for demographic data such as age, sex, clinical features, and results of laboratory tests. There were 48 male and 41 female patients ranging from 8 days to 65 years. Enteric fever was noted year round with peak in August. The most commonly afflicted individuals were 35 students followed by 20 unemployed. Fever was seen in all cases. Apart from fever headache, cough, abdominal pain, chills and diarrhea were preponderant. Pulse-fever disproportion (47.19 %), abdominal tenderness (28.08%), hepatomegaly (16.85 %) and splenomegaly (10.11 %) were the most common physical signs. No rose spots observed. 8% of patients had insignificant titers of less than 1:160 while 91.95% developed significant titers to O and H antigens. Blood cultures were positive in 87 patients, 52.87 % of which became during the first week.

Keywords: Typhoid fever, *S typhi, S.paratyphi A,* Clinical profile, Laboratory Diagnosis

INTRODUCTION

Enteric fever is a systemic infection with the bacterium *Salmonella* Typhi. This highly adapted, human specific pathogen has evolved remarkable mechanisms for persistence in its host that help to ensure its survival and transmission [1]. The clinical manifestations of typhoid fever can be varied, ranging from a mild and non-specific illness to a more severe form characterized by toxemia, internal hemorrhage, perforation, and even death. The

diversity of signs and symptoms and the decreasing incidence of typhoid fever in industrialized countries resulting from the use of safe water supplies and improved sewage disposal, made many physicians unfamiliar with its clinical complications [2], [3]. However, since sanitation has not improved as rapidly in many developing countries like the India, enteric fever continues to be a common public health problem.The present study was undertaken to define the current clinical and laboratory presentation of enteric fever in the Gulbarga region. With these objectives we hope to provide our clinicians with the current clinical and laboratory picture of enteric fever for better diagnostic acuity.

MATERIALS AND METHODS

This was a retrospective study of the available clinical records of patients admitted at the various private and government general hospitals of Gulbarga region from August 2006 to September 2009 diagnosed to have enteric fever with positive *Salmonella* Typhi and *Salmonella* Paratyphi A cultures from blood, urine and CSF regardless of age and sex. A total of 89 cultures proven typhoid patients were studied. 81 of them were positive for blood cultures alone while five were positive for urine and three positive for CSF cultures. Blood and specimens were enriched in bile salt broth selenite F broth respectively and subcultured in Mac-Conkey and Wilson-Blair agar while, CSF samples were subcultured directly in Mac-Conkey and Wilson-Blair agar. The recovered microorganisms were then worked-up for *Salmonella*. Serogrouping was performed once *Salmonella* was isolated. The recovered microorganisms were then identified.

RESULTS

Age and Sex Prevalence

A total of 84 *S.*Typhi and 5 *S.*Paratyphi A were isolated from 1200 samples from suspected enteric fever patients, indicating an incidence rate of 7% and 0.41% respectively. The youngest patient was an 8 days newborn, girl child while the oldest was a 65-year-old male. Highest incidence (30) was in the age group 21-30 year, followed by age group 11-20 year (24) with only 2 patients in the elderly age group (>60 year). (Table 1 and 2). There was no sex predilection. There were 48 males and 41 females.

Seasonal Incidence

The disease was noted all year-round. Highest incidence was recorded in August with 23 cases (25.84 %). This corresponded to peak in rainy season of this region causing an increase in incidence of exposure and ingestion of contaminated food and water. (Table 3)

Occupational Incidence

The most commonly afflicted individuals were 35 students (39.32 %) followed by 20 unemployed (22.47%), housewives, laborers, and office workers in decreasing order of frequency. (Table 4)

Symptoms

Fever was seen in all cases. The temperature was high grade (>40ºC) in 21(23.5%), moderate grade (38.1ºC -40ºC) in 56 (62.92%) patients and low grade in 12 (13.48%). Almost half of these patients experienced chills at some time during the course of illness. Aside from fever, headache was also a common symptom **(**61.79 %).Gastrointestinal manifestation, such as anorexia, nausea and vomiting were preponderant. Abdominal pain was noted in all abdominal quadrants, predominantly in the right lower quadrant region. Among the neuromuscular manifestations

that occurred at anytime during the course of the disease, headache, bodyweakness, myalgia and arthralgia were early symptoms. 51 (57.30 %) patients had non-productive cough. (Table 5)

Signs

The most commonly observed physical finding was pulse-fever disproportion (47.19 %) followed by abdominal tenderness (28.08%). Hepatomegaly was seen in 16.85 % of patients and10.11 % had splenomegaly. No rose spots were observed. (Table 6)

Laboratory Results

Widal test was done in 87 patients. (Table 7) Tests were done during the first– third week of illness. Seven (8%) had insignificant titers of less than 1:160 while 80 (91.95%) developed significant titers to O and H antigens, 17(20.25%) to O antigen, 20(25%) to H antigen and 43(53.75) to both O and H antigens.

Cultures

Positive cultures were obtained in all patients.87 had positive blood cultures alone and 2 had positive CSF culture, one of which also had positive blood culture.52.87 % of positive blood culture patients had this yield during the first week of illness while 43.67 % became positive on the second week. Three had positive blood cultures during the third week of illness.Both of the CSF cultures were positive on the second week of illness. Urine cultures and stool cultures was done in 12 patients and all showed negative results.*Salmonella typhi* was isolated in 84 (96.5 %) cases and *S.paratyphi A* in 5 (5.9 %) cases.

DISCUSSION

In this study, typhoid fever affected all ages and was prevalent in the second and third decades of life. This finding has been consistent with previous published studies [4], [5], [6], [7], [8], [9]. The age factor was related to the opportunities for contacting the offending organism.There was no sexual predilection noted, as also noted by Alora in 1980 [8] and Perez in 1972[7]. In contrast to this observation, male predominance was noted by Lao in 1981[9], Ranoa in 1984[10] and Castanares in 1990[5]. These authors explained that males usually have a higher risk of exposure because of their occupations.Admission rates of typhoid fever were year-round with peak during the rainy season with attendant contamination of drinking water. This seasonal observation was similar to those observed by Quimpo [6]**.**

Fever was usually moderate to high grade and was accompanied by significant pulse-temperature dissociation in about (47.19 %) of patients; a finding noted by Alora [8], Lao, [9] and Quimpo [6] in their respective studies. Headache, cough, abdominal pain, chills and diarrhea were seen in 50% of our patients, similar to those noted by Marcial [11] in contrast to Quimpo [6]**.** Cough commonly seen in our patients can lead to erroneous diagnosis if clinicians are unaware of its presence in typhoid fever. Lucido in 1975 12] considered this symptom to be an important clinical feature of the disease, a finding refuted by separated studies of Tupasi [13] and Alora [14]. Headache was present in 61.79% of patients. A similar study done in Adana, Turkey showed a 60% incidence of headache out of 62 culture proven cases. Another important similarity as observed only by Lao on 1980 [15] was diarrhea, which was more common than constipation. As in most series [7], [13], [16], [17] physical findings were rare. When present, patients manifested abdominal tenderness, hepatomegaly or splenomegaly. These studies were noted in previous studies like those of Quimpo [6]. The characteristic rash of enteric fever was not observed in the present study.Schroeder [18] in 1968 recognized the unreliability of the Widal test based on its characteristics: nonspecific, poorly standardized, often confusing and difficult to interpret. This was based on the fact that *Salmonella* is divided into distinct serologic groups on the basis of somatic (O) antigens. All group D organisms, to which

Salmonella typhi belongs, possess O antigen 9 and out of 78 species in this group, 59 have O antigen 12 of which happened to be present also in groups A and B. Thus a false positive titer can occur in any of these serotypes. Patients with chronic liver disease giving high serum globulin levels as well as several immunological disorders may give false positive titers. On the other hand, it may remain negative despite bacteriological proof of diagnosis. Ismail in 1993 [19] added that it may be affected also by vaccination, is time-consuming and requires paired sera for meaningful interpretation. *Salmonella typhi* was isolated in 84 patients while *S.paratyphi A* was recovered in 5,an observation similar to the finding of Tupasi [13].

CONCLUSION

Enteric fever affected all ages with higher incidence in the age group 21-30 years. There was no sexual predilection. A higher incidence was noted during the rainy season, with students and the unemployed as the most commonly affected group.Fever was the most common chief complaint. Predominance of gastrointestinal manifestations was noted with diarrhea more common than constipation. Pulse-fever disproportion was the most common physical finding.Widal test result had shown significant titer in most of the cases. Blood culture, was the most definitive laboratory tool. Most *Salmonella* isolated were either belongs to serotype group D or group A.

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Table 1. Age Incidence of the 89 Cases of Enteric Fever

Age in years	Number ofcases	Percentage (%)
0-10	11	12.35
11-20	24	26.00
21-30	30	33.70
31-40	15	16.85
41-50	6	6.74
51-60	1	1.12
61-70	2	2.24
Total	89	100

Table 2. Sex Incidence of the 89 cases of Enteric Fever

Age in years	Number ofCases	Percentage (%)
Male	48	53.93
Female	41	46.07
Total	89	100

Table 3. Seasonal Incidence of the 89 cases of Enteric Fever

Month	Number ofcases	Percentage (%)
January	5	6.61
February	8	8.98
March	5	6.61
April	11	12.35
May	8	8.98
June	2	2.24
July	7	7.86
August	23	25.84
September	13	14.60
October	3	3.37
November	3	3.37
December	1	1.12
Total	89	100

Table 4. Occupational Incidence of the 89 cases of Enteric Fever

Occupation	Number of cases	Percentage (%)
Student	35	39.32
Unemployed	20	22.47
Housewives	7	7.86
Laborer	5	5.61
Office worker	9	10.11
Driver	6	6.74
Farmer	4	4.49

Food Handler	3	3.37
Total	89	100

Table 5. Symptoms of the 89 cases of Enteric Fever

Symptom	Number of cases	Percentage (%)
Fever	89	100
Headache	55	61.79
Cough	51	57.30
Abdominal Pain	50	56.17
Chills	47	52.80
Diarrhea	45	50.56
Anorexia	32	35.95
Body weakness	31	34.83
Vomiting	29	32.58
Nausea	8	8.98
Myalgia	6	6.74
Sore throat	6	6.74
Body Malaise	4	4.49
Constipation	3	3.37
Arthralgia	3	3.37
Sweating	2	2.24

Table 6. Signs of the 89 cases of Enteric Fever

Signs	Number of cases	Percentage (%)
Pulse Fever	42	47.19
Hepatomegaly	15	16.85
RLQ Tenderness	14	15.73
RUQ Tenderness	11	12.35
Splenomegaly	9	10.11

Table 7. Laboratory Results (Widal Test) of the 89 cases of Enteric Fever

Week	1	2	3	4	Total
Insignificant Titer	3	2	1	2	7
Significant Titer (1:160)					
O	4	8	5	-	17
H	9	7	4	-	20
O and H	21	13	9	-	43

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RESEARCH ARTICLE

Bioremediation for Removal of Heavy Metals using *Saccharomyces cereviseae* a Novel and Economical Process

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ABSTRACT

Today due to fast development and industrialization and production of different toxic compounds containing heavy metals the environment surrounding the industries are heavily polluted and cause destruction of living ecosystems. An attempt has been made in the present investigations to remove the stress of heavy and toxic metals, environment friendly approach is applied and use of naturally occurring microbe is emphasized by the process of bioremediation. One such microbe used for bioremediation is bakers yeast *Saccharomyces cereviseae* which has natural decontamination process. The removal of heavy metal ions, Mn, Pb, Cd, Cu & Fe from aqueous synthetic solutions using *Saccharomyces cereviseae* was utilized in the present studies. The studies were carried out by varying parameters such as effect of equilibrium time, determination of growth kinetics of *S.cereviseae*, effect of Cystine, effect of initial pH and metal concentrations.The optimum pH ranged from 5-6.5 for different heavy metals. The maximum removal of different heavy metals ranged from 60-75% at an initial concentration 10µg/ml with 2% (v/v) 0f inoculums concentration at 30℃ .The cells and cultures were subjected to immobilization process using sodium alginate. Present investigations also revealed the strong ability for heavy metal removal by process of bioaccumulation was probably due to presence of cystein in the cell walls of *S.cereviseae*. Use of microbial resource coupled with bioaccumulation proved to be one of the most promising, viable and economical strategies for removing environmental pollutants.

Key words - *Bioaccumulation; bakers yeast; Immobilization Saccharomyces cereviseae; cystein;*

INTRODUCTION

Environmental contamination due to anthropogenic and natural sources is increasing day by day because of an increase in population, industrialization and urbanization. The enigma for the public, scientists, academicians and politicians is how to tackle the contaminants that jeopardize the environment. The ideal solution for pollution abatement is Bioremediation, the most effective and innovative technology to come along that uses biological systems for treatment of contaminants (Roy etal., 2013). A variety of physical, chemical and biological technologies have been developed for clean-up operations of contaminated sites. Bio-remediation one of the most sustainable and promising technology to reduce or eliminate environmental hazards, resulting from toxic chemicals and other hazardous waste. Bioremediation can be categorized as a) Phyto-remediation b) Zoo-remediation c) Microbial- remediation depending on utilization of plants, animals, insects, bacteria or fungi. Bio-remediation is utilized as it is economical, safe, environmental friendly, six times cheaper than regular incineration.

In the present investigations *Saccharomyces cereviseae* (budding yeast) has been used as source of bioremediator. Microbial bioremediation technology involves the use of microbes, their metabolic reactions to de toxify, degrade or remove environmental contaminants. Metal contamination is a global problem and the main reason is that 80% of the land is irrigated by waste water generated by industries. Use of microbial resources coupled with other modern techniques is one of the most promising and economical strategies for removing environmental pollutants (Rajendran and Gunashekaran, 2006) Bioremediation is the use of microbial metabolism to remove pollutants. Technology can be generally classified as *in-situ* or *ex-situ. In- situ* bioremediation involves treating the contaminated material at the site, while *ex-situ* involves the contaminated material to be treated elsewhere. Recent advancements have proven successful *via* the addition of matched microbe strains to the medium to enhance the resident microbe population's ability to break down contaminants. Environmental contamination by hydrophobic compounds is widespread, including petroleum spills and industrial solvents.

The objective is to use a combination of transport processes and biological activity in order to eliminate these contaminants permanently and economically.Bacteria and fungi have the ability to grow in a range of hydrophobic materials such as hydrocarbons and organic sulphur and nitrogen compounds, while the objective of yeast cultures is to convert these compounds to Co2 and more yeast cultures we can harness their enzymes to achieve important and interesting reactions.Yeast used in bioremediation to remove heavy metals is of great importance as it is eukaryotic organism, as yeasts are chemo-organotrophs since they utilize organic compounds as a source of energy and do not require sunlight to grow. Carbon is obtained from hexose sugars, such as glucose and fructose or disaccharides such as sucrose or maltose. Some species can metabolise pentose sugars like ribose to alcohols and organic acids. Yeasts grow best in a natural or slightly acidic pH environment. Yeasts grow best from 28°F -113°F . Common media is YEPD/ Yeast Extract Peptone dextrose on solid media or liquid media in broth .Yeasts are often isolated from sugar-rich material especially in the skins of fruits and berries, exudates from plants. The most common type of asexual reproduction ios by budding or by fission. Yeast species are used as alcoholic beverage, baking, bioremediation, bioethanol as non-alcoholic beverages as nutritional supplements as probiotic and Vit B12.

The removal of cations of heavy metals from industrial waste water can be accomplished by biotechnological methods which make use of microorganisms as cation collectors (Babel and Kurniawan 2003). Microbial biomass has been derived from various sources, eg., actinomycetes cyanobacteria, other bacteria, algae moulds and yeasts (Baldrian, 2003).The mechanism of metal binding to microbial biomass can be roughly divided into three main types intracellular accumulation, sorption or complex formation on cell surface and extra cellular accumulation or precipitation .The metabolically independent biosorption of metals by yeast cells occur within several minutes (Volesky , 2003).All the heavy metals contributes markedly to the environmental pollutions,especially of water and soil. Low metal concentrations can be conveniently be removed by low -cost low energy bioaccumulation which is at

the same time, a highly efficient process. The present study aims the removal of heavy metals using live and immobilized cells of *Saccharomyces cereviseae* .

MATERIALS AND METHODS

Microorganisms; media and culture/ cultivation conditions; *Saccharomyces cereviseae* strain was procured from the microbial stock collection, NCL, Pune and it was cultivated in standard Yeast extract/ peptone/ dextrose (YPD/YEPD) rich medium comprising in (W/v) %; dextrose 2; yeast extract 1 and peptone 2 and Agar 2% at room temperature/ pH-5.5. Subcultures were stored at 40℃

Inoculum suspensions of *S.cereviseae* containing 107cells/ml, with the sterilized metal salts being added at different concentrations before autoclaving. The flasks were incubated at 300℃ for different time intervals shaking at 200 rpm.

Estimation of heavy metals (Synthetic solutions of Mn (Manganese sulphate) Pb (Lead nitrate) Cd (Cadmium sulphate) Cu (Copper sulphate) Fe (Ferric chloride) 10µg/ml to 90µg/ml using different metal ion concentrations were carried out . Final metal ion concentrations were determined and estimated by Atomic absorption spectro photometer (AAS).

Immobilization using Na-alginate ; Wet biomass was suspended in the 2% (W/v) concentration of sodium alginate and this was added dropwise to a solution of 2% (w/v) $Cacl_2$. The resulting beads were washed using distilled water and subsequently used for study.

Analysis of the heavy metals; The analysis of specific metal uptake (q) by biomass was calculated as follows (Zang et al. 1998)

Q= vx(ci-cf)/1000w

The data were statistically analysed by using one way analysis of variance (ANOVA) at p= 0.005

RESULTS AND DISCUSSION

A) Maintained the yeast cultures by subsequent subculturing on standard Yeast extract Peptone dextrose (YPD/YEPD) and Nutrient broth of 50 ml YPD was prepared and loopful of yeast cells cultured in 250ml conical flasks at 30o c on a rotary shaker for 24 hrs at 200 rpm, pH of the medium was maintained at 5.5 with the sterilized metal salts are added and incubated at 30℃ at different time intervals shaking at 200rpm (Fig: 1).

B) Removal of heavy metals ions (Cd, Pb, Mn, Cu and Fe) at various initial metal concentrations

Initial metal ion concentration plays a major role in calculating the bioaccumulative capacity of yeast cells/ cultures, as metal concentration increased the removal of heavy metal ions is decreased as shown in (Fig.1-5). There is real danger that these metals may poison the cell and stopping biological activity and growth. It has been inferred in several instances that the accumulation of metal results from the lack of specificity in a normal metal transport system and that, at high concentrations heavy metals may act as competitive substrate in a transport system. Similar results were obtained in the case of *Schizo saccharomyces pombe* for Cu removal as reported by Sai Subhasini *et al.,* 2011 and in case of *Trametes versicolar* for Cu, Pb and Zn removal (Puranik *et al.,*1997).

C) Effect of heavy metal on bioaccumulation at various pH (Fig. 7)

It is evident from the table that the maximum removal of all heavy metals Cd,Mn, Pb, Cu and Fe occurred at a range of pH-6.5 being slightly acidic in conditions. In contrary to this as reported by Sai Subhashini *et al.,* in 2011 the removal of Cu occurred at pH-4.0 and from pH-5.0 the level of removal started decreasing. Normally the yeast cell wall consists of protein coat, which develops a charge by the dissociation of ionizable side groups of the constituent amino acids. The ionic state of ligands such as Carboxyl, phosphate, imidazole and amino groups will promote reactions with the positively charged metal ions. At low pH 3.0 cell wall ligands closely associated with the hydronium ions (H3O+) and restricted the accumulation of Cu=2 ions as a result of repulsive forces. Removal of Copper and Lead by *Micrococcus letues* (Leung et al 2000) also showed the same trend. The increasing trend from pH 3 to 5 is due to strong relations of bioaccumulation to the number of surface negative charge , which depends on the dissociation of functional group. At higher alkaline pH values (8) and above, a reduction in the solubility of metals contributes lower uptake rates (Hasan et al 2000) reported the variation of adsorption of nickel at various pH is on the basis of metal chemistry in solution and the surface chemistry of the sorbent.

D) Effect on cystine on metal accumulation in Yeast cells (30µg/ml) (Fig.6)

It is very significant that the presence of Aminoacid Cystein in this present investigation has proved to be ideal for heavy metal accumulation by yeast cells and the effect of concentration of cystine and removal of heavy metal also varies for all heavy metals. In the present studies the concentration of cystine for the removal of copper was 2-4mg/l, Fe- 5-6mg/l, Pb, Mn and Cd- 6mg/l. The microorganisms have an independent metabolism. The capacity of binding or accumulation of heavy metals to living/dead cells, capsules, slime layer, extracellular polysaccharides or to any of the amino acid (Cystine). The presence of an amino acid (histidine) and bioaccumulation by S.cereviseae has also been reported by Pearce and Sherman (1999). Low operating cost, effective in dilute solutions generates minimum effluent/sludge, dead or immobilized biomass is also preferred. Microbial biomass binds large amounts.

E) Effect of equilibrium time for heavy metal removal

The effect of incubation time or contact time on heavy metal removal also was studied at regular time interval of 24 hrs ; (48, 72,96,120,144) as fungal cultures take certain period to grow. The equilibrium reaches 96 hrs for maximum removal of heavy metals. For many heavy metals the d orbital of their atoms are not completely filled and react with other compounds in the form of their oxides or sulphides and cause production of free radicals and cellular damage. Humans are exposed to heavy metals since long back, however within recent 50 years it has been augmented due to rapid development of industry. Heavy metals enter into water or soil and then to human food chain. At this level bioremediation of heavy metals is an effective and efficient methodological measures for protecting environmental health.If yeast is to be used for bioaccumulation of heavy metals then it must be immobilized. One possible method is gel immobilization. In the present investigations gel immobilization is an important attribute as when accumulating metal cations Na-alginate is used with calcium chloride,$2H_2O$ (Calcium alginate systems) are used which is similar to the reports of Brady and Duncan,1994).

CONCLUSION

This experimentation of heavy metal accumulation by yeast cultures and cells can be relevant in removing contaminated soils and waters caused by industrialization or pollution revealed a positive indication for its role in bioremediation of Cd,Pb,Mn,Cu and Fe in contaminated samples of waters and soils. Microorganisms may be used to remediate waste-waters contaminated with heavy metals. Microorganisms accumulate metals by a number of different process such as uptake by transport, biosorption to cell walls and entrapment in extracellular Capsules, precipitation and oxidation – reduction reactions (Das *et al.,* 2008; Vijayadeep and Sastry, 2014). Some or all of these processes may be invoked by viable (living) microorganisms to accumulate or immobilize soluble metal ions.

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Fig. 1.Effect of heavy metals on growth of *S. cerevisiae after 48 hrs*

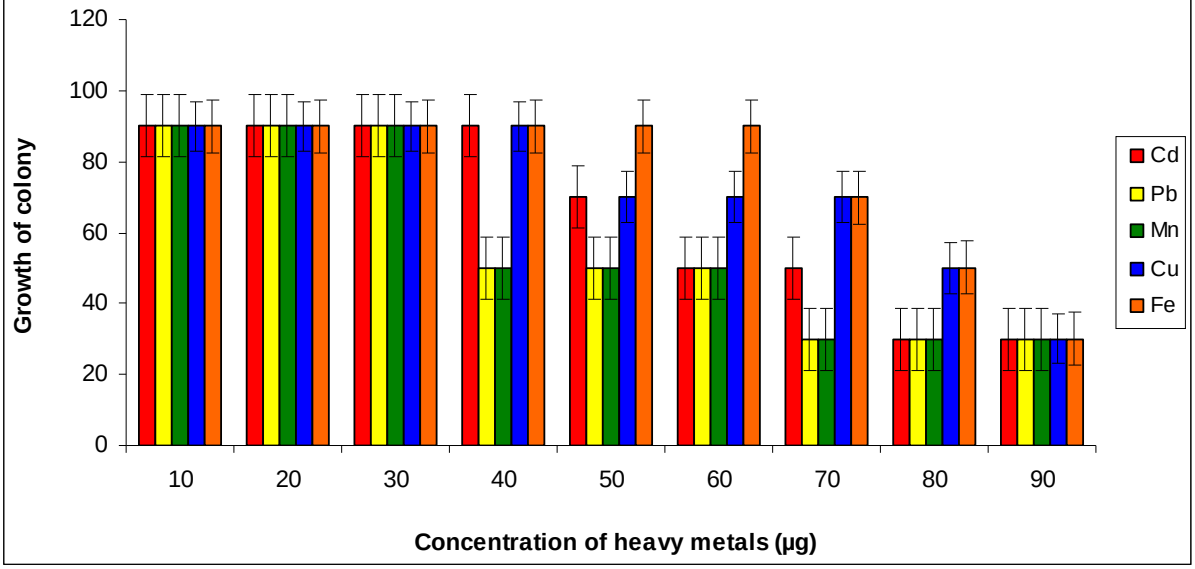


Fig. 2. Effect of heavy metals on growth of *S.cerevisiae* after 72 hrs

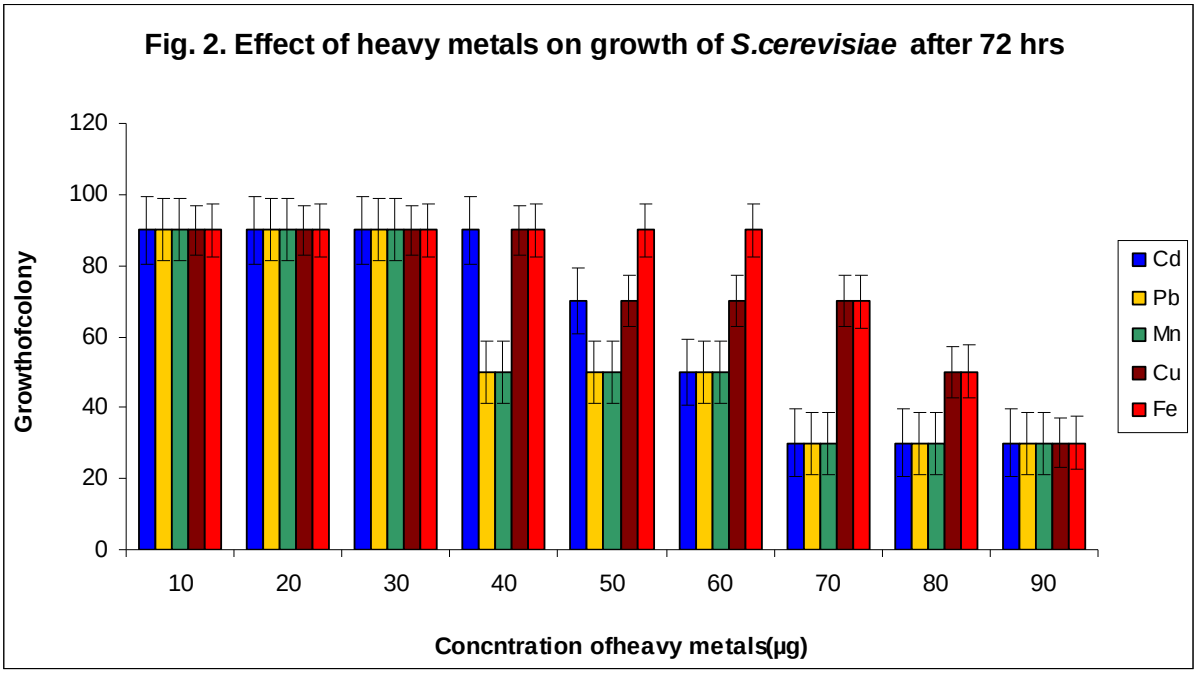


Fig. 3. Effect of heavy metals on growth of *S.cerevisiae* after 96hrs

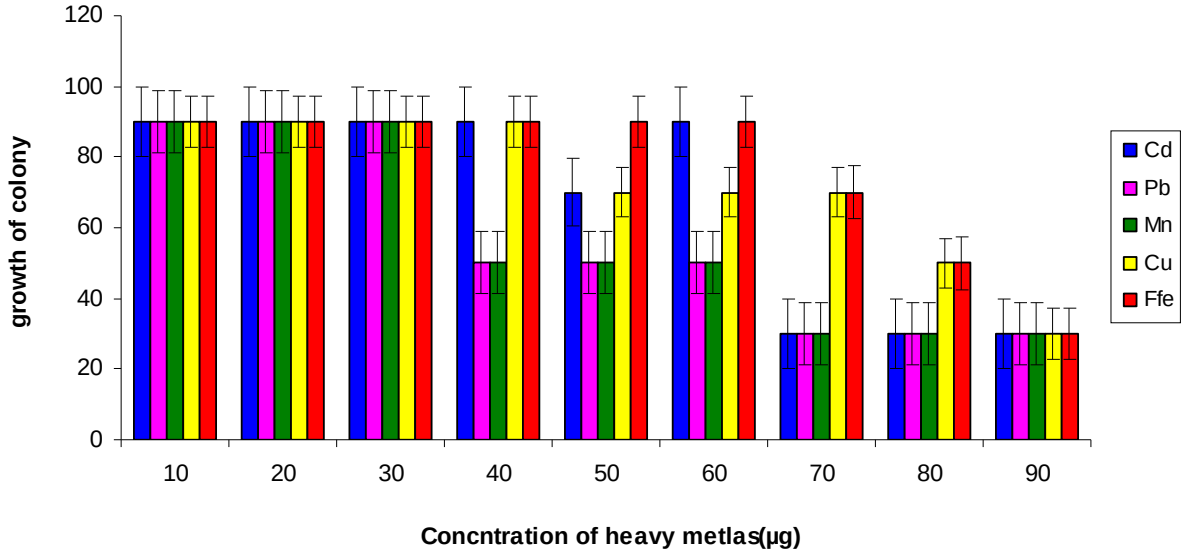
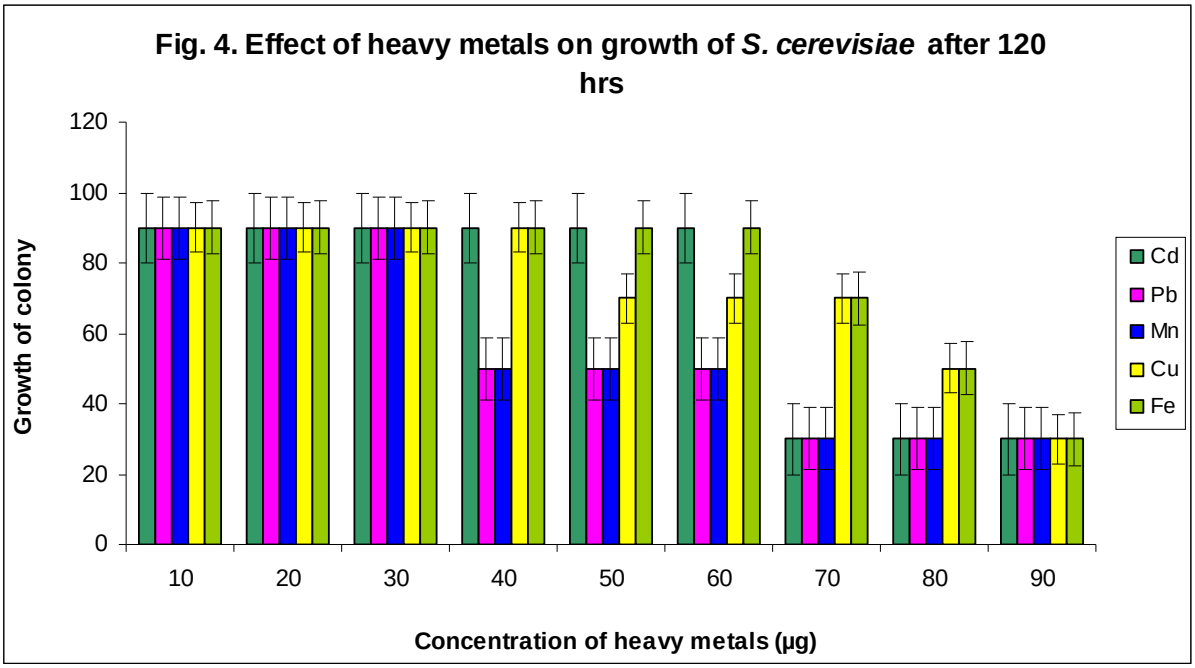


Fig. 4. Effect of heavy metals on growth of *S. cerevisiae* after 120 hrs



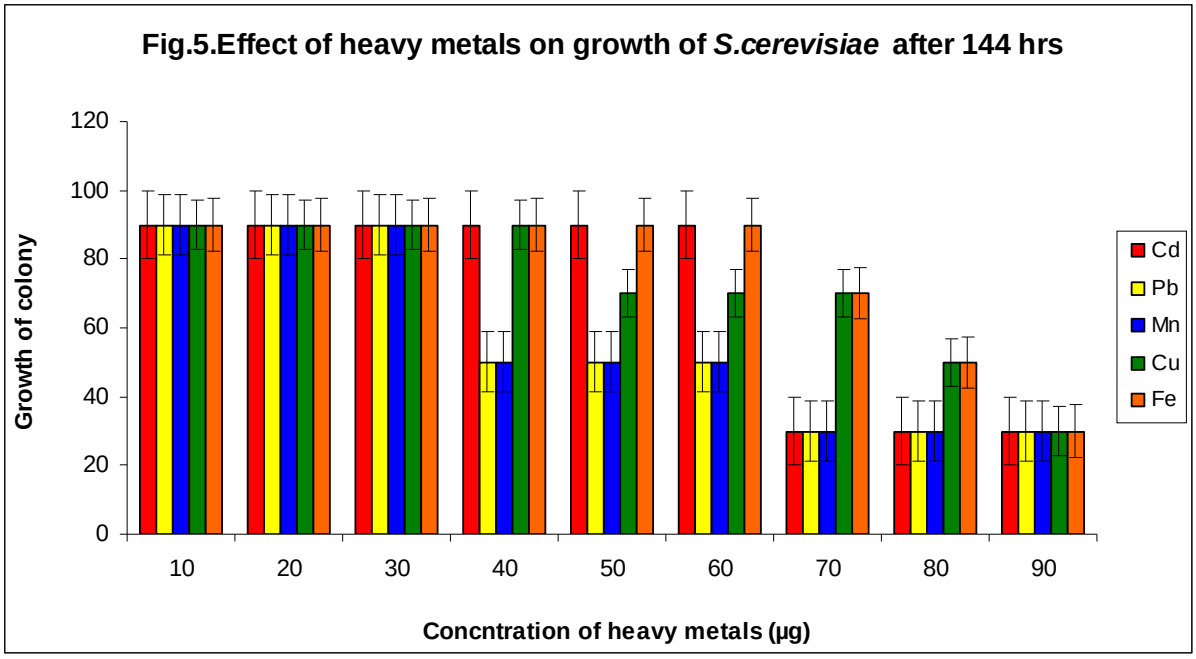


Fig.5.Effect of heavy metals on growth of S.cerevisiae after 144 hrs
Growth of colony
120
100
80
60
40
20
0
10
20
30
40
50
60
70
80
90
Concntration of heavy metals (µg)
Cd
Pb
Mn
Cu
Fe

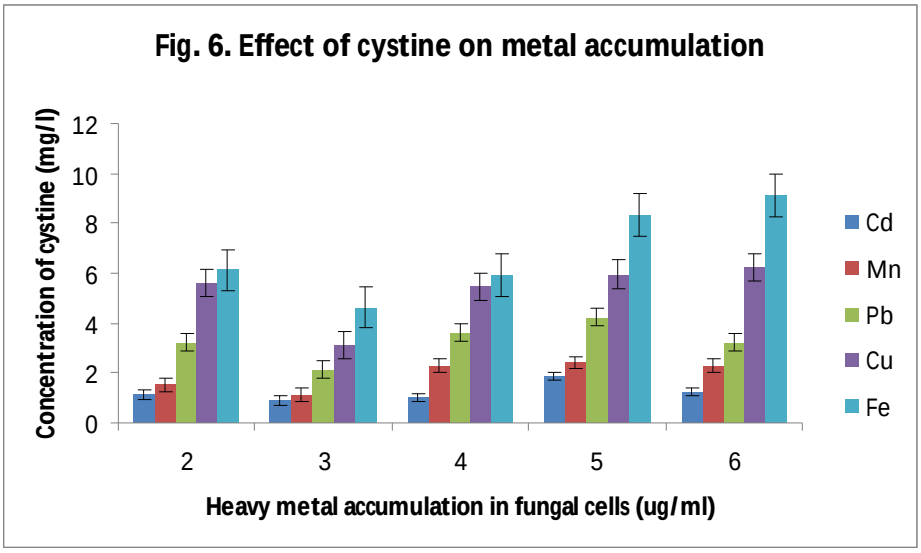


Fig. 6. Effect of cystine on metal accumulation
Concentration of cystine (mg/l)
12
10
8
6
4
2
0
2
3
4
5
6
Heavy metal accumulation in fungal cells (ug/ml)
Cd
Mn
Pb
Cu
Fe

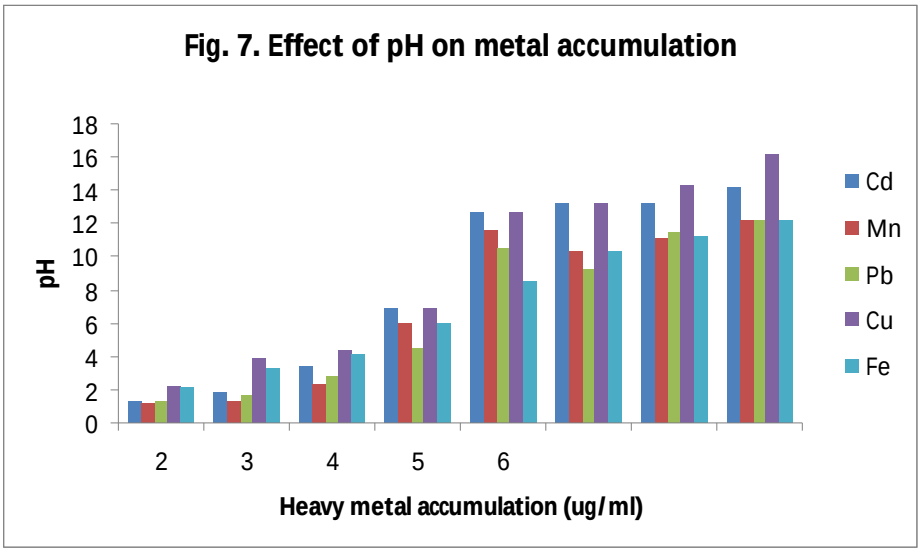


Fig. 7. Effect of pH on metal accumulation
18
16
14
12
10
8
6
4
2
0
pH
2
3
4
5
6
Heavy metal accumulation (ug/ml)
Cd
Mn
Pb
Cu
Fe

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RESEARCH ARTICLE

In vitro Synergistic Effect of Indian Plants Extracts against *Escherichia coli* Associated with Infectious Diarrhea

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ABSTRACT

Diarrhea is an important clinical problem and a leading cause of mortality in human beings in world wide. Increased multidrug resistance (MDR) is a serious threat to treatments and increases mortality and health care costs. The drug combinations are one such approach to fight against MDR effectively. Therefore, screening of three plants (*Terminalia chebula*, *Punica granatum*, *Camellia sinensis*) were evaluated as antimicrobial agent against diarrhea causing *E.coli*. The results revealed that, all the tested plants of different extracts possess different degree of antimicrobial activity alone and combination of antibiotic. MICs and MBCs of all plant extracts, antibiotics and combinations were studied using checker board assay. FIC index was calculated for the combination of plant extract and antibiotic to interpret the synergism. Among the three plants, ethanol extract of *Terminalia chebula*, *Punica granatum* combination with antibiotic showed the FIC index of 0.5 to 1.0 indicates that both have the potential synergistic antimicrobial activity against diarrhea causing *E.coli*. *C.sinensis* (green and black tea) showed better antimicrobial activity alone, but not in combination with antibiotic.

Keywords: MDR *E.coli*, synergistic effect, *Terminalia chebula*, *Punica granatum*

INTRODUCTION

Diarrhea is one of the leading causes of morbidity and mortality in humans in developed and developed countries. Furthermore, increased resistance to antibiotics has resulted in serious challenges in the treatment of this infectious disease world wide. Therefore, there exists a need to develop alternative nature or combination therapy [1]. Rising

antibiotic resistance is serious public health threat, the drug combinations are such approach to fight against the multi drug resistant bacteria (MDR) effectively [2]. Diarrhea is an intestinal infection responsible for death in the elderly in developed countries and responsible for death of 3-4 million infants and young children each year [3]. Due to poor hygienic conditions in developing countries in both hospital and community, enteric bacterial infection caused by resistant strains of *E.coli*, *Shigella* and other members are more problematic and of major public health problem [4,5].

India is fortunate as it has one of the richest flora of the world with about 120 families of plants comprising 1, 30,000 species. The use of about 2400 of these plants are mentioned in traditional system of Indian medicine for treating aliments such as wounds, leprosy, skin diseases, diarrhea, dysentery, jaundice, cough and cold, etc. [6]. Novel antibacterial actions of plant extracts or phytocompounds have been documented which include inhibition of MDR-efflux pump [7] anti-antibiotic resistance properties [8]. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body [9]. Recently there are lot of attraction towards the natural based herbs as an antimicrobial agent because of its eco friendly and health hazardless nature [10]. The traditional Indian systems of Ayurveda and Siddha medicines support the importance of medicinal plants to treat disease [11]. The fruit of *Terminalia chebula* is considered as the "king of medicine" by Tibetans and second-to-none by ayurvedic apothecaries, and also held in high regard by other folk medicinal practitioners [12]. *Punica granatum* is a plant with a wide variety of activities. The whole plant has medicinal values and is investigated by various authors [13]. Tea is one of the supreme consuming beverages all over the world, which possesses several health benefits as a result of different phytochemicals which contain important medicinal activities [14]. Herbal compound extracts can be combined with antibiotics to have an effective therapy. Screening crude extracts for synergistic interaction with antibiotics is expected to provide bioactive compounds to be used in combinational therapy. Therefore, the efficiency of plant extracts to inhibit MDR *E.coli* was determined in this study. Antimicrobial activity, synergism between plant extracts and antibiotics against the MDR *E.coli* will also be evaluated in order to minimize the adverse effects and maximize the antibiotic effect against MDR bacteria.

MATERIALS AND METHODS

Bacterial strains and antibiotic susceptibility test

Fourteen *E.coli* were obtained from the clinical specimens of diarrhea samples from the private hospitals, Erode dt, Tamilnadu, India and subjected to disc diffusion method using antibiotics [11]. The antibiotic discs (HiMedia, Mumbai) Streptomycin (10mcg), Ampicillin (10mcg), Chloramphenicol (30mcg), Tetracycline (30mcg), Penicillin (10mcg), Gentamycin (10mcg) were used. Briefly the zone of inhibition in mm interpreted sensitive and resistant using the chart supplied by HiMedia, Mumbai (India). *E.coli* k12 was used as standard bacteria.

Plant materials

Terminalia chebula fruits were collected from Siddha Medical Centre of Tirupur Dt, Tamilnadu, India. *Punica granatum* peels collected from departmental store of Erode Dt, Tamilnadu, India. *Camellia sinensis* leaves (green and black tea) were obtained from a Green shop, Johnson square, Nilgirie Dt, Tamilnadu, India.

Preparation of plant extracts

All collected plant materials surface sterilized with 10% sodium hypo chloride solution and rinsed with sterile distilled water, air dried at room temperature. The dried plant parts of *Terminalia chebula* fruits, *Punica granatum* rind, *Camellia sinensis* leaves (green and black tea) were used for further extractions.

Aqueous extraction

10gm of each plant materials *Terminalia chebula* fruits, *Punica granatum* rind, *Camellia sinensis* leaves (green and black tea) dried powder was suspended in 60 ml of cold distilled water. The mixture was allowed to soak for 24 hrs, at the end of the extraction it was passed through the Whatman filter paper No.1. The filtrate was dried in water bath at 80˚C for 2 hrs. The dried crude extracts were stored at 4˚C for further use.

Solvent extraction

20gm of each plant materials *Terminalia chebula* fruits, *Punica granatum* rind, *Camellia sinensis* leaves (green and black tea) dried powder was suspended in 100 ml of solvent (70% ethanol, 97% methanol and acetone) separately and soak for 48 hrs. The mixture passed through the Whatman no. 1 filter paper, filtrate were concentrated under rotary evaporator at 40˚C and then stored at 4˚C for further study. The crude extract was prepared by dissolving known amount of dry extract in DMSO, to have stock solution of 500mg/ml.

Antibacterial Activity of Crude Plant Extracts

Plant crude extracts were screened for their inhibitory activity against MDR *E.coli*. The well diffusion assay was performed by dipping sterile cotton swab in to bacterial culture approximately 10^8 CFU ml^{-1} and the bacteria were swabbed on MHA plate. The wells of 7mm diameter were made using sterile gel puncture. 100µl of corresponding plant extracts were loaded into the wells and incubated at 37˚C for 24hrs. The diameter of the inhibition zone was measured.

Checker board assay

Checker board assay [15] was performed to find the value of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of plant extracts. Microtitre plate wells from each column in row 1 were marked and 100µl (500mg/ml) of plant extracts (aqueous, ethanol, methanol, and acetone) and antibiotics (100mg/ml) (chloramphenicol and penicillin) was added, 50 µl of sterile distilled water was added to the rows 2-12. Two fold serial dilutions were performed by transferring 50 µl of solution from the row 1 to row 2, using multi channel micro pipette. This was repeated for the row 2 to 12. 40 µl of double strength nutrient broth and 10 µl of bacterial culture were added to all the wells in separate column, so that the final concentrations of the inoculums obtained in all the wells. To prevent dehydration and contamination, the titre plate was covered with paraffin film and incubated at 37˚C for 24hrs. The bacterial growth was determined after addition of 40 µl tetrasolium red (0.2mg/ml). The MIC of the isolate was taken as the lowest concentration of the dilution that inhibits the growth of bacteria. MBC was defined as in the MIC studies that did not show any turbidity of the bacteria were determined for MBC. The suspension was streaked onto NA plates and incubated at 37˚C for 24hrs. The dilution not showed single colony on NA plates that was taken as MBC.

Evolution of synergistic effects

The interaction between plant extracts and antibiotics was estimated by calculating Fractional Inhibitory Concentration (FIC index) of the combination. The concentration of the individual compound in the combination of plant extracts and antibiotic in which the growth of bacteria is completely inhibited is taken as MIC of individual compound in the combination. The checkerboard method is often combined with calculation of the FIC index to test the antimicrobial potencies of drugs in medical laboratories [16]. FIC value for each agent was calculated using the formula:

FIC of compound a (FIC_a)= MIC of compound a in combination / MIC of compound a alone

FIC of compound b (FIC_b)= MIC of compound b in combination / MIC of compound b alone

The sum of Fractional Inhibitory Concentration (FICs) indices of two compounds in the combination was calculated as follows:

$$FIC_s = FIC_a + FIC_b$$

Synergism was defined as a synergistic if the FIC indices were ≤ 1, additive if the FIC indices were=1, indifferent if the FIC indices were between 1 and 2, antagonism if the FIC indices were ≥2.

Phytochemical Analysis

Phytochemical analysis of the test solution was done according to published methods [17,18,19].

Test for Alkaloids

Dragendroff's test

Dissolve various extract of the herbal drug in chloroform and acidify the residue by adding few drops of Dragendroff's reagent (Potassium Bismuth Iodide). Appearance of orange red precipitate indicates presence of alkaloids.

Test for Flavanoids

Ferric chloride test

To the alcoholic solution of the extract add few drops of neutral ferric chloride solution. Appearance of green color indicates presence of flavanoids.

Test for Triterpenoids

Brieskorn and Binar test

To chloroform extract, add few drops of chlorosulphonic acid in glacial acetic acid. Appearance of red colour within five minutes indicates presence of triterpinoids.

Test for Phenolic Compounds and Tannins

Ferric chloride test

Take 2 ml of extract in a test tube and add ferric chloride solution drop by drop. Appearance of bluish black precipitate indicates presence of phenolic compounds and tannins.

Test for Saponins

Foam test

A small amount of extract taken in a test tube with little quantity of water. Shake vigorously. Appearance of foam persisting for 10 minutes indicates presence of saponins.

Test for Glycosides

Keller-Killiani test

Extract+ 1ml of glacial acetic acid+ few drops of ferric chloride solution + Conc. H_2SO_4 (slowly through the sides of the test tube). Appearance of reddish brown ring at the junction of the liquids indicates the presence of de-oxysugars.

Test for Carbohydrates

Molisch's test

Mix the extract with Molisch reagent and add Conc. H_2SO_4 along the sides of the test tube to form layers. Appearance of reddish violet ring the interference indicates the presence of carbohydrates.

Test for Proteins

Ninhydrin test

Few drops of Ninhydrin added to the extract. Appearance of blue color indicates presence of amino acid where as proteins may rarely give positive result.

Steroids

Liebennann- Burchard reaction was performed for checking the presence of steroids. Few drops of concentrated H_2SO_4 was added down the sides of the test tube with extract. A blue green ring indicated the presence of steroids

.

RESULTS AND DISCUSSION

Bacterial strains and antimicrobial susceptibility

Antibiotic sensitivity of 14 diarrhea causing *E.coli* were showed multidrug resistance (MDR). Among 14, randomly selected four highly MDR profiled *E.coli* (named Ec 1, Ec 2, Ec 3, Ec 4) for further antimicrobial and synergistic study. The Ec 1 and Ec 3 were showed antibiotic resistance profile of Str-Amp-Ch-Pen-Tet and Ec 2 and Ec 4 showed profile of Str-Amp-Ch-Pen. *E.coli* K-12 was used as a negative control, showed sensitivity to all the antibiotics except Nalidicxic acid. The MIC of the pencillin, ampicillin, chloramphenicol, streptomycin and tetracycline ranged from 50 to 100mg/ml. MDR bacteria represent an enormous challenge to modern health care systems. Although some new agents have been induced in the last 10 years (e.g., linezolid, deptomycin, and tigecycline) [20, 21], the wide spread emergence of bacterial resistance to a large member of antimicrobial agents poses major health problems because of difficulties in treatment [21]. The indiscriminate use of antimicrobial agents in the treatment of bacterial infections has led to the emergence and spread of resistant strains, and it resulted in a great loss of clinical efficacy of previously

effective first-line antimicrobials which results in shifting of antimicrobial treatment regimen to second-line or third-line antimicrobial agents that are often more expensive with many side effect [22].

Antimicrobial activity of plant crude extracts

Three traditionally used Indian plant crude extracts of *T. chebula* fruits, *P. granatum* rind, *C. sinensis* leaves (green and black tea) demonstrated broad spectrum activity against all tested four MDR isolates presented in table 1. Among the extracts, *T. chebula* fruits, *P. granatum* rind crude extract were showed highest mm of zone against the MDR *E.coli.* Plant extracts were extracted into acetone, ethanol, methanol, and cold water. The zone of inhibition (mm) in well diffusion assay was measured for different extraction of all four plant materials. Varying level of antimicrobial activity was observed in all extractions. Zone of inhibition ranges from 10-32 mm. The results obtained from the analysis of antimicrobial activity exhibited by studied plant extracts were summarized in table 2. Among the extracts, ethanol extracts showed highest antimicrobial activity than the other extracts. The ethanol extracts of *T. chebula* showed inhibition zone ranged from 26-33mm, *P. granatum* showed 20-29mm, *C. sinensis* (green tea) 14-20mm and *C. sinensis* (black tea) 12-16mm. Similar results were found in previous study, tested bacterial strains were susceptible to ethanolic and methanolic extract of green and black tea but aqueous extracts of tea samples were not effective to kill bacteria *E.coli* as well as other bacterial strains showed resistant to aqueous axtracts to some extent. Methanolic extracts of green tea was more potent against Gram positive and Gram negative bacteria [23, 24]. Aqueous extract from *T. chebula* was found to be effective on MBL which were produced by eleven isolates of *Pseudomonas* and eight isolates of *Acinetobacter* [25]. Vishal Jain *et al*., 2011 reported that the methanol extract of *Punica granatum* showed high antibacterial activity. Aslo Lali *et al*., 2012 reported that the methanol extract of *Punica granatum* showed antibacterial activity against shiga toxin producing *E.coli*.

Minimum Inhibition Concentration and Minimum Bactericidal Concentration

The MIC/MBC of water, acetone, methanol and ethanol extraction of *T. chebula*, *P. granatum*, *C. sinensis* (green and black tea) studied against diarrhoea causing *E.coli* strains. The MIC of ethanolic plant extract was found in the range of 0.24-7.81 mg/ml in *T. chebula*, 7.81-15.62mg/ml in *P. granatum,* 15.6 - 62.5mg/ml in *C. sinensis* (green tea), 62.5 - 125mg/ml in *C. sinensis* (black tea). The MBC value of the extracts was equal to value of MIC or higher than the MIC value as depicted in table 2. The potency of (MIC/MBC) extracts was in order of cold water ≤ acetone ≤ methanol ≤ ethanol. Different range of antimicrobial potency was observed in ethanolic plant extracts against MDR *E.coli* strains. These findings indicate that the high potency was exhibited by *T. chebula*, *P. granatum*. The *C. sinensis* (green and black tea) showed lowest potency when compare to other plant extracts.

Synergistic study

Similarly, the invitro synergistic interaction between ethanolic extracts of *T. chebula, P. granatum* and penicillin, *C. sinensis* (green and black tea) and chloramphenicol were evaluated separately against the strain of Ec 2 and 4. Table 3 revealed that the greatest synergism was found in the combination of *T. chebula* and penicillin could effectively inhibit strain Ec 2 (FIC index was 0.5), followed by *P. granatum* and penicillin against Ec 4 (FIC index was 1.0). Indifference was observed in *C. sinensis* (green and black tea) extracts and chloramphenicol (FIC index fell between 1.125 to 1.625). Combination therapy has earlier been reported to increase activity and prevent the development of resistance. Science phytochemicals are structurally different from the antibiotics and often have different mode of action, they provide novel means of studying the mechanisms of bacterial control at molecular level. Synergism testing using various combinations of phytochemicals with antibiotics could be powerful tool to aid selection of appropriate antibiotic therapy [28]. Synergism of natural products and antimicrobial agents is a thrust area of phytomedicine research, developing novel perspective of phyto-pharmaceuticals. The synergism of plant derived compounds and antimicrobial agents have been evaluated previously against pathogenic microorganisms [29, 30]. Synergistic effect of curcumin and antibiotics against bacteria associated with diarrhea was reported by Nishanth *et*

al., 2014. In this study significant synergistic effect of *T.chebula* and antibiotic was observed against bacteria causing diarrhea.

Phytochemical analysis

In present investigation, phytochemical screening showed the presence of following constitutions: ethanolic extracts of *T. chebula* fruits (Flavonoids, Terpenoids, Tannin, Glycoside, Carbohytrates), *P. granatum* rind (Flavonoids, Tannin, Saponins, Carbohytrates, Steroids), *C. sinensis* green tea (Alkaloids, Flavonoids, Terpenoids, Tannin, Saponins, Glycoside), and *C. sinensis* black tea (Alkaloids, Flavonoids, Terpenoids, Tannin, Saponins, Glycoside, Steroids) (table 4). The results of green and black tea were found to be similar with previous study [23]. Manogar *et al*., 2012 performed preliminary phytochemical screening and found that the ethanol extract of *T.chebula* posses phytosterols, triterpenoids, carbohydrates, glycosides, phenolic compound, and tannin. This suggests that, it may posses anti-inflammatory, analgesic, antidiarrhoeal, antimicrobial, antioxidant, immunomodulatory, antihelmintic, antitumour, hepatoprotective activities. The three different extracts of *P.granatum* peel were found to contain Flavonoids, Triterpenoids, Tannin, Glycoside, Saponins, Carbohytrates, Steroids and Vitamin C [32].

CONCLUSION

In the study we looked at the effects of combining the plant extracts of *T. chebula* fruits, *P. granatum* rind, *C. sinensis* leaves (green and black tea) with antibiotics demonstrated broad spectrum activity against all tested MDR *E.coli*. In the presence study plant extracts alone recorded antimicrobial activity. Ethanolic extracts of *C. sinensis* leaves (green and black tea) showed antimicrobial activity but showed indifference in synergism. *T. chebula* combination with antibiotic recorded significant synergistic effect followed by *P. granatum*. These data suggested that *T. chebula* and *P. granatum* in combination with antibiotic could be useful option for the treatment of complicated bacterial infection. In addition to achieving these synergistic effects, the combinations of two or more compounds are essential for the following reasons: to prevent or suppress the emergence of resistant strains, to decrease dose related toxicity, as a result dosage, and to attain a broad spectrum of activity.

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Table: 1 Antibacterial activity of crude extracts against diarrhea causing MDR bacteria *E.coli*

S.No	Name of Plant (family)	Part used	Common Name	Crude Extract Zone of Inhibition (mm)				
				EC1	EC2	EC3	EC4	EC K12
1	*Terminalia chebula* (Combretacae)	Fruit	Harir	24	27	24	27	19
2	*Punica granatam* (Punicaceae)	Rind	Anar	19	23	21	21	20
3	*Camellia sinensis* Green Tea (Theaceae)	Leaves	Tea Tree	13	15	12	14	17
4	*Camellia sinensis* Black Tea (Theaceae)	Leaves	Tea Tree	12	15	12	13	16

EC- *E.coli* K12-control strain

Table 2: Antibacterial activity of plant extracts against diarrhea causing *E.coli*

S. No.	Name of Plant	Extracts	Zone of Inhibition (mm), MIC and MBC (mg/ml)														
			EC1			EC2			EC3			EC4			K12		
			A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	*Terminalia chebula*	Ethanol	27	7.81	7.81	32	0.24	0.24	26	7.81	7.81	31	0.24	0.48	33	0.24	0.24
		Methanol	23	7.81	7.81	30	0.24	0.24	22	7.81	7.81	25	0.48	0.48	30	0.24	0.24
		Acetone	20	7.81	7.81	26	1.95	1.95	18	15.6	15.6	21	7.81	15.6	28	0.48	0.48
		Cold water	18	15.6	15.6	24	7.81	7.81	14	31.2	62.5	18	15.6	15.6	24	1.95	1.95
2																	
	Punica granatam	Ethanol	20	15.6	15.6	25	7.81	7.81	20	15.6	31.2	21	15.6	15.6	29	3.90	3.90
		Methanol	16	15.6	15.6	25	7.81	7.81	18	15.6	15.6	17	15.6	15.6	28	3.90	3.90
		Acetone	13	15.6	15.6	20	15.6	15.6	13	31.2	31.2	12	31.2	31.2	21	7.81	7.81
		Cold water	13	62.5	62.5	20	15.6	15.6	11	31.2	31.2	12	125	125	20	7.81	7.81
3																	
	Camellia sinensis	Ethanol	20	15.6	15.6	20	31.2	31.2	14	62.5	125	16	62.5	62.5	20	15.6	31.2
		Methanol	15	62.5	62.5	14	62.5	62.5	11	125	125	11	62.5	62.5	14	31.2	62.5
	Green Tea	Acetone	11	62.5	62.5	11	62.5	62.5	11	125	125	11	62.5	62.5	12	62.5	62.5
		Cold water	11	125	125	10	125	125	11	250	250	10	125	125	10	62.5	62.5
4																	
	Camellia sinensis	Ethanol	15	125	125	16	62.5	62.5	14	125	125	12	125	125	16	31.2	31.2
		Methanol	13	125	125	16	125	250	11	125	250	10	125	125	15	31.2	31.2
	Black Tea	Acetone	13	125	125	14	125	125	11	250	250	10	1252	1252	12	62.5	62.5
		Cold water	13	125	125	12	125	125	11	250	250	10	50	50	10	62.5	62.5

A: Zone of inhibition (diameter in mm), B: MIC (mg/ml), C: MBC (mg/ml).

Table 3: *In vitro* Interaction effect of ethanolic plant extracts and antibiotics

Ethanol plant extracts	Test organism	MIC of plant extract alone	MIC of plant extract in combination	MIC of antibiotic alone	MIC of antibiotic in combination	FIC A	FIC B	FIC Index	Interpretation
Terminalia chebula	EC 2	0.24	0.12	100	0.12	0.5	0.0012	0.5012	Synergistic
	EC 4	0.24	0.24	100	0.24	1	0.0024	1.0024	Synergistic
Punica granatam	EC 2	7.81	7.81	100	7.81	1	0.0781	1.0781	Synergistic
	EC 4	15.62	7.81	100	7.81	0.5	0.0781	0.5781	Synergistic
Camellia sinensis Green Tea	EC 2	31.25	1.25	100	31.25	1	0.325	1.325	Indifferent
	EC 4	62.5	62.5	100	62.5	1	0.625	1.625	Indifferent
Camellia sinensis Black Tea	EC 2	62.5	62.5	100	62.5	1	0.625	1.625	Indifferent
	EC 4	125	62.5	100	62.5	0.5	0.625	1.125	Indifferen

Table 4: Phytochemical analysis of ethanolic extracts of plants

Ethanolic extracts	Phytochemicals								
	Alkaloids	Flavonoids	Terpenoids	Tannin	Saponins	Glycoside	Carbohytrates	Proteins	Steroids
Terminalia chebula	-	+	+	+	-	+	+	-	-
Punica granatam									
Camellia sinensis Green Tea	-	+	-	+	+	-	+	-	+
Camellia sinensis Black Tea	+	+	+	+	+	+	-	-	-
	+	+	+	+	+	+	-	-	+

+ Present, - absent.

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RESEARCH ARTICLE

Shell Shape Variation of Lucinidae Species *Anodontia edentula* (Linnaeus, 1758) and *Austriella corrugate* (Deshayes, 1843) using Landmark-based Geometric Morphometric Analysis

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ABSTRACT

Landmark based geometric morphometric analysis was used to generate ventral shell shape variations of *Anodontiaedentula* and *Austriellacorrugata*. A total of 300 samples of clams were collected in ZamboangaSibugay, Western Mindanao, Philippines.Sexes of the clams were determined through direct gonadal examination.Canon EOS 1100D was used to photograph each sample for imaging and landmarkings. Images were processed using geometric thin-plate spline grids (TPS), partial warps (PW) and relative warps (RW) software. The generated relative warp scores were subjected to statistical analysis using the PAST software. The shape variationwithin male *A. edentula* samples was found on the ventral margin (46.27%) and the left posterior margin (38.15%) within females.The shape variations within male *A. corrugata* were found on the ventral and posterior margin (43.50%) and upper right posterior margin (47.01%) for females. Variations occurred on the ventral margin for both pooled species of *A. edentula* (40.73%) and *A. corrugata* (47.74%). Among the populations, only female *A. corrugata* exhibited highly significant distribution of the variations found in RW2 confirmed through normality test. DFA was more successful in categorizing male and female *A. corrugata*with 85.33% correctly classified than the *A. edentula* samples with only 74.67% correctly classified. MANOVA revealed that only the pooled *A. corrugata* samples have statistically significant mean shape variations between males and females. The shape changes are probably related to the clam's mode of living and feeding habits.

Keywords: Geometric Morphometrics, gonads, landmarks, variation,*Austriella corrugata,Anodontiaedentula*

INTRODUCTION

There are over 150 different edible species of clams in the world. Among these species, the mangrove clams face the most problem when it comes to long term sustainability because their demand as delicious seafood for human consumption is increasing day by day even though they are considered to be a poor man's diet (Ingole et al.,2002). In the Philippines, collectors already complained of decrease of sizes and numbers of mangrove clams (imbao) due to destruction and overexploitation of mangroves that serve as their habitat and abuse in the gleaning of the clams (De la Rosa, 2004). There are two congeneric species abundant in local Philippine markets: *Anodontiaedentula* (imbaobayi) and *Austriellacorrugata* (imbaolaki). As listed in the World Register of Marine Species, *Anodontiaedentula*(Linnaeus, 1758)and *Austriellacorrugata*(Deshayes, 1843) belong to Phylum Mollusca, Class Bivalvia, Subclass Heterodonta, Order Lucinoida and Family Lucinidae (Bouchet and Taylor, 2015). They can be distinguished from each other through the remarkable difference in their external shell remarkably in their growth lines. *Anodontiaedentula* shells are very inflated, globose, and the outer surface has irregular concentric growth lines while the shells of *Austriellacorrugata* are moderately inflated, not globose, and the outer surface has sharp concentric ridges and fine concentric grooves in the interspaces (FAO, 2009).

Bivalves have important ecological roles in marine and freshwater ecosystems, and are of economic importance to humans (The BivAToL Project 2007). Researchers from SEAFDEC are already studying the potential of these clams to clean-up sediments and make polluted waters habitable again because they harbor symbiotic bacteria in their gills that can oxidize sulfur and clean the water of sulfur in the process. Due to the decline in population of mangrove clams, scientists are already helping the mangrove clams spawn. There is a need for biopsy which involves sacrificing a number of ripe adults to determine sex because these mangrove clams are dioecious (De la Rosa, 2004). Sexual dimorphism seemed to be apparent in the population of Imbaobayi wherein shells of males have a more pointed, triangular umbo while females have a more curved umbo (Millarez, 2005). Geometric Morphometric Analysis which uses a set of landmarks to describe shape is easier when it comes to data collection and provides a more precise biological representation in determining shell differences (Echem, 2015).

MATERIALS AND METHODS

Collection site

Anodontiaedentula and *Austriellacorrugata* samples were collected in SubaNipa, Olutanga, ZamboangaSibugay (7.33º N, 122.83ºE). These mangrove clams were shipped and sold in San Roque Wet Market, Zamboanga City (Figure 1).

Collection of samples

A total of 300*Anodontiaedentula*and *Austriellacorrugata* samples ranging from sizes 45mm to 50mm were collected from Olutanga, ZamboangaSibugay (7.33°N, 122.83°E) that were sold in San Roque Wet Market, Zamboanga City. The samples were brought to College of Science and Mathematics Laboratory, Western Mindanao State University, Zamboanga City for direct gonadal examination and shell preservation.

Gonadal inspection

Gonadal examination adapted from the gamete stripping procedure of Borne and Helm (2004) using a MOTIC photomicrograph and a compound microscope was done to determine the sexes of *Anodontiaedentula* and *Austriellacorrugata* based on the appearance of the gametes. The clams were placed in separate ice packed containers for 1 hour until the shells were opened. A dissecting scalpel was used to cut the muscles attaching the ventral and the dorsal shell. The shells were then pried open manually. The mantle was cut and pushed aside to separate it from the

gills. A sample tissue of the gonad found beneath the gills was collected and spread on a glass slide and covered with a cover slip to be viewed under a microscope to examine the sexes by the appearance of the gametes.

Sample preparation for imaging

A DSLR camera (Canon EOS 1100D) with a focal length of 50 mm was placed on a tripod to photograph the clam placed on a flat surface with red background, displaying the ventral shell and umbo at a constant distance of 50 cm.

Landmarks Selection

Twenty-five (25) landmarks consisting of five (5) true landmarks and twenty (20) pseudolandmarks were assigned for ventral shell with emphasis on the umbo structure (Figure 4).

RESULTS

The males *Anodontiaedentula*showed 3 significant relative warps gaining a total variation of 77.25%. Results revealed that there was variation manifested at the ventral margin in RW1 with 46.27% variance. At RW2, the variation is found on the left posterior margin 18.69% variance. At RW3, the curvature of the ventral margin is affected with 12.29% variance (Table 1).Histograms and box plots are constructed to graphically represent the data gathered for the species to further aid in visually identifying where the variation exists based on the description of the deformations of the grids (Figure 5).

Normality test revealed that there is no significant distribution of variation in the 3 significant relative warps of male *A. edentula*since the computed values are higher thanα=0.05 (Table 2).The female *Anodontiaedentula* accounts for 3 significant relative warps gaining a total variation of 80.91%. Results revealed that there was variation manifested on the ventral margin in RW1 with a 38.15% variance. At RW2, the variation is found on the left posterior margin with 30.58% variance. At RW3, 12.18% variance accounts for the change in size for females(Table 3).The histograms and box plots of the significant relative warps of femalepopulation of *A. edentula*revealed shape variations as visualized in the deformation of grids (Figure 6).

Normality test revealed that there is no significant distribution of variation in the 3 significant relative warps of the female *A. edentula*since the computed values are higher thanα=0.05 (Table 4).The males*Austriellacorrugata* accounts for 4 significant relative warps with total variation of 80.57%. Results revealed that at RW1, there was variation on the posterior and ventral margin with 43.50% variance. At RW2, there was variation on the left curve of posterior margin and umbo tip with 17.00% variance. At RW3, variation is concentrated on the ventral margin with 11.85% variance. At RW4, variation is found on the orientation of the upper left posterior margin with 8.22% variance (Table 5).

The histograms and box plots of the significant relative warps of the male population of*A. corrugata*showed shape variations as visualized in the deformations of the gridsof the relative warps (Figure 7).Normality test revealed that there is no significant distribution of variation in the 3 significant relative warps of the female *A. edentula* since the computed values are higher than α=0.05 (Table 6).The female *Austriellacorrugata* accounts for 3 significant relative warps with a total variation of 75.2%. Results revealed that at RW1, 47.01% variation is found on the right posterior margin. At RW2, 16.35% variance can be found on the ventral margin. At RW3, 11.84% variance is found on the size of the shell (Table 7).Normality tests revealed that there is a significant distribution of variation in RW2 of the female *A. corrugata*since the computed *P*-value (0.0012) is less than α=0.05 (Table 8).

Pooled male and female *A. edentula* accounts for 3 significant relative warps gaining a total percent variation of 78.92%. Results revealed that there was variation at the ventral and upper left posterior margin with 40.73% variance in RW1. At RW2, variation is manifested on the left posterior margin with 25.92% variance and at RW3, 12.27% variance relates with the shell width.The shape variations of the pooled*A. edentula* as visualized in the deformations of grids.Figure 9 show the spread of the individual specimens of the pooled *A. edentula* using the relative warp scores.Discriminant Function Analysis (DFA) correctly classified males and females at 74.67% using variables (Table 10).The DFA graph of the pooled *A. edentula*shows the frequency of the overlapping between males (blue) and females (red) and the discriminants showing where the sexes share common characteristics (Figure 11).Multivariate Analysis of Variance (MANOVA) reveals the significance of the difference between the mean shapes of males and females. There is no significant shape difference between groups of *A. edentula*since the P-value (0.23) is greater thanα=0.05 (Table 11).

Pooled male and female *Austriellacorrugata* accounts for 4 significant relative warps gaining a total percent variation of 81.06%. Results revealed that there was variation on the posterior and ventral margin at 47.74% variance for RW1, width at 14.22% variance for RW2, angling of the sides of the ventral margin and the right posterior margin at 10.66% variance for RW3 and over-all shape of the ventral margin at 8.44% for RW4. The shape variations of the pooled *A. corrugata* as visualized in the deformations of grids.Figure 12 show the spread of the individual specimens of the pooled A. corrugata using the relative warp scores.

It can be observed in the histogram of the shape characters of the pooled *A. corrugata*that the bins overlap at some point implying no complete separation of data sets. This entails that there is a variation between male and female *A. corrugata*. However, there are shared characteristics between the two because of the overlapping bins (Figure 14).MANOVA revealed that α=0.05, there is a highly significant shape difference between groups of *A. corrugata* since the P-value (8.26E-06) is less than α=0.05 (Table 14).

DISCUSSION

The male *Anodontiaedentula* tissue samples are milky in appearance and are sometimes observed to be sticky when spread on a slide. When viewed under a microscope, the sperms are sometimes motile and are tightly packed within a sticky material. The female species have the same color of tissue sample, but with rougher texture and when spread, visibly show the numerous circular structures which are the egg cells. The egg cells are sometimes stalked and pear shaped. The fertilized egg cells possess an irregular spherical shape when grouped together with other fertilized eggs. The rare hermaphroditic *A. edentula* samples have a unique appearance of both the sticky, whitish material reminiscent of condensed milk and some large circular structure with an observable dot in the middle. Both the egg cells and groups of sperm were observed under a microscope. The gonads of *Austriellacorrugata*are distinct from each other. The male gonads are whitish in color and when the sample tissue is placed on a slide, the substance is observed to be runny and packed with motile sperms. The female species on the other hand has a gonad that is darkly colored, ranging from dark green to black. It has a rougher texture compared to that of the male species with a characteristic foul odor similar to the odor associated with canals. The egg cells are more circular in appearance compared to that of the pear shaped egg cells of *Anodontiaedentula*.

The significant relative warps for the male and female are based on the acceptable cumulative frequency of 75%. Each relative warp shows the direction of shape change from the mean form. Shape variations are attributed to the ventral margin in male *Anodontiaedentula*. The scatter plot displaying the spread of the male samples shows areas of overlapping which signify that there is a weak amount of variation present but the individuals that are isolated exhibit variation that exists within the populations.According to Çetinkaya-Rundel (2014), prominent peaks determine the modality and the box plots are further visualization of the skewness and symmetry of the distributions. The box length in the box plot gives an indication of the sample variability and the line across the box shows where the sample is centered. Male samples have a multimodal distribution with skewness to the left for the

first significant relative warp. The variations in the female samples occur on the upper corner of the left posterior margin. The areas where overlapping is observed in the scatter plot is denser for female *A. edentula* samples compared to the males samples. The samples also have a multimodal distribution with skewness to the right for the first 2 significant relative warps. Most of the extreme values signifying high correlation are in the positive direction. Normality test confirmed that the distribution of variation is normal for the female samples.

The male *Austriellacorrugata* samples are also affected on the ventral margin. Most of the samples are centered in the scatter plot but few shapes with extreme variations can be observed. The distribution is multimodal with the first significant relative warp being skewed to the left and the rest of the significant relative warps having an almost symmetric distribution. In the PCA scatter plot, the extreme values are mostly near the x-axis or PC2 which means that variables are highly correlated with PC2 but normality test reveals that the variations are normally distributed.
The female *A. corrugata* samples have mostly warping for upper right of the posterior margin to the wing. There are only very few shapes with extreme variations which can be observed in the scatter plot. The distribution is multimodal with only the first significant relative warp displaying skewness to the left and the rest are already almost symmetric in distribution. Most of the extreme values are farther away from the PC2 and there is a noticeable spread in the values unlike the scatter plots of other populations sampled.

The pooled *Anodontiaedentula* samples also have variations on the ventral margin. In the scatter plot, there is a dense overlapping for the two sexes but more females exhibit extreme shape variations. The distribution is multimodal with the first two significant relative warps being skewed to the left. In the PCA scatter plot, most of the spread show the extreme values for the male samples leaning towards the positive direction while the female samples are leaning towards to the negative direction. DFA computes the percent correctly classified in predicting the category (male or female) membership of the pooled samples based on the variables. The DFA graph of the pooled *A. edentula* shows the overlapping of male (blue) and female (red) population where the samples share common characteristics. MANOVA determines whether the shapes of the shells differ in mean shape between males and females and the results show that it is not significant for pooled *A. edentula.* The variations for the pooled *Austriellacorrugata* are also mostly found on the ventral margin. In the scatter plot, there seems to be an equal amount of shells with extreme shapes for both sexes with the females exhibiting the higher values. The distribution is multimodal with all significant relative warps having almost symmetric distribution. In the PCA scatter plot, extreme values for the male are mostly leaning towards the negative direction while the females are mostly leaning towards the positive direction. DFA is more successful in correctly classifying males from females and the DFA graph shows lesser frequency in the discriminants where common characteristic is shared by both sexes. MANOVA reveals that there is a highly significant difference in the mean shape between males and females.

CONCLUSION AND RECOMMENDATIONS

Males and females were categorized through direct gonadal examination. *Anodontiaedentula* species have gonads that are not distinguishable from each other while *Austriellacorrugata* samples have significant differences in the appearance of their gonads. *Anodontiaedentula* egg cells are pear shaped while *Austriellacorrugata* egg cells are more circular in form. Results from the study showed shape variations in the two species of clams studied using landmark-based geometric morphometric analysis. The variations in the male *Anodontia edentula* samples are mostly found on the ventral margin (46.27%) and left posterior margin (38.15%) for females. The variations in male *Austriellacorrugata* are found on the ventral and upper right posterior margin (43.50%) while the variations for females are found on the upper right of the posterior margin (47.01%). Both pooled *Anodontia edentula* and *Austriella corrugata* have variations on the ventral margin with 40.73% and 47.74% variations, respectively.

Multivariate statistical analysis using principal component analysis (PCA), Normality test, DFA andMANOVA further supported the data. With the normality tests, only female *Austriellacorrugata* exhibited highly significant distribution of the variations found in RW2. DFA was more successful in categorizing between populations of

*Austriellacorrugata*with 85.33% correctly classified. MANOVA revealed that the difference between the mean shapes of males and females for pooled *Anodontiaedentula* isnot significant while for pooled *Austriellacorrugata* samples, the mean shape variations are considered to be statistically significant. The shape changes are probably related to the clam's mode of living and feeding habits. It is recommended that further studies regarding geometric morphometric analysis will be employed on the species of *Anodontiaedentula*and *Austriellacorrugata* particularly concentrating on the umbo area to try to establish an identification index to discriminate sexes as *Austriellacorrugata* showed a promising result in exhibiting sexual dimorphism. It is also recommended to correlate the tissue biometric measurements with the shell.

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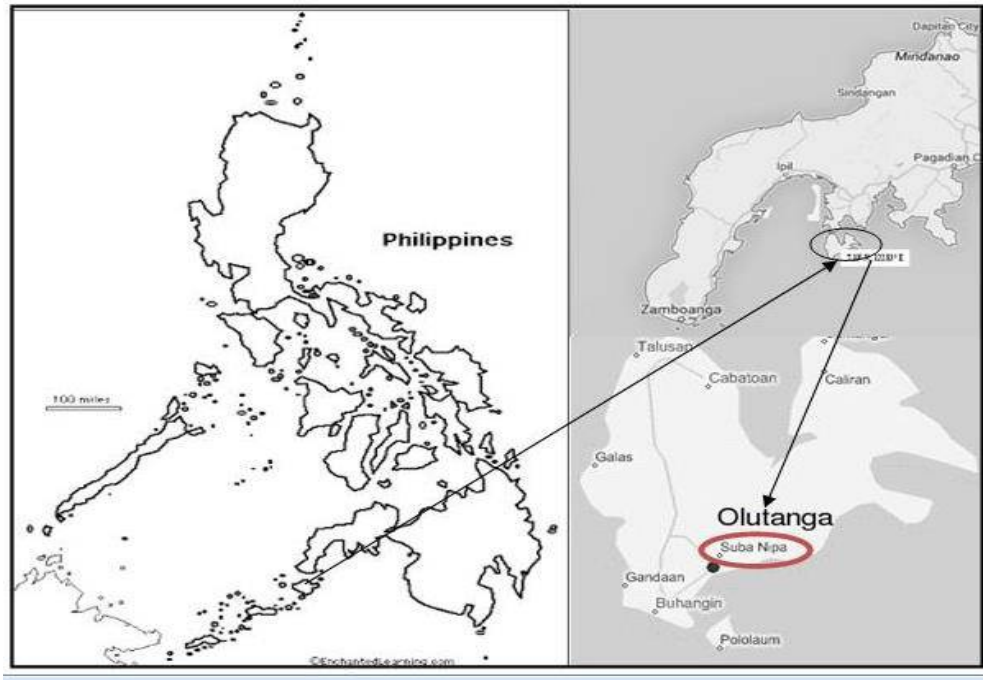
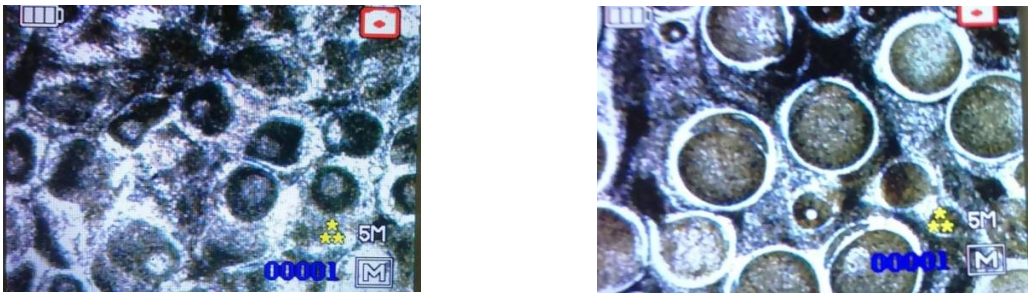


Figure 1. Map showing the Municipality of Olutanga, ZamboangaSibugay

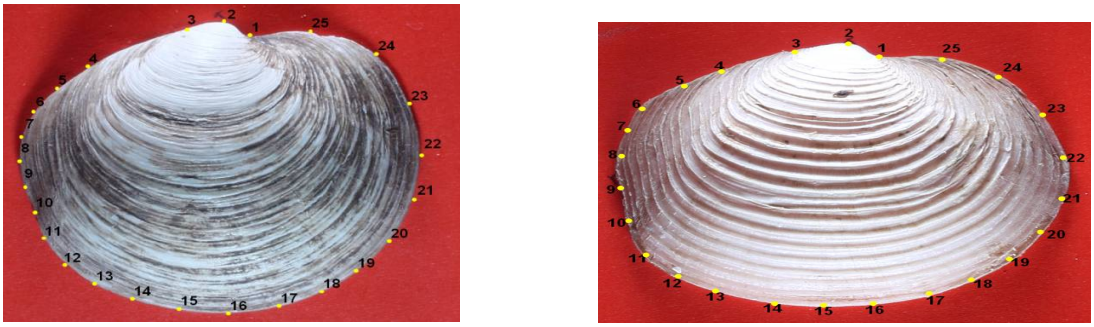
(A)(B)

Figure 2 (A-B). Sperm cells of *Anodontia edentula* (A) and *Austriella corrugata* (B) viewed under a microscope (400x)



(A) (B)

Figure 3 (A-B). Photomicrograph of the oocytes of (A) *Anodontia edentula* and (B) *Austriella corrugata* (40x).



(A) **(B)**

Figure 4. Landmarks on the ventral shell of (A)*Anodontiaedentula*and ventral shell of (B) shell of *Austriellacorrugata*

Table 1.Variability in the ventral shell of male *A. edentula.*

Relative Warp	% Variation	Description of the variation
1	46.27 %	There is a substantial narrowing in the ventral margin for –extremes and widening for + extremes.
2	18.69 %	Twisting out of shape is observed on the curvature of the left posterior margin for the – extremes while expansion is observed on the left posterior margin for + extremes.
3	12. 29%	The landmarks in the ventral margin are squeezed together for the - extremes while it is farther apart in the + extremes.
Total	77.25%	

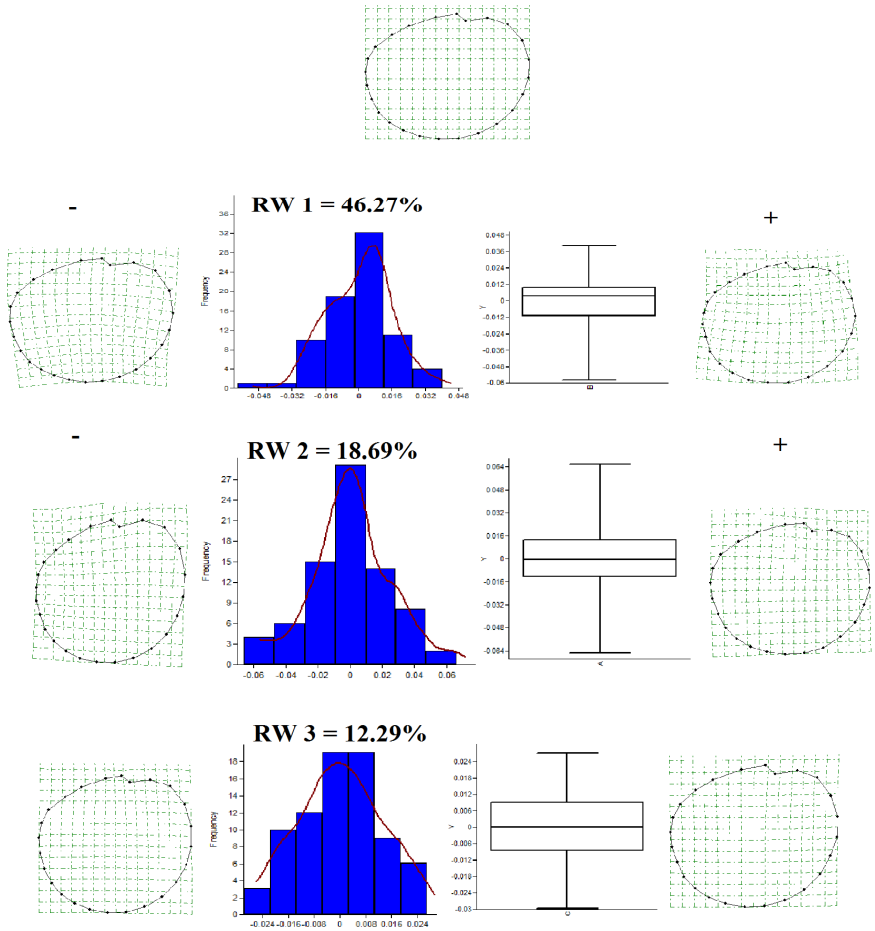


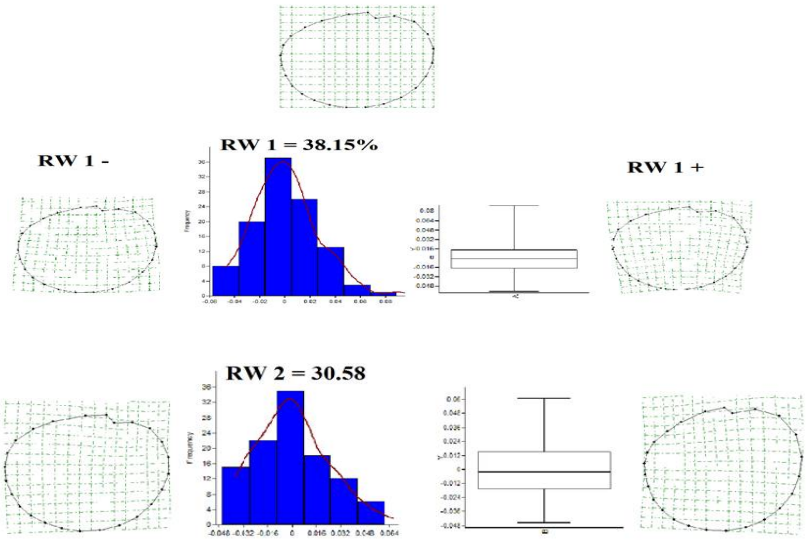
Figure 5. Histograms and box plots of the significant relative warps of male *A.edentula*

Table 2. Normality test for the distribution of variations in the significant relativewarps of male *A. edentula*

	RW 1	RW 2	RW 3
Shapiro-Wilk W	0.98	0.98	0.99
P(normal)	0.42	0.13	0.70

Table 3.Variability in the ventral shell of female A. edentula.

Relative Warp	% Variation	Description of the variation
1	38.15%	Constriction in the upper corner of left posterior margin is observed for the – extremes and narrowing in the ventral margin is observed for + extremes.
2	30.58%	Lengthening in the upper corner of the left posterior margin is observed for – extremes. For the + extremes, there is a compression of the curve on the left posterior margin.
3	12.18%	Pronounced enlargement is observed for – extremes while for the + extremes, the shells are observed to be reduced in size.
Total	80.91%	



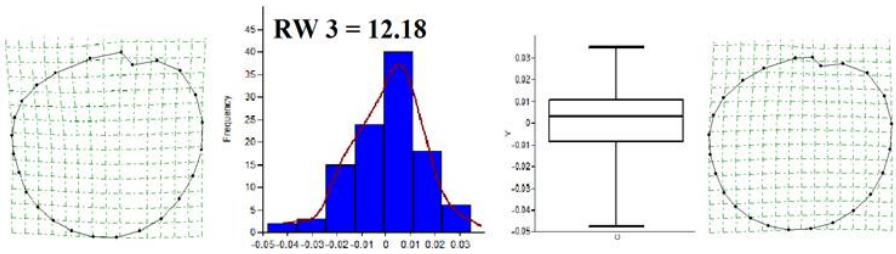


Figure 6. Histograms and box plots of the significant relative warpsof female *A. edentula*

Table 4. Normality test for the distribution of variations in the significant relativewarps of female *A. edentula.*

	RW 1	RW 2	RW 3
Shapiro-Wilk W	0.98	0.98	0.99
P(normal)	0.42	0.13	0.70

Table 5.Variability in the ventral shell of the male *A. corrugata*

Relative warp	% Variation	Description of the Variation
1	43.50 %	Twisting on the left and right upper posterior margins including the wing for the - extremes is observed while the + extremes shells have a diminished ventral margin.
2	17.00 %	The left curve of posterior margin is bent towards the left posterolateral edge of umbo for - extremes while the anterior left of the umbo tip is noticeably extended for the + extremes.
3	11. 85 %	The ventral margin is misshapen for the - extremes and does not assume an oval shape while for the + extremes, the sides of the ventral margin are angular.
4	8.22%	For the upper left posterior margin, it is slightly curvy in the – extremes while it is almost linear for the + extremes.
Total	80.57%	

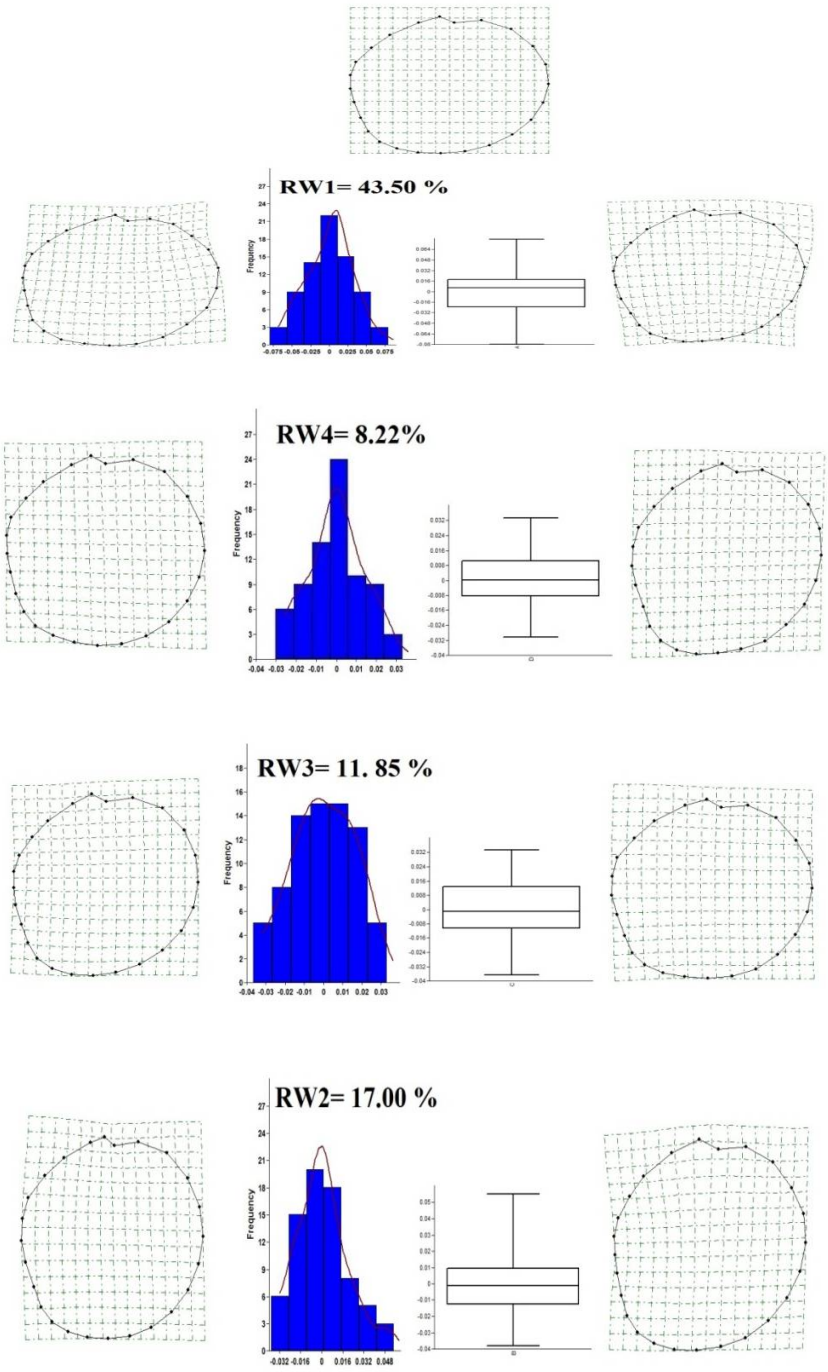


Figure 7.Histograms and box plots of the significant relative warps of male *A corrugata*

Table 6.Normality test for thedistributions of variations in the significant relativewarps ofmale *A. corrugata.*

	RW 1	RW 2	RW 3	RW 4
Shapiro-Wilk W	0.99	0.98	0.99	0.99
P(normal)	0.73	0.20	0.55	0.75

Table 7.Variability in the ventral shell of female *A. corrugate*

Relative Warp	% Variation	Description of the Variation
1	47.01%	There is skewing on the upper right posterior margin and wing for – extremes and prominent curving in the upper right posterior margin for + extremes.
2	16.35 %	Shrinkage is observed for the shells in the – extremes while the opposite is observed in the shells of the + extremes with undeviating first few points of the curve of the ventral margin.
3	11.84 %	The expansion is horizontal for - extremes and vertical for + extremes making it narrower.
Total	**75.2%**	

Table 8.Normality test for the distributions of variations in the significant relative warps of female *A. corrugata.*

	RW 1	RW 2	RW 3
Shapiro-Wilk W	0.98	0.95	0.99
P(normal)	0.29	**0.0012**	0.73

Table 9. Variability of pooled *A. edentula*

Relative Warp	% Variation	Description of variation for pooled data
1	40.73%	The shells in the - extremes have a tapered ventral margin which is manifested by males while the landmarks in the upper left posterior margin are closer together in the + extremes which is manifested by females.
2	25.92%	For the shells in the – extremes, the left posterior margin is slanted towards the left posterolateral edge of the umbo which is seen in the – extremes of males and + extremes of females. For the shells in the + extremes, there is a spacing out in the first few points of the left posterior margin which is seen in the + extremes of both males and females.

3	12.27%	Shells are narrower in the – extremes which is manifested in the + extremes of females while the shells are wider in the + extremes which is observed in the + extremes of males (RW1) and – extremes of females.
Total	**78.9%**	

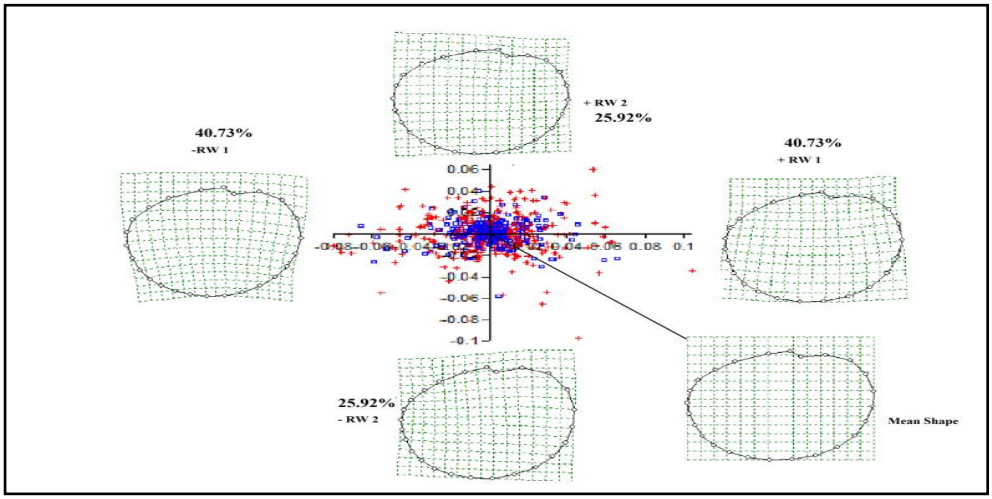


Figure 9. Scatter plot of the relative warps of pooled *A. edentula.*

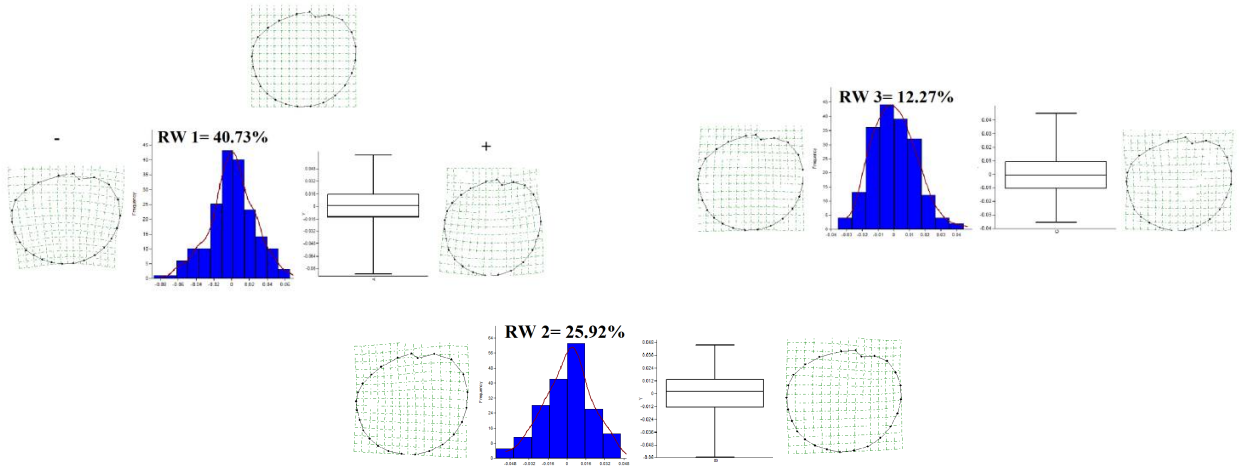


Figure 10.Histograms and box plots of the relative warps of pooled *A. edentula.*

Table 10. DFA of pooled *A. edentula*

DFA	MALE	FEMALE
Male	71	0
Female	0	79
Percent correctly classified		74.67%

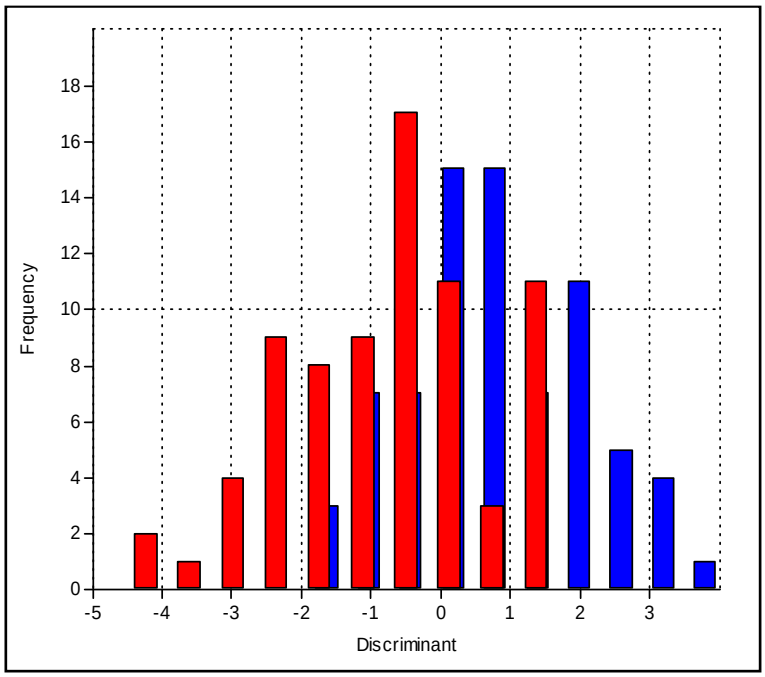


Figure 11. DFA graph of pooled *A. edentula*

Table 11. MANOVA of pooled *A. edentula*

MANOVA	Wilks' lambda	Df1	Df2	F	P(same)
	0.65	46	103	1.19	0.23

Table 12.Variability of pooled *A. corrugata.*

Relative Warp	% Variation	Description of variation for pooled data
1	47.74 %	Distortion in the upper left and right posterior margin is observed for the shells in the - extremes which is commonly observed in males while for the + extremes, the landmarks of the ventral margin are compacted which is also manifested by males.

2	14.22 %	The shapes are rounded for both – and + extremes but shells in the + extremes have considerably thinner width which is manifested by females.
3	10.66 %	The sides of the ventral margin in the - extremes are sharply angled which is manifested by males whereas in the + extremes, there is a slight pull on the upper right posterior margin for + extremes which is manifested by females (RW1).
4	8.44%	Ventral margin for - is angled instead of oval shaped while for the + extremes, the landmarks before the central point of the ventral margin are compressed and therefore don't assume an oval shape and these are all manifested by males (RW3).
Total	**81.06%**	

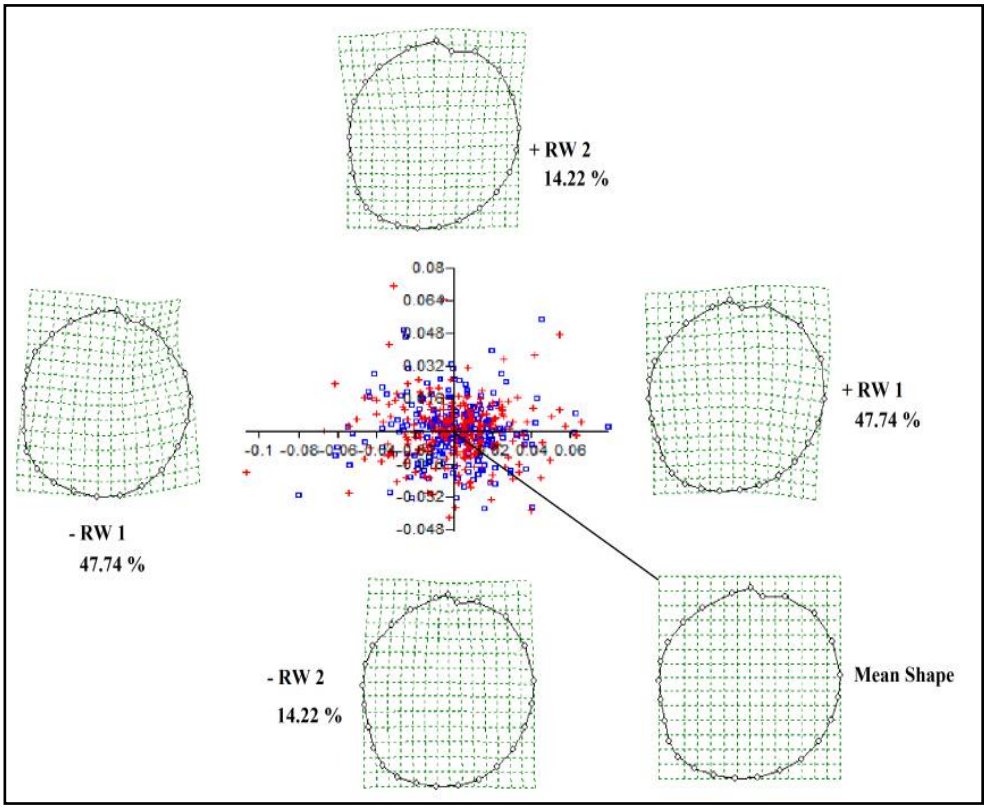


Figure 12.Scatter plot of the relative warpsof pooled *A.corrugata.*

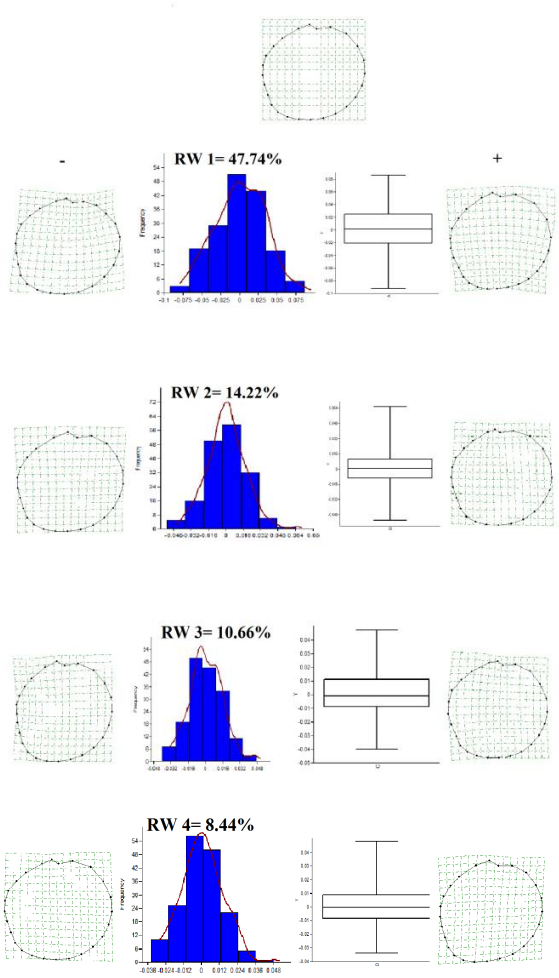


Figure 13. Histograms and box plots of the significant relative warps of pooled *A. corrugata.*

Table 13. DFA of pooled *A. corrugate*

DFA	Male	Female
Male	73	0
Female	0	77
Percent correctly classified	85.33%	

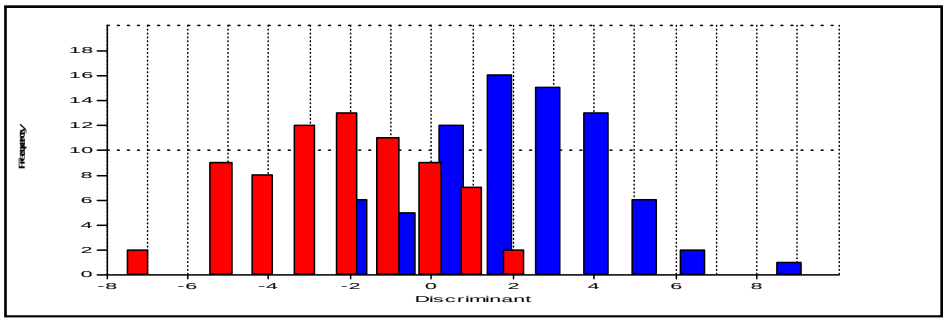


Figure 14.DFA graph of pooled *A.corrugata.*

Table 14.MANOVA of pooled *A. corrugata.*

MANOVA	Wilks' lambda	Df1	Df2	F	P(same)
	0.44	46	102	2.80	8.26E-06

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RESEARCH ARTICLE

Cerebellar Cortical Layer Atrophy in the Aging Human- A Post-Mortem Observation

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ABSTRACT

The change in the thickness of cerebellar cortex layers with increasing age is evident change of number and morphology of cells of the cerebellar cortex that causes disorders of fine movement, equilibrium, hypotonia and postural changes. The present study was done to see the histological changes of cerebellum in different age groups of Bangladeshi people. This cross sectional descriptive type of study was designed and done in the Department of Anatomy, Dhaka Medical College, , which was performed on the cerebellum of 28 Bangladeshi people, collected during autopsy examination of unclaimed dead bodies, from Department of Forensic Medicine. Paraffin blocks of cerebellum were cut at 5mm thickness and stained with routine Harris' Haematoxylin and Eosin (H & E) stain. The samples were divided into four different age groups Group-A (20-29 years), Group-B (30-39 years), Group-C (40-49 years) and

Group-D (> 50 years). Histological studies revealed that gray matter was more prone to shrieked with age that the white matter. The age related change of molecular layer and granular layer start earlier than Purkinje cell layer but Purkinje cell layer more prone to change with age than other two layers of cerebellar cortex.The thickness of cerebellar gray matter and white mater and the three layers of cerebellar cortex were decreased with age and were statistically highly significance. Further study on morphological changes of Purkinje cell and granular cell with electron microscope was recommended.

Keywords: white matter, Cerebellar cortex, age.

INTRODUCTION

Cerebellum literally means "little brain" as it is containing as many neurons as all of the rest of the central nervous system [3]. Age-related changes in brain morphology are apparent in both postmortem histological and in vivo magnetic resonance imaging (MRI) studies but the ratio of age-related atrophy of cerebellum shows less alternation if we compare with other areas of the brain [[9] [11]. In healthy adults, age-related volume reduction is more pronounced in gray matter especially prefrontal, but shrinkage of sensory and entorhinal cortices were very few [2]. The majority of post-mortem studies age-related alteration observe decline in total brain weight, cortical thinning and gyral atrophy that is particularly accelerated during the sixth and seventh decades [9]. Some other study shows that shrinkage of the cerebellum less from young to middle adulthood, and more shrinkage occur from middle adulthood to old age [2]. Cerebellar degeneration also as a complication of chronic alcoholism [4]. A quantitative histological study on cerebellar cortex on chronic alcoholics male was found that there was shrinkage of molecular layer 11-39%, granular layer 9-10% and loss of Purkinje cell layer 21% [10].

There are also several diseases that effect on the cerebellum and may also affect the metastatic complication of a distant carcinoma. In Alzheimer disease significantly affect the three layers of cerebellar cortex, the molecular layer decrease 24% and granular layer decrease 22%, as there were 30% reduction of total number of granular cells and 32% decrease in the total number of Purkinje cells [12].The Purkinje had be suggested to be more prone to change then the granular cell that causes cerebellar atrophy [7]. The neonatal period is the active period of neuronal proliferation, migration, and synaptogenesis in the cerebellar cortex. Exposure to toxic substances during this vulnerable period may affect the cytogenesis, morphogenesis, of the cerebellar cortex which could lead to impairment in some of the brain functions [6].

Neurological diseases also influence the rapid degeneration of cerebellar cortex, the average thickness of the cerebellum cortex decreased from 1175.82 μm in the normal aging process and microscopically destruction of Purkinje cells with preservation of the basket fibers was found. But the morphometric examination of the cerebellum of the patient having neurological disease, revealed significant atrophy, with a decrease in the volume of the molecular layer by 24% and of the granular layer by 22% [4].The objective of the study was to examine the histological changes of cerebellar cortical layer thickness in each decade of life and to correlate this cerebellar depletion in cortical layer with the process of aging.

MATERIALS AND METHODS

Materials

A Cross-sectional descriptive type of study was carried out in the Department of Anatomy, Dhaka Medical College, and Dhaka, Bangladesh. The present study was performed on 28 human cerebellum collected from the morgue of

Dhaka medical college, Dhaka. Among them lowest age was 22 years and highest was 58 years. The collected samples were divided in to four groups Group-A (20-29 years), Group-B (30-39 years), Group-C (40-49 years), Group-D (>50 years).

Methods

Preservation of brains

After collection of whole brain, 100ml of 40% formaldehyde solution was injected by using a 50cc syringe into the brain through the surfaces (super lateral and inferior surfaces). Then it was preserved in 40% formaldehyde solution (Origin Germany) for 15 days. After 15 days the cerebellum was collected from the preserved brains and fixed in 10% formol saline solution which Composed 37-40% formaldehyde (100cc), Sodium chloride (9gm), tap water (900cc).

Preparation of the slide

Tissue blocks were made through the cerebellum parallelwith the median plane divided the folia at right angle and were fixed in 10% formol saline in a plastic container for 1 week. The tissues were washed in running tap water, dehydration was done with ascending grades of alcohol, cleared with xylene, infiltrated and embedded in paraffin. Paraffin blocks were cut along the long axis of the folia at 5mm thickness and were stained with routine Harris' Haematoxylin and Eosin (H & E) stain. For measurement of the thickness of the cerebellar cortex, 7 slides were selected from group each group and total 28 slides were examined. The light compound microscope was used for the microscopic measurement which was OLYMPUS CHB, made in Tokyo, Japan.

Estimation of thickness of the gray matter and white matter and proportions of the three layer of cerebellar cortex

For the study of histological parameters, the stained tissue section on the slide was divided into three equal parts by a computer generated transparent sheath; three different fields were chosen from each slide for measuring the thickness of the gray matter and white matter and thickness of three layers of cerebellar cortex. The thickness was measured by using a stage micrometer and ocular micrometer, slides were examined under x 40 objectives x 1o eyepiece. The stage micrometer calibration was focused under the objective to be used and ocular micrometer calibration was superimposed on them in such a way that starting mark on the ocular micrometer matched exactly with the starting mark on the stage micrometer, then determination of how many of the smaller division of the ocular micrometer correspond with how many smaller division of the stage micrometer.
In high magnification (x 40 objective x 10 eyepiece):

4 smallest division of ocular micrometer match with 1 smallest division of stage micrometer.
So, 1 smallest division of ocular micrometer matched with ¼ or 0.25 smallest divisions of stage micrometer.
As 1 stage micrometer division=10 µm
Therefore 1 ocular micrometer division=0.25x10 µm
=2.5 µm
The thickness was then multiplied by 2.5 to get the value in µm. Total three measurements were taken for each slide and the average was recorded.

Ethical clearances

This research work was approved by the Ethical Review Committee of Dhaka Medical College, Dhaka.

RESULTS AND DISCUSSION

The generalizability of our findings is conditioned by the desire to examine the histological changes of cerebellum with aging nevertheless to see the efforts of disease to the healthy participants. The observed pattern of brain aging may not represent a normal physiological course. Life style disease such as vascular risk factors, toxic material act as negative modifiers of cerebellar aging.

Thickness of gray matter and white matter of cerebellum

According to table 1 the mean ± SD thickness of gray matter and white matter were found 510.28±52.98 µm and 240.71± 33.31 µm in group A, 668.00±36.29 µm and 311.42± 16.64 µm in group B, 579.16±11.68 µm and 208.33± 16.47 µm in group C, 427.60±17.35 µm and 159.00± 7.87 µm in group D respectively. The highest mean of thickness of gray matter and white matter were found in group B and lowest were found in group D (>50years). Age related degeneration occur between 50-60 years of age, was found Ellis et al 1920[19],Hall et al 1975 [20],Torvik et al 1986[19]. The differences in mean thickness of gray and white matter of cerebellum in different age groups were statistically significant in all groups except group A and group C.Pakkenberg et al (2003) and Anderson et al (2003) conducted a stereological study on 19 normal Caucasian male cerebellums, age ranges from 19-85 years and revealed that global white matter was loss with age [13] [14]. The MRI study done by Naftali Raz on 2005 showed the significance age related shrinkage of the cerebellum especially above the 50 years. In the present study gray matter was more prone to shrieked with age that the white matter and shrinking started mainly from age group B (Age 30-39years). Anderson 2009 done study on multiple sclerosis, found that Gray matter atrophy more prominent than the white matter atrophy [15]. Total cerebellar volume and total grey matter volumes was not atrophic in first episode of schizophrenia, but patients exhibited increased total white matter volumes and smaller global grey to white matter ratios that may linked to symptoms that expressed in established schizophrenia [16].

Thickness of three layer of cortex of cerebellum

According to table 2 the mean ± SD thickness of molecular layer was found 299.85 ± 20.95 µm in group A, 260.42 ±18.46 µm in group B, 221.66±10.72 µm in group C, 134.20 ± 18.39 µm in group D respectively. The highest mean of thickness of molecular layer was found in group B and lowest were found in group D. The mean ± SD thickness of Purkinje cell layer was found 59.28 ± 5.25 µm in group A, 56.28 ±6.47 µm in group B, 48.83±4.44 µm in group C, 42,60 ± 2.30 µm in group D. And the mean ± SD thickness of granular cell layer was found 251.14 ± 31.21 µm in group A, 251.57 ±31.80 µm in group B, 235.50 ± 8.04 µm in group C, 220.80 ± 31.31 µm in group D. The highest mean thickness of cerebellar cortex were found in group A(molecular layer and granular layer) and group B(Purkinje cell layer)but lowest were found in group D in all three layers of the cerebellar cortex that may cause development of progressive ataxia together with other associated neurological signs during increasing age. The differences in mean thickness of molecular layer of cerebellum in different age group were statistically significant in all groups except group A & group C.The granular layer thickness highly significant only Group A & B and group B & D but compares of other groups nonsignificant or very few significant. Mean difference of Purkinje cell layer thickness were statistically significant and the difference started from group A & group C. As the thickness of molecular layer and granular layer were statistically highly significant in group A and group B that means the changes of these layers start first than the Purkinje cell layer.Emma Gheorghe et all (2006)state that neural degeneration affects all the three layers of the cerebellum cortex mainly the molecular and ganglionic layer of the cerebellum, and Purkinje neurons disappear on large territories, while the granular cells only decrease in number. Andreas R Luft et al (1999) state that the although the loss of PC reported by different authors in mice and humans. PC contribute very little in cerebellum volume but their axons comprise the major part of cerebellar white matter especially of folial white matter, reduction of these significantly contribute to the loss of overall volume.In group B (age 30-39 years) showed the reduction of cell populations and thickness and presence of vacuoles within the granular layer which may be due to Chronic perinatal lead exposure or may be due to toxic effect on the cerebellar cortex.

CONCLUSION

The age related atrophy more affects in the cerebellar cortex then the white matter of cerebellum. The degeneration affects all the three layers of the cerebellum cortex and Purkinje cell layer thickness affect more with increasing age than the molecular and granular layer. Further study with larger sample size and observations of the morphological changes of the Purkinje cell. Granular cell, Basket cell, and the Golgi cells are recommended. Stereological analysis of cell of cerebellum by using the electron microscope and computer imaging are also recommended.

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Table-1: Thickness of Gray matter and White matter in different age groups

Thickness of Gray matter and White matter (µm)			
Group(n)	**Gray matter Mean ± SD**	**White matter Mean ± SD**	**P value**
Group A (20-29 years)	510.28 ± 52.98	240.71 ± 33.31	< 0.001**
Group B (30-39 years),	668.00 ± 36.29	311.42 ± 16.64	< 0.001**
Group C (40-49 years),	579.16± 11.68	208.33 ± 16.47	< 0.001**
Group D (>50 years)	427.60 ± 17.35	159.00 ± 7.87	< 0.001**
Compare between the groups- P value			
A vs. B	< 0.001**	< 0.001**	
A vs. C	< 0.05*	> 0.05^{ns}	
A vs. D	< 0.05*	< 0.001**	
B vs. C	< 0.001**	< 0.001**	
B vs. D	< 0.001**	< 0.001**	
C vs. D	< 0.001**	< 0.05*	

Table-2: Thickness of three layers of cerebellar cortex in different age groups.

Thickness of three layer of cerebellar cortex (µm)				
Group(n)	**Molecular layer Mean ± SD**	**Purkinje cell layer Mean ± SD**	**Granular layer Mean ± SD**	**P value**
Group A (20-29 years)	299.86 ± 20.95	59.28±5.25	251.14 ± 31.21	<0.001**
Group B (30-39 years)	260.42 ± 18.46	56.28±6.47	251.57 ± 31.80	< 0.001**
Group C (40-49 years)	221.66 ± 10.72	48.83±4.44	235.50 ± 8.04	< 0.001**
Group D (>50 years)	134.20 ± 18.39	42.60±2.30	220.80± 31.31	< 0.001**
Compare between the groups- P value				
A vs. B	< 0.001***	> 0.10^{ns}	< 0.001***	
A vs. C	> 0.10^{ns}	< 0.05*	< 0.05*	
A vs. D	< 0.001***	< 0.001***	> 0.10^{ns}	
B vs. C	< 0.05*	> 0.05^{ns}	> 0.10^{ns}	

B vs. D	< 0.001**	< 0.001**	< 0.001***	
C vs. D	< 0.001***	> 0.10^{ns}	< 0.05*	

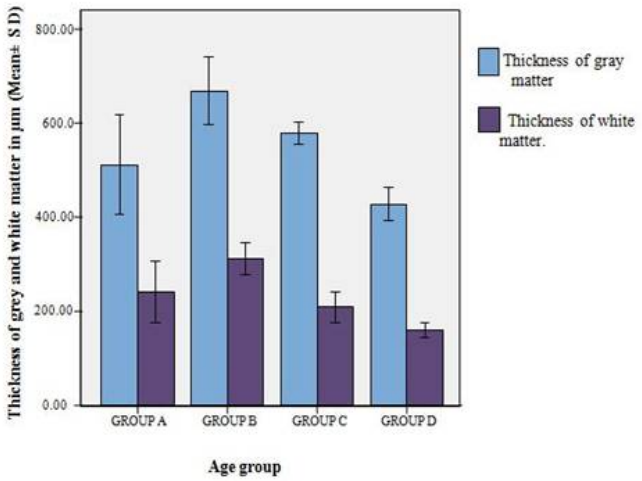

Fig-1: Thickness of gray matter and white matter in different age groups.

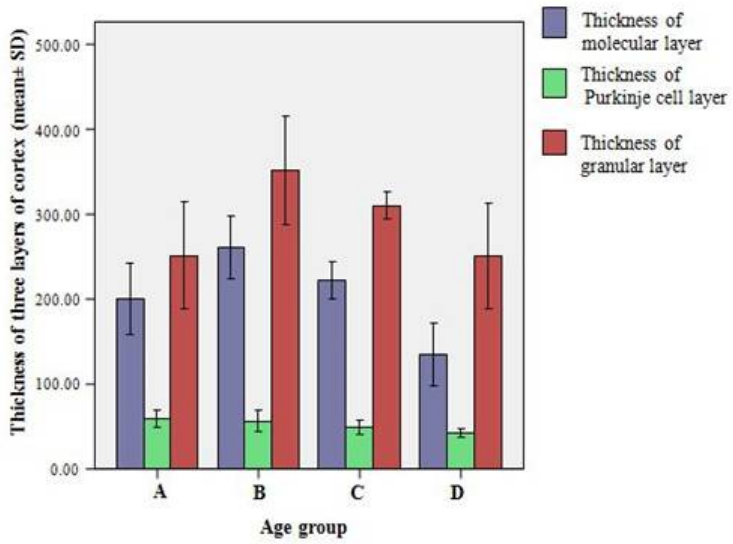

Fig-2: Thickness of three layers of cerebellar cortex in different age groups

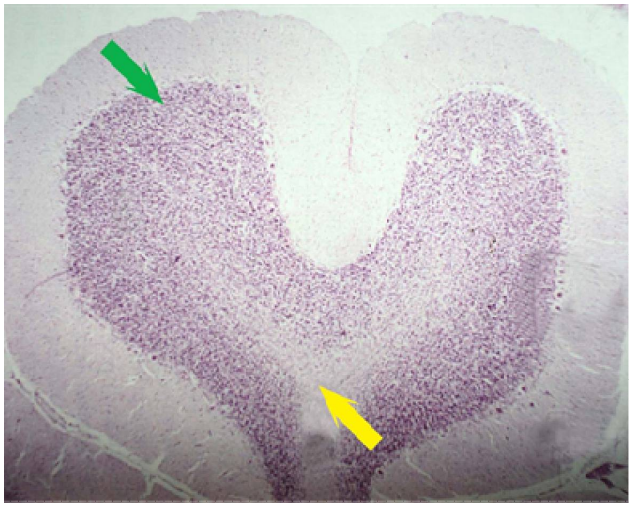

Fig-3: The sectional view of cerebellum showing gray matter (green arrow),white matter (yellow arrow) taken from group B (30-39 years) under light microscope (x 10 objective x 10 eyepiece in H &E stain).

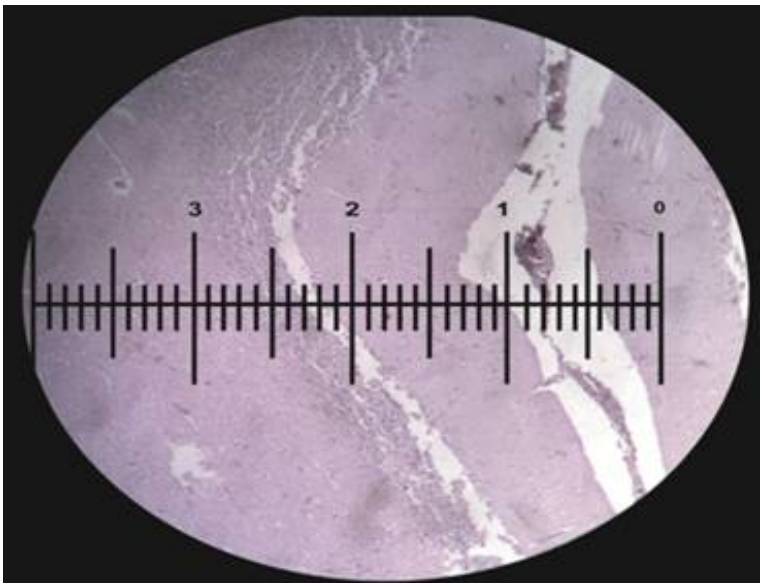

Fig 4: Showing measurement of three layers of cerebellum by using the ocular micrometer (Under light microscope x40 objective x 10 eyepiece).

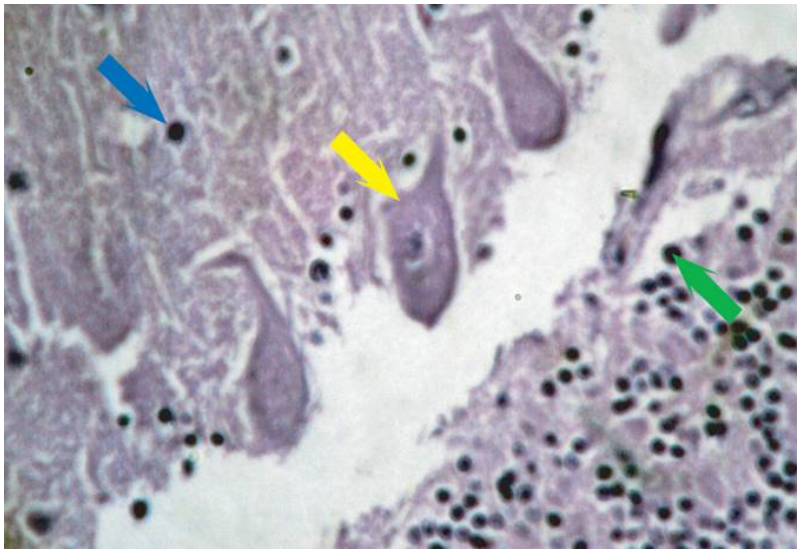

Fig-5: The sectional view of cerebellum showing Purkinje cell (yellow arrow) taken from group B (30-39 years) under light microscope (x 40 objective x 10 eyepiece) in H &E stain.

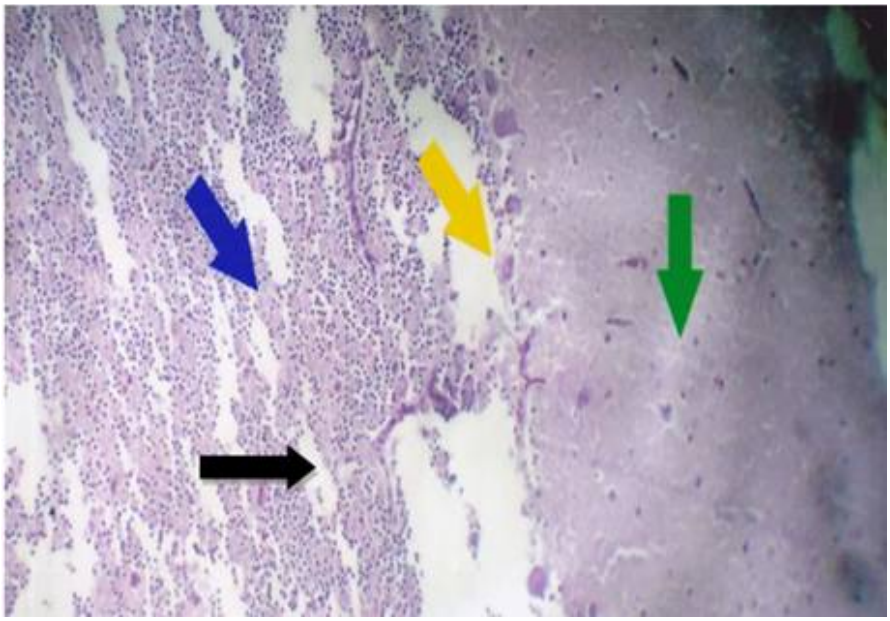

Fig-6: The sectional view of cerebellum showing molecular layer (green arrow), purkinje cell layer (yellow arrow) and granular cell layer (blue arrow) and empty spaces (black arrow) taken from group B (30-39 years) under light microscope (x 10 objective x 10 eyepiece) in H &E stain

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RESEARCH ARTICLE

The Effects of Transfection with Baculovirus on Sf9 Cell Line and Expression of Recombinant S1 Domain of the Porcine Epidemic Diarrhea Virus Spike Protein

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ABSTRACT

Porcine epidemic diarrhea virus (PEDV) is the causative agent of porcine epidemic diarrhea, a highly contagious enteric disease of swine. The spike (S) protein of PEDV is a type 1 transmembrane envelope glycoprotein and consists of the S1 and S2 domains, which are responsible for virus binding and fusion, respectively. Since S1 domain is involved in a specific high-affinity interaction with the cellular receptor and induction of neutralizing antibody in the natural host, it is a primary target for the development of effective vaccines against PEDV. In the present study, PEDV-S1 was expressed on *Spodoptera frugiperda* (Sf9) cell lines as a host cell, and the expression of PEDV-S1 was obtained by a baculoviral expression system. The plasmid production, optimum multiplicity of infection, optimum transfection time, infected and uninfected cell size and cell behavior during transfection were also determined. For maximum PEDV-S1 gene, optimum multiplicity of infection (MOI) and time were 0.1 and 72 h, respectively. The size of infected cells increased significantly after 24 h post infection ($P<0.001$). The results indicated that Sf9 cell line was applicable to the large scale for PEDV-S1 expression by using optimized parameters (infection time and MOI) because of its high productivity.

Key words: Baculovirus, PEDV-S1 protein expression, *Spodoptera frugiperda* (Sf9), Transfection

INTRODUCTION

Porcine epidemic diarrhea (PED) is a devastating swine disease that is characterized by acute enteritis and lethal watery diarrhea followed by severe dehydration leading to death, with a high mortality rate in piglets 1,2. The disease was initially recognized in England in 1971, but the causative agent of this disease, PED virus (PEDV), was later identified in 1978 3. PED epidemics were first reported in Asia in 1982, and since then, PED has continued to threaten swine health, causing substantial economic losses in the Asian swine industry 4-6. In 2013, PED outbreaks suddenly occurred in the United States and have swept through the pork industry across the country, raising concerns about control measures for PED prevention 7,8. In Taiwan, Porcine epidemic diarrhea virus (PEDV) appeared in 2013 9; however, a retrospective study indicated that the virus had been present as early as 2013 10. Although periodic vaccination strategies have been implemented nationwide to control PED in Taiwanese swine herds, PEDV has continually emerged, causing tremendous harm to the productivity of Taiwanese pig farms.

PEDV, a member of the genus Alphacoronavirus within the family Coronaviridae of the order Nidovirales,is a large, enveloped virus possessing a single-stranded, positive-sense RNA genome of approximately 28 kb with a 50 cap and a 30 polyadenylated tail 3. The PEDV genome is composed of the 50 untranslated region (UTR), at least seven open reading frames (ORF1a, ORF1b, and ORF2 through 6), and the 30 UTR 11. The two large ORFs 1a and 1b make up the 50 two-thirds of the genome and encode the non-structural replicase genes. The remaining ORFs in the 30 terminal region code for four major structural proteins: the 150-220-kDa glycosylated spike (S) protein, the 20–30-kDa membrane (M) protein, the 7-kDa envelope (E) protein, and the 58-kDa nucleocapsid (N) protein 12,13.The S protein of PEDV is a type I membrane glycoprotein composed of 1,383 to 1,386 amino acids (aa), depending on the strain. It contains a putative signal peptide (aa 1-24), a large extracellular region, a single transmembrane domain (aa 1,334-1,356), and a short cytoplasmic tail. Although PEDV has an uncleaved S protein because it lacks a furin cleavage site, the S protein can be divided into S1 (aa 1–735) and S2 (736-the last aa) domains based on homology with S proteins of other coronaviruses 14-17. Like other coronavirus S proteins, the PEDV S protein is known to play a pivotal role, interacting with the cellular receptor to mediate viral entry and inducing neutralizing antibodies in the natural host 18,19. More precisely, previous studies have shown that the S1domain includes the main neutralizing epitopes and the receptor-binding region 20,21. Furthermore, along with the full-length S gene, the S1 portion is known to be a suitable region for determining genetic relatedness among the different PEDV isolates and for developing differential diagnostic assays 22,23. Considering these molecular and biological features of the S1 domain, it would be an appropriate target for developing effective vaccines against PEDV.

In the current study, the expression of PEDV-S1 protein was performed by using baculovirus infected insect cell line (*Spodoptera frugiperda*, Sf9). Monitoring the effect of transfection reactions on the cell characteristics is scarce in the literature. Therefore, the efficiency of transfection, optimum multiplicity of infection (MOI), optimum transfection time, cell behavior and cell size were monitored to determine optimal harvest time for large scale production of Sf9 cell line and expression of PEDV-S1 protein.Determining the characteristics of transfected Sf9 cells might be useful to find out the point of peak protein production and to predict the maximum protein yield, thus stable and economical recombinant protein expression can be achieved. These characteristics may be differentiated according to cell line. Therefore, there are no certain signals about the transfection and/or infection effect on the insect cells. This current study may be a resource for those interested in a large scale production of recombinant PEDV-S1 protein on Sf9 cell line.

MATERIALS AND METHODS

Cell culture and Viruses

Spodoptera frugiperda (Sf9) cell lines were cultured at 27°C in TNMFH medium (PAN Biotech GmbH, Germany) with 10% heat-inactivated fetal bovine serum (PAN Biotech GmbH) and gentamicin (50 µg/ml) (PAN Biotech GmbH). Cell density and viability were assessed by Trypan blue staining. Cell viability was calculated on the basis of the percentage of living cells with respect to the total number of cells at various times post-infection. The Sf9 cells, which were cultured in suspension, were infected in spinner flasks (80 ml of culture media) at a cell density of 2 x 10^6 cells/ml. Cell viability at the time of infection was above 95 % and 99 % for monolayer and suspension, respectively. In this study, the PEDV-S1 gene was provided at Vaccine and Adjuvant Laboratory (Instutite of Animal Vaccine Technology, National Pingtung University of Science and Technology, Pingtung, Taiwan) and was used for amplification of the PEDV-S1 gene.

Plasmid construction

The pFastBacHTb plasmid was constructed by cloning the nucleotide sequence of the gene encoding for the S1 protein. The sequence was PCR-amplified from a previous vector, using the primers: F_EcoRI (5'-GGGGGAATTCATGATTTCTTTTGTTACTCTGC-3'), and R_NotI (5' TTTTGCGGCCGCC GCTGTAGAACATCCGT CT 3'). The PCR condition for the amplification by following: 5 minutes initial denaturation at 94ºC, followed by 30 cycles, each containing of 30 seconds at 94ºC, 30 seconds at 60ºC, and 1 minute 30 seconds at 72ºC, and a final extension at 72ºC for 10 seconds. PCR was carried out in 10 µl volume containing 1 µl of DNA template, 5 µl of 2x Master mix, 0.5 µl of each primer, 3 µl of ddH_2O. The PCR products were loaded to agarose gel (1.2 %, w/v) for confirmation. The PCR products containing the PEDV-S1, were digested with *Bam*HI and *Hin*dIII restriction enzymes in order to insert the DNA, in frame, into the pFastBacHTb transfer vector (Gibco BRL, USA) of the Baculovirus Expression System (Invitrogen, USA). The recombinant plasmids were used to transform competent *E.coli* DH10Bac (containing the wild-type baculovirus genome in bacmid form) to generate the corresponding recombinant bacmids upon transposition. Cloning steps were moitored by using sequence analysis and controlled by Genetyx-Win Program.

Transformation of baculoviral pFastBacHTb_PEDV-S1 to *E.coli* DH10Bac™

One vial of DH10Bac competent cells (100 µl) were thawed on ice for each transformation. 3 µl pFastBacHTb plasmid DNA were added to the cells and mixed gently. The cells were incubated on ice for 30 min. Heat shock was applied to the cells for 45 s at 42^{0}C without shaking and immediately transferred the tubes to ice and chilled for 2 min. LB (Luria Broth) medium was added 900 µl at room temperature and shake at 37^{0}C at 225 rpm for 4 h. Serial dilutions of the cells (10^{-1},10^{-2},10^{-3}) was prepared with LB (Luria Broth) medium.

Each dilution was plated on LB (Luria Broth) agar that contained 50 µg mL^{-1} kanamycin, 7µg mL^{-1} gentamicin, 10 µg mL^{-1} tetracycline, 100 µgmL^{-1} X-gal and 40 µgmL^{-1} IPTG. The plates were incubated for 48 h at 37^{0}C. White colonies, which include recombinant bacmid *E.coli* clones, were picked by blue/white-screening (Invitrogen).

Production of recombinant baculovirus

After transformation of *E. coli* DH10Bac cells, *Spodoptera frugiperda* (Sf9) cells were cultured at 27^{0}C in TNMFH medium, supplemented with 10% FBS. For transfection, 9 x 10^5 cells were plated in 6 well tissue culture dishes and incubated for 1 h in 2 mL TNMFH medium containing 10%FBS (without antibiotics) to allow adhesion of the cells to surface. Recombinant bacmid DNA had been preincubated for 45 min at room temperature with Cellfectin II (8 µl).

Cells were incubated with the liposome-DNA complex for 5 h at 27^{0}C. The transfection medium was removed and 2 mL of TNMFH medium, containing antibiotics was added. The DNA was transfected into Sf9 cells. Transfected cells were incubated at 27^{0}C for 144 h allowing baculovirus production. The recombinant virus was amplified twice to obtain virus stocks of the highest titer and harvested. Classical plaque assay was applied for virus titration 24.

Optimization of PEDV-S1 protein expression and transfection

Sf9 cells in 6 well plates (TNMFH medium, 10 % v/v heat-inactivated FBS and gentamicin (w/v, 50 µgmL^{-1}) were infected by recombinant virus with MOI of 0.1, 0.25, 0.5, 0.75, 1, and 10 plaqueforming units per cell. Cells were harvested at 24, 48, 72, 96, 120, and 144 h post transfection and centrifuged for 5 min at 100 g. The cells were washed three times with phosphate-buffered saline (PBS) and then resuspended on ice in 300 µl of sonication buffer (20 mM Tris–HCl pH 8.0, 300 mM NaCl, 10 % (w/v) glycerol, IPEGAL CA-630) for 9 x 10^5 cells mL^{-1}. After sonication three times at 20 s pulses with a Bandelin model sonicator at 45 % power, samples centrifuged at 4^{0}C for 10 min at 12,000 g. PEDV activities measured to determine optimum MOI with the maximum PEDV-S1 expression.

Determination of virus replication and cytopathic effects

Viral transfection may be cytopathic effects and structural changes in the host cell. The effects of transfection with MOI 0.1 on the cells measured by using mitochondrial tetrazolium test [MTT, 3-(4,5-dimetiltiazol-2-il)-2,5- difenil-tetrazolium bromide assay] in every 6 h for 72 h post transfection 25.

Measuring of Sf9 cell size during transfection

Transfection may cause changes in cell properties by the introduction of DNA, and the most distinctive change is the size of infected cells. After transfection with the MOI 0.1 and 100 µl samples were placed into cover glass, and the cell sizes were measured under an inverse phase microscope (Olympus, Japan) for 100 cells from 10 different areas.

Monitoring PEDV-S1 protein expression and baculovirus replication with immunofluorescence method

The immunofluorescence method was used to detect the location and relative abundance of baculovirus. 6x rabbit polyclonal antibody (GTX115045; Gene Tex, USA) and its used as primer antibodies, and also goat antirabbit (594; Invitrogen, UK) IgG was seconder antibodies.

Determination of protein concentration and western blot

After transfection, cells were harvested at 24 h intervals for Western blot analysis. Western blot samples (1.5 mL) were centrifuged at 16,000g for 2.5 min. After removing the supernatant, the cell pellets were stored at -20^{0}C. The protein concentration was determined with the BCA Protein Assay Reagent Kit (Pierce, USA).

Statistical analysis

The viability of uninfected and infected cells was calculated, and the results were compared to the Student's t test. The arithmetic averages of the cell sizes were calculated and the results were compared with the Student's t test. A *P*-value of <0.05 was considered significant by using computer software SPSS ver. 16.0 (SPSS Inc., Chicago IL, USA).

RESULTS

Optimization of PEDV-S1 protein expression and transfection parameters Production of recombinant baculovirus

The plasmid construct was successfully transfected into *E.coli* DH5α and then DH10Bac, which resulted in the generation of the bacmid DNA. A detail of the cloning strategy and nucleotid sequence of PEDV-S1 is described in Fig. 1. Sf9 cells were transfected with this bacmid DNA by using cationic lipid agent for protein expression. Transfection was monitored daily by measuring the PEDV activity for post transfection. Expression of PEDV-S1 protein was similar in uninfected and infected Sf9 cells for 24 h, in contrast the expression of PEDV-S1 protein increased after 48 h post infection. Finally, the highest protein activity was determined for 96 h post infection. The expression of this virus slightly decreased after 120 h post infection (Fig. 2). Changes in cell shape, cell size and density of the cells are indicated in Fig. 3. The cells became bigger and more rounded while the number of cells decreased, and cells lost adherence ability to the culture plate obvious after 72 h post transfection. Similar report by Radner, et al. [26] that typical changes in the morphological properties of cells at post transfection were reported in the literature.

The maximum viral activity was obtained at 96 h post transfection. However, when the transfection productivity (viral activity per hours) consider, it can clearly be seen that 72 h post transfection (4.03 mU mL^{-1} h^{-1}) could be better than 96 h (3.62 mU mL^{-1} h^{-1}) for optimum transfection. Transfected Sf9 cells were harvested and loaded into Western blot gel. The Western blot results implied that the expression of recombinant PEDV-S1 on infected Sf9 cells was increased at 72 h post transfection, whereas, expression of PEDV-S1 had insignificant changes in uninfected cells (Fig. 4). The molecular mass of pFastBacHTb_PEDV-S1 was about 55 kDa according to the marker on the gel. This molecular mass had been predicted from the amino acid sequence of PEDV-S1 (1,503 bp) 27. The result was in consistence with previous studies 28-30.

The viral activities of the samples, which were loaded to the Western blot, for uninfected cells were 2.62 ± 0.1 mU mL^{-1} at 24 h, 7.88 ± 0.24 mU mL^{-1} at 48 h and 12.79 ± 2.01 mU mL^{-1} at 72 h, while the activities for infected cells at 24, 48 and 72 h increased to 2.86 ± 0.14, 8.36 ± 0.34 and 291.91 ± 5.73 mU mL^{-1}, respectively.

Addition, the transfection time for standard recombinant enzyme production, MOI was another important parameter. The first, a logarithmic pattern was formed for MOI as 0.1, 1, and 10 for 24, 48, 72, 96, 120 and 144 h post transfection. PEDV-S1 expression levels were monitored by measuring the viral activity. The viral activity time course show in Fig. 5.

In Fig. 5 show that the expression of PEDV-S1 with MOI 0.1 was similar to MOI 1 and three times higher than MOI 10. These results indicated that the range of MOI 0.1–1 should be studied further for the maximum PEDV-S1 expression. Therefore, different ratios of MOI were performed as 0.1, 0.25, 0.5, 0.75, and 1 at 24, 48, 72, 96, 120, and 144 h post transfection, respectively (Fig. 6). The amount of expressed PEDV-S1 from infected Sf9 cells was found similar to MOI at 0.1 and 1. The amount of expressed PEDV-S1 from infected Sf9 cells was found similar from MOI 0.1 to 1.

Baculovirus replication and cytopathic effects

The transfected Sf9 cells showed that the typical cytopathic effects such as ceasing of cell growth. As a result the decreasing of the infected cell viability. The viability of infected and uninfected cells were in Table 1. There was a significant difference between the number of viable uninfected and infected cells ($P<0.001$). Moreover, irregular cell shapes, increased cell size and lost attachment were observed under inverse phase microscope (Olympus, Japan). At the end of the 30 h post infection, 60 % of adherent cells lost attachment capability to the culture surface. Similarly, at 66 h post transfection the 80–90 % of the cells were detached and formed clusters.

The effect of transfection on Sf9 cell morphology

The most distinctive effect of transfection on Sf9 cell morphology was the cell size. The Fig. 7 show that the size of uninfected cells was 18.0 ± 0.67 µm. There were significant differences between uninfected and infected cell sizes after 24 h post infection ($P<0.001$). The infected cells (MOI 0.1) were monitored by using immunofluorescence method for 72 h post infection (Fig. 8). The immunofluoresence images revealed that the percentage of baculovirus increased gradually after 24 h post infection, and PEDV-S1 was expressed from the transfected Sf9 cells after 48 h post infection.

The relationship between the replication of baculovirus and the expression of PEDV-S1 were detected by using fluorescence intensity of infected cells and uninfected cells. By this compare, fluorescence intensity was proved that increased 20 % in baculovirus infection, whereas, the PEDV-S1 expression increased 3% after 24 h post infection. Expression of the PEDV-S1 from infected Sf9 cells rose gradually until 72 h post transfection before slightly increasing at 96 h post transfection.

The results indicated that the transfection efficiency in the percentage of transfected Sf9 cells in the population at 81 %. Moreover, the ratio of the number of infected cells (MOI = 0.1),which expressed PEDV-S1 that the total infected cells at 80 % (Fig. 9).

DISCUSSION

Recently, baculovirus expression system and Sf9 cell line have widely used for recombinant protein production. The PEDV-S1 gene was successfully expressed in the baculovirus-insect cell system in a related study 31. Examined parameters, MOI and infection time, were expanded to determine optimum MOI value (0.1, 0.25, 0.5, 0.75, 1 and 10) and harvest time (0, 24, 72, 96, 120 and 144 h) in this study.The entire process was monitored to observe the effects of this approach. In addition to PEDV activity and MOI against time, productivity of PEDV-S1, and yield of the virus determination of the change in the cell size were also carried out. These parameters have been considered as potentially useful items for monitoring infected cell cultures to determine the maximum protein production and to calculate the maximum productivity.

The most critical parameter for maximizing the production is to find an optimal harvest time for transfection of the cells. Therefore, productivity of PEDV-S1 had been used to determine the time of maximum protein production (PEDV-S1 activity), and optimal harvest time hereby. As mentioned above, since productivity of PEDV-S1 from infected Sf9 at 72 h was 1.11 fold higher than at 96 h. Therefore, post infection of 72 h was performed as an optimum harvest time (Fig. 2). Related manuscript have been supported the data 32,33.Monitoring methods may indicate some correlations between cell density, PEDV-S1 activity or cell size and infection rate. The data represented that density of the infected cells was similar to the uninfected cells at 0 h. However, the infected cell density decreased gradually until 144 h (Fig. 3). It is thought that density of the infected cell may be related to recombinant baculovirus replication in the cells or the infected cells may change their metabolism in order that expressing of PEDV-S1 instead of their growth. Hence, the results indicate that there has been an opposite correlation between cell density and infection, namely protein production in infected cells.Whereas 32 have supported the data, 34 reported no strong connection between the cell density and infection.

Although a similar pattern was found for infected Sf9 cell line (with MOI = 0.1-1) in Fig. 6, the yields of PEDV-S1 per MOI were measured as 2.69 and 0.3 Ucell.pfu^{-1} mL^{-1} for MOI = 0.1 and 1 respectively. Therefore, MOI = 0.1 value was performed as optimum MOI for expression of PEDV-S1 from Sf9 cells. As it is well known, using low MOI is preferable for industrial production because of the low passage effects.After infection with MOI = 0.1, infected cell viability was checked by MTT assay and any sharp decrease was not observed in viability of infected cells. Therefore, it was thought that baculovirus could not be a lytic virus, but could be a budded virus. The baculovirus species is the

most frequently used for baculovirus studies. 35 remarked it as a non-lytic virus.The average diameter of the cells in the uninfected cultures was 18.0 ± 0.67 µm at the beginning of infection. The average size of uninfected Sf9 cells was reported by 36 as 18.5 ± 1.5 µm, which is in correlation with the current study.

In the current study; Sf9 cells infected with low MOI (0.1) swelled rapidly and reached to a point at average 23 lm diameter at 72 h likewise the study by 36. The average diameters were similar at the beginning of infection for uninfected and infected cells, but the diameter of infected cells increased by 42 % at 72 h post infection (Fig. 7). 34 reported the increased diameter of infected cell as 28 % with MOI = 0.5 at the same time. It is clearly understood that the cell size of Sf9 could be considered as one of the infection signs.Increased cell diameter leads to lack of membrane resistant and becomes more fragile against hydrodynamic forces like shear stress. Therefore, it is important to measure the uninfected and infected cell diameters, and calculate possible shear stress as preliminary calculations before large scale production.

The immunofluorescence images supported the findings from the cell diameter and cell density experiments, and also showed baculovirus infected Sf9 cells and expressed PEDV-S1. In this study proliferation of transfected Sf9 cell ceased, and cell size increased after 24 h post infection. These findings indicate that viral replication gained acceleration after 24 h post infection, and this situation cause increasing in the number of infected Sf9 cells, which has been also supported by 37.A success in the expression studies depends on the efficiency of infection. 26 found infection efficiency approximately 20-30 % of Sf9 and Sf21 cells at 72 h, while another related study gave the infection efficiency as 40-60 % [38]. In the present study, the highest transfection efficiency of recombinant PEDV-S1 was achieved by 80% transfected Sf9 at 72 h. High infection efficiency and the short incubation period have offered fast and high yield recombinant protein production by large scale infection of Sf9 cells.

CONCLUSION

In summary, the findings about optimum harvest time, productivity, infection efficiency, also some negative or positive correlations between cell density, PEDV-S1 activity and cell size have been manifested. The phenomenons might be a guide for baculoviral PEDV-S1 producers from Sf9 cells. The results of research that are described in this report will be performed in the following study that is based on scaling up PEDV-S1 expression from infected Sf9 cell line.

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Conflict of interest

The authors do not have any conflict of interest.

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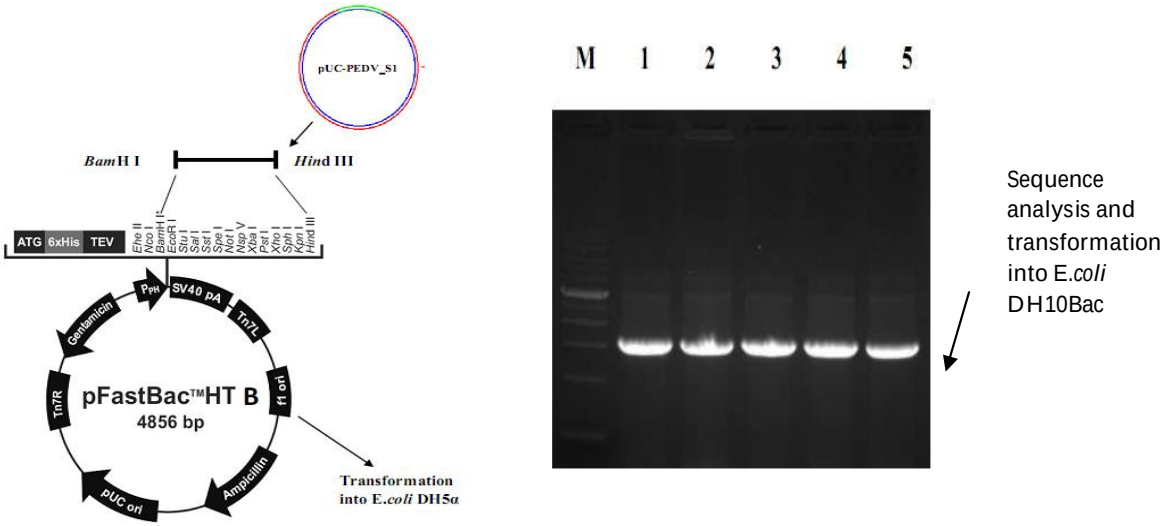

Fig. 1- Shematic of the cloning strategies and nucleotide sequence of PEDV-S1. a) The PEDV-S1 gene was obtained from pUC_PEDV-S1 inserted into the pFastBacHTb cloning system, and contructed plasmid was transformed *E. coli* DH5α. b) PCR screening of the positive *E. coli* DH10Bac colonies containing the pFastBacHTb_PEDV-S1 plasmid.

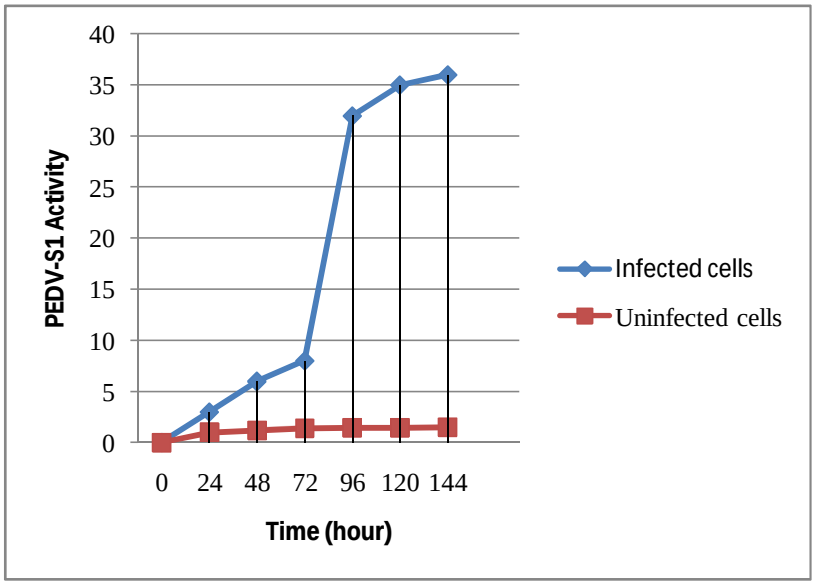

Fig. 2- Comparison of PEDV-S1 expression with bacmid DNA by using cationic lipid agent

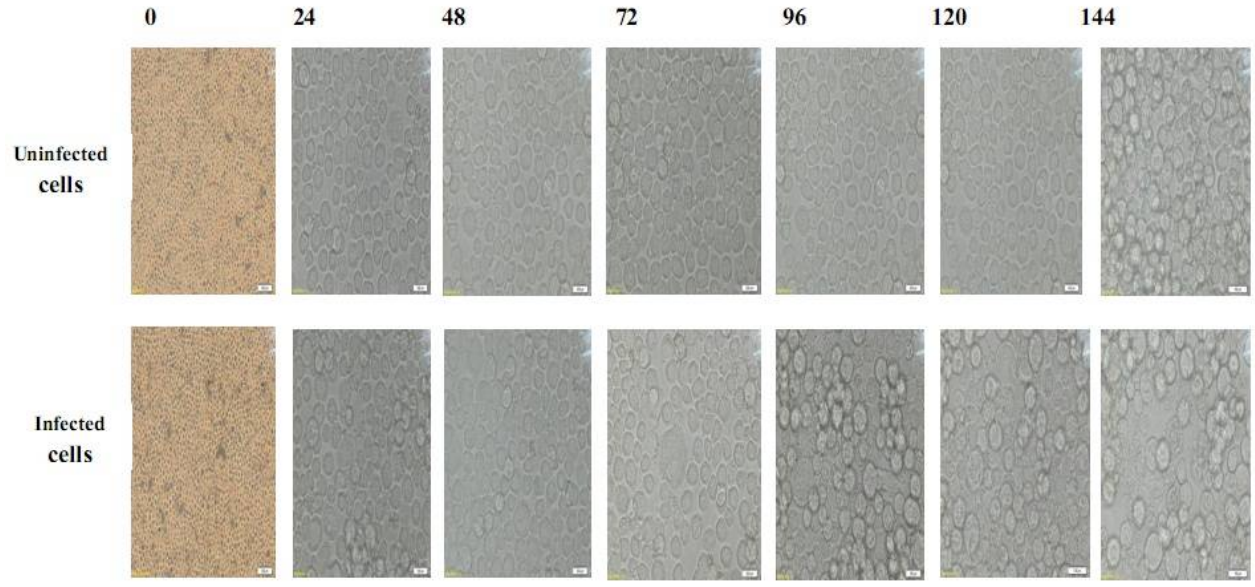

Fig. 3- Time dependent morphological differentiations of insect cells after transfection

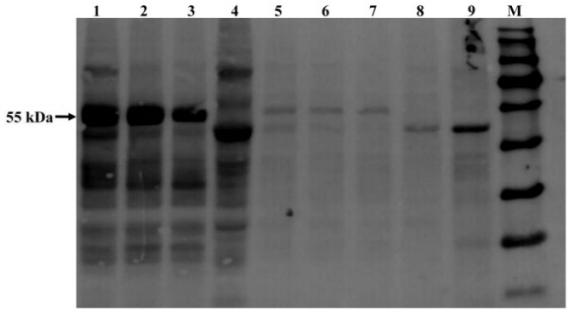

Fig. 4- Western blot analysis of PEDV-S1 expression from uninfected and infected cells; (sample: 1->3, 5->7; 4,8: Cell only (control); 9: E2 virus Pellet (Positive); M: Protein marker).

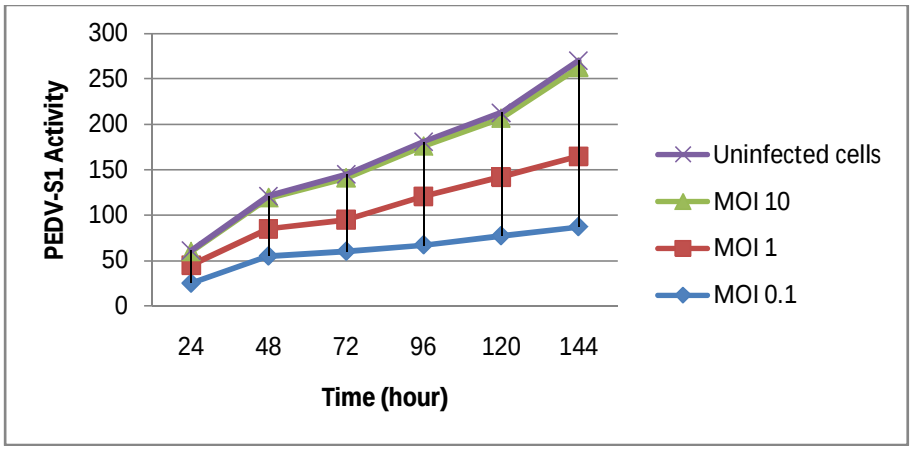

Fig. 5- Transfection of Sf9 cells with MOI 0.1, 1 and 10

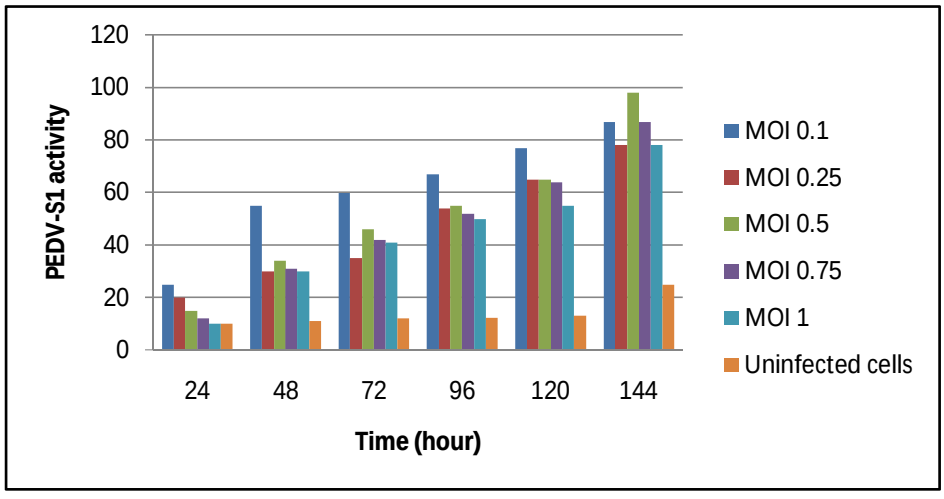

Fig. 6- Transfection of Sf9 cells with MOI 0.1, 0.25, 0.5, 0.75 and 1

Table 1- Measurement of the viability of infected (MOI 0.1) and uninfected Sf9 cells by MTT assay (p<0.001)

Time (hour)	Number of the viable (cell. 10^4. mL^{-1})	
	Uninfected Sf9 cells	Infected Sf9 cells
0	1	1
24	5.12	2.86
48	9.36	2.67
72	15.03	2.82
96	20.57	1.96
120	24.84	2.58
144	29.05	2.64

P < 0.001

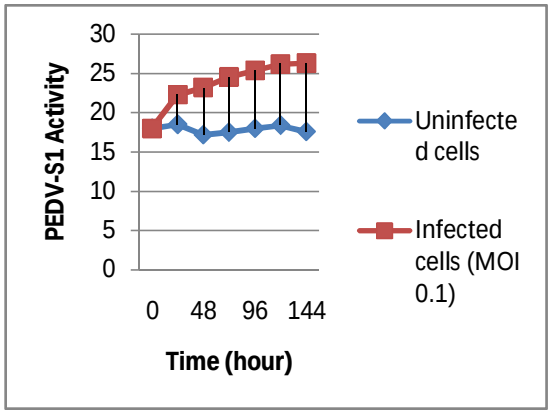

Fig. 7- Effect of transfection on Sf9 cell size

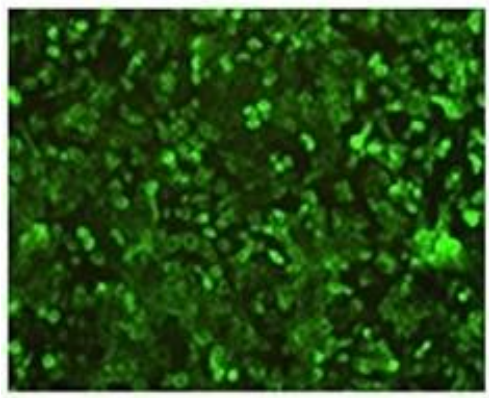

Fig. 8- Immunofluorescence images of baculovirus infected Sf9 cells

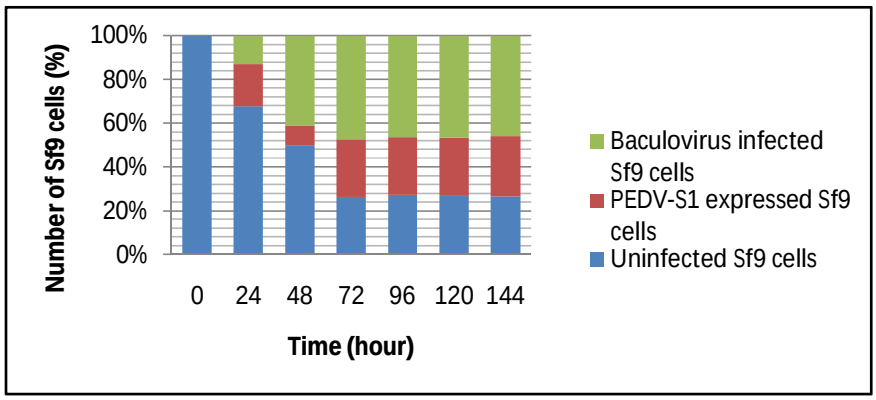

Fig. 9- Correlation between uninfected Sf9 cells, baculovirus infected Sf9 cells and expression of PEDV-S1 (from infected Sf9 cells) by using immunofluorescence images

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RESEARCH ARTICLE

Phytotoxic Evaluation of Flavonoid Isolated from *Portulaca oleracea* L

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ABSTRACT

In this study, the aerial part of *Portulaca oleracea* Lhas been investigated to evaluate Phytotoxicity effect, the results obtained reveals that the plant found to contain flavonoids as part of their secondary metabolites, this observations agrees with the few available reports conducted by many researchers on the presence of flavonoids in *P. oleracea* L. The flavonoids were extractedfrom dried powdered leaves of *P. oleracea* L with Soxhlet extraction method. The crude extract was partially purified.Qualitative tests were conducted to confirm the presence flavonoids in the *P. oleracea* L extract. Frothing test, as a follow up was also done on the extract to ensure the absence of saponins which are usually abundant as secondary metabolites in plants. The allelopatic investigation of the partially purified extract on green gram seed germination, shoot and root elongationhas showed that the flavonoids of P. *oleracea* L acts as phytotoxic. The inhibition of green gram seed germination, radicle and coleoptile growth was observed in the present study was dose-dependent manner when compared with control group.

Key words: Phytotoxicity,*Portulaca oleracea,* flavonoids

INTRODUCTION

Flavonoids are established as natural anti-oxidants, which have captured a fast growing interest among consumers and scientists in medical, pharmaceutical, chemical and agricultural industries. They belong to the group of poly-phenolic compounds diverse in chemical structure and characteristics, found ubiquitously as secondary metabolites or chemical constituents in plants either consumed as foods or used byman in folk-lore medicine. They occur

naturally in fruit, vegetables, nuts, grains, seeds, flowers, roots, stems and bark of plants; and are integral part of the humandiet [10, 14]. Literature reports on the presence of flavonoids and other active chemical substances such as Tannins, Terpenes, Saponins, Xanthones and glycosides as phytochemicals in different tropical plants which serve basically as foods and medicinal herbs abound and are daily on the increase following continuous scientific investigations [22]. The phytochemicals present in these plants are largely responsible for the medicinal functions associated with them.

Overuse of synthetic herbicides to control weeds lead to an increased risk of herbicide resistant weed biotypesand harsh environmental pollutions [11, 1, 16, 18],Alternative weed management strategies that are ecofriendly and cost-effective are therefore a time demanding issue throughout the world. In this backdrop, phytotoxic plants might help in resolving the problems created by synthetic herbicides as they possess growth retarding substances. Recently, there has been an increasing interest shown by the researchers on phytotoxic medicinal plants [6, 8, 17].This interest on medicinal plants could be due to either (i) the easier screening process of phytotoxic compounds from medicinal plants [7] or (ii) the possibility to have more bioactive compounds in medicinal plants than other plants. These phytotoxic plants could beused in several ways to control weeds, for example, (i) sowing/transplanting them as relay or cover crops with main crops, (ii) direct application of their crude extracts as bio-herbicides, or (iii) isolation and characterization of their active substances and using them as a tool for new natural and biodegradable herbicides development.*P. oleracea* L is a medicinal plant belongs to the family *Portulacaceae*and generally used as a vegetable in the preparation of curries. Hot water extract of dried leaves are taken orally as a diuretic and for liver diseases. Leaves and shoot are cooked as vegetableto use as a food, seeds are taken orally as a vermifuge, and seeds stepped in wine are taken orally, as an emmenagogue. In Ayurvedic and Unani medicines, the seeds are taken orally as a vermifuge. Shoots are also used as food. In west indices hot water extract of aerial parts and seeds are taken orally to provoke menses. Hot water extract of leaves is taken orally for painful menstruation. The main constituents of the plant are alkaloids and γ-linolenic acid, aspergic acid, Folacin, fructose glucose, Histidine, Saponins, tannin etc.The flavonoid extract of plant is subjected for Phytotoxicity test to evaluate the effect on seed germination and also the effect of root and shoot growth further to screen the presence of phytochemical constituents.

MATERIALS AND METHODS

Plant Material

Healthy aerial part of the plant of *P. oleracea* L. was collected from around Gulbarga University campus during the month of June 2014. The plant material was identified and authenticated from the Department of Botany, Gulbarga University, Kalburgi, Karnataka (India); voucher specimen (Number HGUG-5013) has been deposited in herbarium of the same department

Chemicals

Methanol, Ethanol, Ethyl acetate, Petroleum ether, Diethyl ether, H_2SO_4, Chloroform, HCl, KOH, Hexane, Silica gel 60–120 mesh, Tween 80, Phosphate buffer saline, FCR reagent, all the chemicals, solvents, and reagents were analytical grade and were obtained from Hi Media.

Isolation of Total Flavonoids by Soxhlet Extraction Method

Before extraction, *P. oleracea* L .was crushed into powder by versatile plant pulverizes. The powder of the sample was degreased by Soxhlet extractor with petroleum ether until the color of elute becomes colorless. The same powder sample was accurately weighed and placed in Soxhlet extractor by adding 80 mL of ethanol: water (70: 30) solvent,

followed by the extraction for up to 5 h, and then extract solution was concentrated. The extract was centrifuged at 11000 rpm for 30 min; supernatant was taken for further use [24].

Qualitative Test for Flavonoids

Two different methods were used to determine the presence of flavonoids in the extract; 5 mL of dilute ammonia solution was added to a portion of total flavonoid extracts followed by addition of concentrated H_2SO_4[22, 9].

Phytotoxic assay

Germination Bioassay

Different concentration of isolated compound solution was prepared as 0.25 mg/ml, 0.5 mg/mL, and 1 mg/mL and four sterilized petri-dishes were divided into 4 groups for culture of green gram seeds. Clean cotton was placed at the bottom of each petri-dish, and were labeled as group 1-4 and among these, Group-I, is treated with 10 mL of distilled water and served as control, Group-II, treated with 10 mL of 0.25 mg/mL of compound, Group-III, treated with 10 mL of 0.5 mg/mL of compound, Group-IV, treated with 10 mL of 1 mg/mL of compound. 10mL of distilled water was added to each petri-dish every day from the second day of the experiment. This is to compensate the water loss through evaporation. The germination pattern was observed regularly for seven days.

Growth Bioassay

The Petri dishes and the isolated compound were prepared as described above. Ten seeds ofGreen gram were placed on filter paper in petri dishes. The root and shoot lengths of each seedlingswere measured by a thread and a ruler method after incubation period of 7 days. The recorded values were compared with those of the control groups to determine the index of inhibition of growth. Control Petri dishes were maintained as germination bioassay.

Statistical analysis of Data

The bioassay experiments were conducted as completely randomized design (CRD) with three replications. The experiments were repeated thrice to avoid any experimental error. The data generated in each experiment were analyzed using one way ANOVA analysis. Results are expressed as Mean ± SEM, the *p-value* of 0.05 or less was considered as significant for all experiments.

RESULTS

Qualitative Test for Flavonoids

The yellow coloration is observed after adding $H_2SO_4$and it disappeared on standing. Few drops of 1% aluminum solution were added to a portion of flavonoid extract and again yellow coloration was Observed, this indicates the presence of flavonoids

Phytotoxic assay

Germination and Growth Bioassay

The data generated in this present study is showing that the treatment of flavonoid of *P. oleracea* L to green gram seeds at various concentrations such as 0.25, 0.5 and 1.0 mg/mL has caused significant (p<0.01)reduction in the growth and development of shoot and radicle elongation when compared to the control group.

The germination of green gram seeds has occurred faster in the control relatively to those treated with flavonoid, indicating that the flavonoid from *P. oleracea* L has inhibited seed germination. Reduction in the growth due to flavonoid treatment proves that the green gram seeds were more susceptible to the phytotoxic effects of the compound. Moreover, the inhibitory effects on the germination of green gram seeds and on their radicles and coleoptiles were observed to increase with increase in the concentration of the compound. This implies that the inhibitory activity of the *P. oleracea* L compound is dose dependent. In the present study the *P. oleracea* L compound treatment at various concentrations of 0.25%, 0.5% and 1.0% to green gram seeds has reduced the growth profile of green gram seedlings in dose dependent pattern. There is a clear indication that the compound retarded seed germination and seedling growth by inhibiting certain endogenous growth hormones such as Gibberellic acid (GA3), Indolacetic acid oxidase (IAA-oxidase) and indo-3-acetic acid (IAA). Thus, their application in agriculture could be considered in weed control.

DISCUSSION

In the present study the isolated flavonoid from *P. oleracea* L was treated at different dose level against germination, radical and shoots elongation of green grams. The treatment of flavonoid of *P. oleracea* L at various concentrations such as 0.25, 0.5 and 1.0 mg/mL were caused significant (p<0.01) decreasein the growth between the shoot and root lengths.The higher concentration of 1.0 mg/mL flavonoid treatment on green gram seeds has shown significant decrease when compared to control seedlings. The germination of seeds has occurred relatively to faster in the control compared to those treated with flavonoid, it indicates that the flavonoid of *P.oleracea* L is inhibitedthe seed germination. Reduction in the growth of root and shoot length is due to flavonoid treatment proves that the green gram seeds were more susceptible to the phytotoxic effect caused by the compound, the available literaturesurvey reports thatthe flavonoids arethe allelechemicals. Among available 44 phenolic compounds these flavonoids have inhibited radish germination andradical growth in cress and wheat. Kaempferol, diosmethin, phloridzin, rutin, morin, and quericetin pentaacetate havestimulated radish germination and inhibited the radical growth of cress. the flavone, chrysin, and the flavanone, hesperetin, are significantly inhibited the germination and hyphal growth at all applied concentrations on vesiculararbuscularmycorrhizaglomusmosseae and alfalfa plants [21]. Flavonols isolated from the leaves of *Pluchealanceolata* were tested at a concentration of 10-4M and 10-3M against asparagus bean seedlings, resulting in an inhibitory activity [5].

The *Castanea sativa* Millleaves contain the flavonoids such as Quercetin, Rutin and Apigenin that are inhibited seed germination and epicotyl and root growth in *R. sativus*.Some flavonoids are potent inhibitors of energy metabolism, blocking mitochondrial and chloroplast functions [3]. Moreover, these compounds are considered as potent allele chemicals inhibiting the mitochondrial oxygen uptake. Flavonoids appear to act primarily as germination and cell growth inhibitors, possibly through interference with the energy transfer system within the cell. Flavones have been shown to interfere with ATP formationin plant mitochondria. Specific structural requirements for particular flavonoids to act as stimulators of destruction of Indolaceacetic acid via IAA oxidase, which results in the inhibition of ATP formation, were reported by several researchers . The arrangement of the B-ring in the flavonoid structure has been proposed as responsible for the biological activity. Furthermore, in the intracellular medium, flavonoids assume a negative charge at neutral p^H [12, 13, 23, 15, 4, 2, 19].In low concentrations, these compounds can promote cellular growth, perhaps due to more effective utilization of cellular enzymes, proteins and electron carriers.High concentrations of flavonoids, on the other hand, could act as membrane hyperpolarizes, altering the ATP pump, making the flavonoids toxic for the cells, andtherebyreducing their growth[15, 19]. Therefore, in the present investigation the growth inhibition in green gram seed after treatment of flavonoid may be due to their ability to interfere with enzyme activity.

CONCLUSION

Weed management is one of the most challenging tasks in crop production. Overuse of synthetic herbicides causes severe environmental pollution besides being developed herbicide resistant weed biotypes. Plant product based natural herbicides could serve as an alternative to synthetic herbicides that are biodegradable and environment friendly, In this regard, *P. oleracea L*acts as a promising role in eradicating weeds . Isolation and characterization of phytotoxic substances from *P. oleracea L*may promote the development of plant product based natural herbicides.

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Plant Classification

Kingdom- Plantae (Plants)
Sub kingdom- Tracheobionta
Division- Spermatophyta
Sub Division- Angiospermae
Class- Dicotyledoneae
Order- Caryophyllales
Family- Portulacaceae
Genus- Portulaca
Species- *P. oleracea* L.

Fig: 1. Areal part of *P. oleracea* L

Control **0.25mg/ml** **0.5mg/ml** **10mg/ml**

Fig 2: Effect of various concentration ofFlavonoid treatmenton Germination of Green gram seeds.

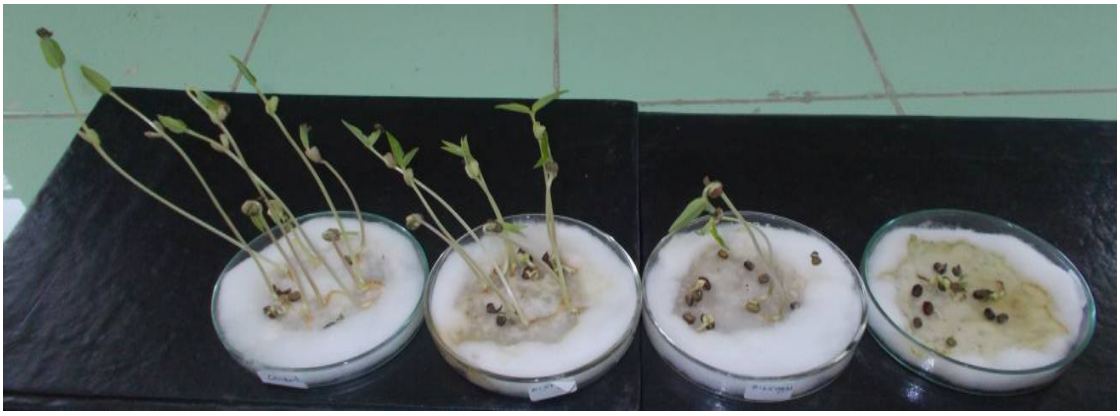
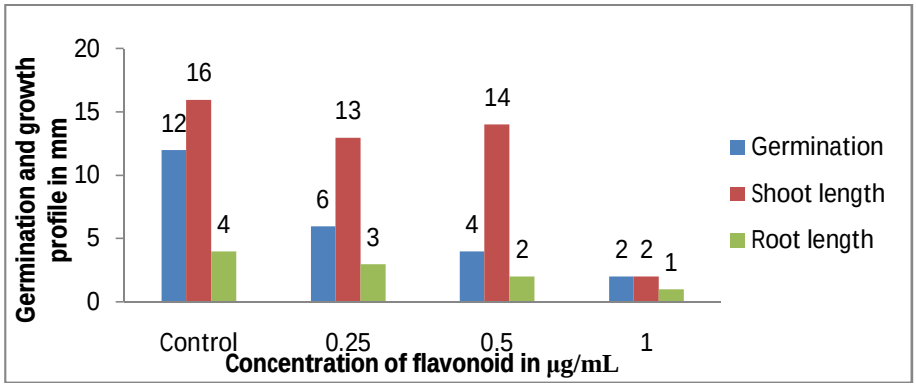


Fig 3: Effect of various concentration of Flavonoid treatment onSeed germination and Growth of Green gram.



Graph 1: Effect of flavonoid on germination, shoot and root growth profile of green gram in phytotoxic assay.

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RESEARCH ARTICLE

Analytical and Numerical Analysis of Two Dimensional Transient Heat Flux

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ABSTRACT

This paper presents the analytical and numerical investigation in two dimensional transient heat flux with platinum plate. The objective of this work is to present the method of simple and accurate measurement of temperature with time varying heat flux. The methodology of measuring temperature with space and time coordinates satisfies a criterion which is developed in this paper. The simple transient 1-D semi-infinite solution has been used to generate extremely accurate values for temperature and heat flux at any point of a finite rectangle in a convolution time with Green function and Laplace technique. A complex 2D semi-infinite problem is solved explicitly and evaluated numerically as part of the analysis. A one-dimensional transient heat transfer modelling is used to infer surface heating rates from the closed form temperature solutions. The analytical results are compared with MATLAB and ANSYS and it is seen that these results have a very good agreement with MATLAB and ANSYS.

Keywords: Surface heat flux, semi-infinite analysis, inverse analysis

INTRODUCTION

The word heat transfer mainly depends upon the temperature and flow of heat. The temperature defines the amount of thermal energy available, while the flow of heat represents the movement of thermal energy from place to place by virtue of temperature difference. In thermodynamic energy transfer it is mainly governed by three fundamental laws. The zeroth law states that 'no heat transfer' takes place when two bodies are in thermal equilibrium, i.e. the bodies are at the same temperature. Heat is transferred by three mechanisms: conduction, convection and radiation. Conduction is the

transfer of energy from higher energy to lower energy as a result of intraction between molecules. Convection is the transfer of energy between a solid surface and the adjacent moving fluid. Radiation is the transfer of energy due to electromagnetic wave or photon. The rate of heat transfer per unit surface area is called heat flux. Heat transfer is the exchange of thermal energy from a body at a high temperature to another body at a lower temperature. This transfer of thermal energy may occur under steady or unsteady state conditions. Under steady state conditions the temperature within the system at any particular point does not change with respect to time. Conversely, under unsteady or transient state conditions the temperature within the system does vary with time. The accuracy of high transient temperature used fast response temperature sensors such as thin film gauges(TFG) and the heat flux may be obtained.

Transient heat transfer is the most effective role in many industrial and environmental problems. Such as in fact there is no direct method by which the heating rates can be measured during a process. Rather, transient temperatures are used different types of temperature sensors, and then heating rates are analyzed from temperature data [1-2]. Using very small time solutions at the heated surface of some basic one-dimensional geometries is investigated and discussed. Also, two-dimensional cases for various heated regions on the surface of the semi - infinite body are analyzed. The solutions are presented in the context of the finite difference form of two-dimensional unsteady heat conduction equation (FEM) [3-4]. Two -dimensional transient and steady- state heat conduction solutions when the time-partitioning method is applied [5,6,7].

Mathematical formulation and solution

The basic two-dimensional unsteady heat conduction equation is

$$\frac{\partial^2 T}{\partial x^2}+\frac{\partial^2 T}{\partial y^2}=\frac{1}{\alpha}\frac{\partial T}{\partial t} \qquad (1)$$

Transient temperature data can also be obtained from analytical formulation for the present heat transfer gauge configuration from constant heat flux by using two-dimensional heat conduction equations with semi-infinite assumption for backing material. This formulation can be obtained by solving two-dimensional unsteady heat conduction equation using Green's functions technique (Taylor &Francis 2011)

$$\frac{\partial^2 T}{\partial x^2}+\frac{\partial^2 T}{\partial y^2}=\frac{1}{\alpha}\frac{\partial T}{\partial t} \quad (x>0,y>0,t>0) \qquad (2)$$

$T(x=0,y,t) = 0$ $(y>0,t>0)$ (3)

$$-K\left(\frac{\partial T}{\partial y}\right)_{y=0}+hT\left(x,y=0,t\right)=f_0 \quad (x>0,\ t>0) \qquad (4)$$

$T(x,y,t=0) = 0$ $(x>0,\ y>0)$ (5)

Where 'h' is the heat transfer coefficient at y=0 and f_0 is equal to hT_∞ where T_∞ being the ambient temperature. (In general f_0 can also include a prescribed heat flux). The exact two-dimensional transient temperature for this problem is derived in an integral form using Green's functions (GFs) and Laplace-Technique [8-9], which can be presented as the following in the equation

$$T(x,y,t)=(hT_\infty)\frac{\alpha}{K}\int_0^t erf\left(\frac{x}{\sqrt{4\alpha u}}\right)\frac{e^{-\frac{y^2}{4\alpha u}}}{\sqrt{\pi\alpha u}}du \qquad \text{.....(6)}$$
$$-(hT_\infty)\frac{\alpha}{K}\frac{h}{K}\int_0^t erf\left(\frac{x}{\sqrt{4\alpha u}}\right)e^{-\frac{y^2}{4\alpha u}}rerf\left(\frac{y}{\sqrt{4\alpha u}}+\frac{h\sqrt{\alpha u}}{K}\right)du$$

$rerf(z)=e^{z^2}erfc(z)$ Is so called scale complementary error function. After using laplace technique the above equation reduces to

$$T-T_\infty=\frac{2(q_s/A_s)\sqrt{\frac{\alpha t}{\pi}}}{K_1}\exp\left(\frac{-x^2}{4\alpha t}\right)-\frac{(q_s/A_s)x}{K_1}\left(1-erf\frac{x}{2\sqrt{\alpha t}}\right)$$
$$\frac{2(q_s/A_s)\sqrt{\frac{\alpha t}{\pi}}}{K_2}\exp\left(\frac{-y^2}{4\alpha t}\right) \qquad \text{..... .....(7)}$$
$$-\frac{(q_s/A_s)y}{K_2}\left(1-erf\frac{y}{2\sqrt{\alpha t}}\right)$$

Where, $K_1=K_2=K$ is the thermal conductivity of platinum plate.

Using the same nodal points as those used the finite-difference form of this equation [8-9]

$$\frac{1}{\alpha}\frac{T_{m,n}^{p+1}-T_{m,n}^{p}}{\Delta t}=\frac{T_{m+1,n}^{p}+T_{m-1,n}^{p}-2T_{m,n}^{p}}{\Delta x^2}+\frac{T_{m,n+1}^{p}+T_{m,n-1}^{p}-2T_{m,n}^{p}}{\Delta y^2} \qquad \text{..... (8)}$$

$$\Delta x=\Delta y$$
$$Fo=\frac{\alpha\Delta t}{\Delta x^2}$$

Explicit finite difference equation for 2-D unsteady heat transfer equation becomes

$$T_{m,n}^{p+1}=Fo\left(T_{m+1,n}^{p}+T_{m-1,n}^{p}+T_{m,n-1}^{p}+T_{m,n+1}^{p}\right)+(1-4Fo)T_{m,n}^{p} \qquad \text{..... (9)}$$

Non – Dimensional equation for 2-D unsteady heat transfer equation becomes

$$T_{i,j}^{n+1}=\frac{Fo\left[T_{i+1,j}^{n+1}+T_{i-1,j}^{n+1}+T_{j+1,i}^{n+1}+T_{j-1,i}^{n+1}\right]+T_{i,j}^{n}}{1+4Fo} \qquad \text{.....(10)}$$

It has been analysed here with two conditions, first one is keeping constant heat flux with neglecting dimensionless parameter at the center and second one is the applied dimensionless parameter and neglecting heat flux at the center.

RESULTS AND DISCUSSION

Analysis with Matlab codeing with Non-Dimensional Parameter

Fig.1 shows the isotherm contour with respect to position of nodes in the two-dimensional unsteady state. The maximum temperature 310K is at the centre of plate while other surfaces are maintained at 300K. This contour is in elliptical form because of used equation (9) temperature is totally dependent on position with fixed value of Fourier number (F_o) and Thermal diffusivity (α).Fig.2 shows the variation of temperature (K) with respect to position (mm) inside plate. The temperature gradually decreases with increase in position. Fig.3 shows the isotherm contour in the same condition of plate by ANSYS. Fig.4 shows the variation of temperature with respect to time(Sec) which is of parabolic nature because of very small time duration.

Analysis with Fortran plateform with Non - Dimensional parameter

Fig.5 represents heat flux distribution throughout the plate with atmospheric boundary conditions at temperature 300K. Fig.6 represents the variation of temperature with respect to time and curve is of parabolic in nature due to very small time interval. Fig.7shows the temperature variation with respect to time through MATLAB using equation (7).

Validation of Result

Fig.8 shows the validation of the graph through ANSYS and MATLAB. This graph presents the good agreement between the results of MATLAB and ANSYS. Also the analytical results are justified by the above two results as shown by the fig 9 and 10.

CONCLUSION

After forgoing entire analysis of the above results, following conclusion comes into the picture that the response time of platinum plate gauge material is very high for constant temperature at centre as compare to the constant heat flux at the centre because of very low temperature gradient between the centre and surface boundary. Thus it is seen that the influence of constant heat flux is very less as compare to the constant temperature at the centre. So it can be said that platinum plate gauge material can be used as a semi – infinite body (SFB) in any transient analysis when constant heat flux is provided at the centre.

Nomenclature

A :Surface area
h :Heat transfer coefficient
k :Thermal conductivity
q :Heat flux
T :Temperature
T_∞ :Ambient temperature
ΔT :Temperature difference
$\propto$:Thermal diffusivity
F_o :Fourier number
erfc :Complementary error function

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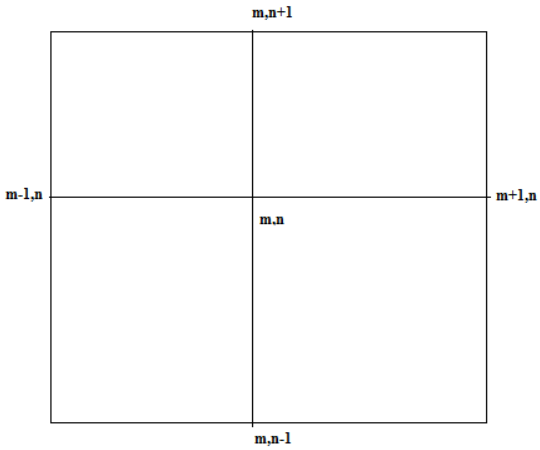

Fig.1:- shows the isotherm contour with respect to position of nodes in the two-dimensional unsteady state

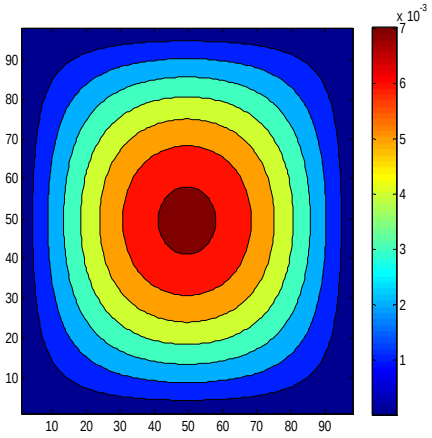

Fig.2:- Isotherm Contourf of 2D unsteady conduction equation

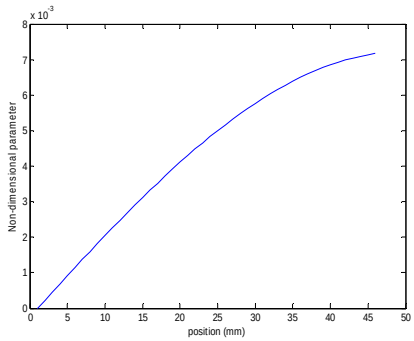

Fig.3:- Variation of Non-dimensional parameter with respect to position (mm).

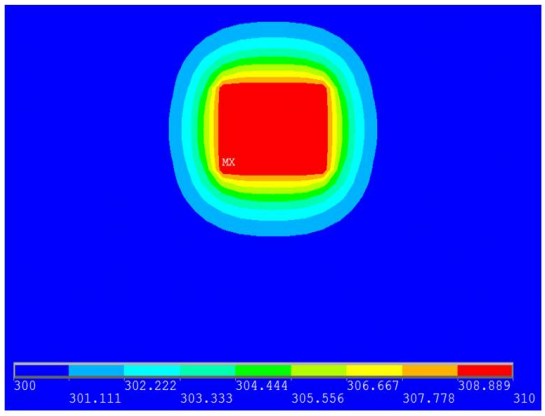

Fig.4:- Temperature distribution in plate through ANSYS

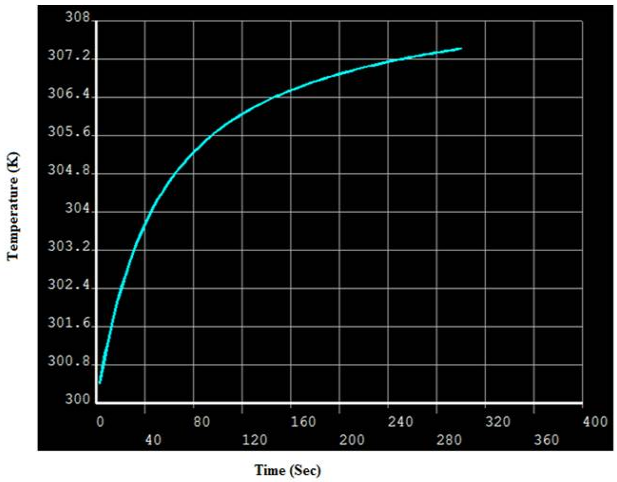

Fig.5:- Variation of Temperature (K) with respect to time (sec)

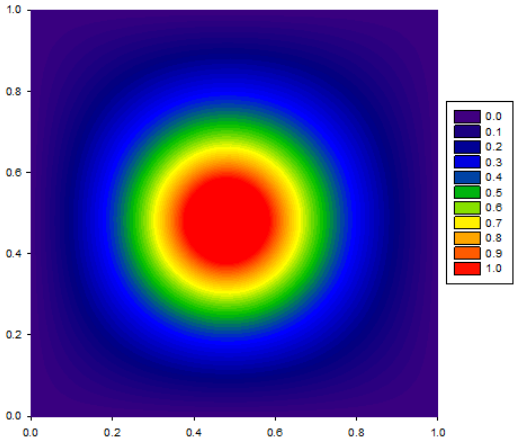

Fig.6.Analysis of constant heat flux at the centre

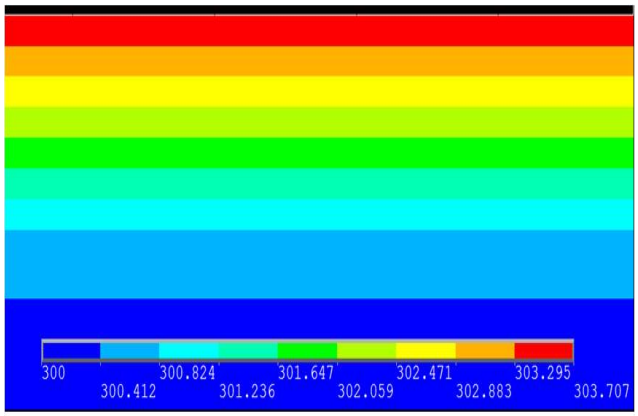

Fig.7.:- Heat flux distribution through the plate

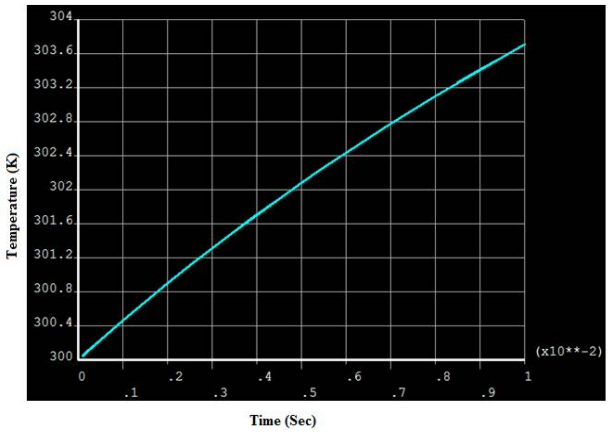

Fig.8:- Variation of Temperature (K) vs time (sec)

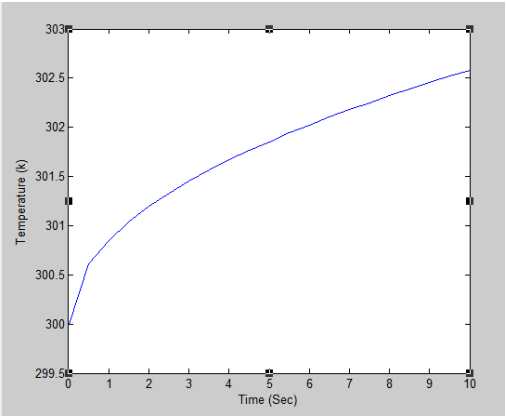

Fig.9:- Variation of Temperature (K) with respect to time (sec)

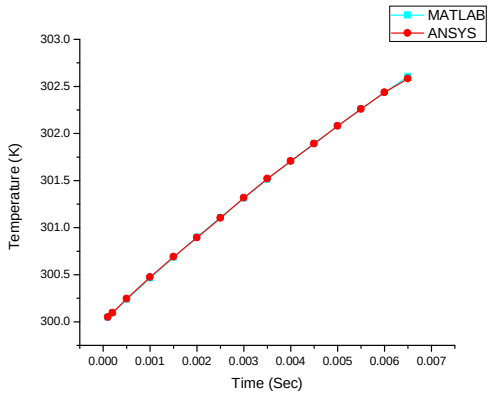

Fig.10:- Validation of graph through MATLAB and ANSYS

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RESEARCH ARTICLE

Study of Tensile Strength, Impact Strength, and Thermal Stability of Epoxy – TEOS Hybrids

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ABSTRACT

In this research prepared hybrid organic – inorganic (epoxy – TEOS) using sol- gel method with different ratios of Tetraethylorthosilicate (TEOS) (0,4,6,8,10)% .Make mechanical tests the tensile strength and impact strength to find out carrying hybrid component with tensile , impact and the best ratio in applications To find the best ratio (4%) compared with other ratios the good bond between epoxy and inorganic material. To choose the best ratio 4% to study thermal gravimetric analysis (TGA). The thermal degradation of hybrid(epoxy + 4% TEOS) take place at higher temperatures than the epoxy pure.

Key words: - Tensile strength, Impact strength , TGA, Epoxy , TEOS

INTRODUCTION

The mechanical behavior refers to the response of materials to forces. Under load, a material may either deform or break. The factors that govern a materials resistance to deformation and very different from these governing its resistance to fracture [1]. Fractures can classify in several ways. A fracture is described as ductile or brittle depending on the amount of deformation that precedes it failures may also be described as intergranular or transgranular depending on the fracture path [2].Failure in a tensile test of a ductile material occurs well after the maximum load is reached and a neck has formed [2]. In this case, fracture usually starts by nucleation of voids in the center of the neck, where the hydrostatic tension is the greatest [2]. Impact test are often used to assess the toughness of materials. The most common of this is the charpy test. A notched bar is broken by a swinging pendulum. The energy absorbed in fracture is measured by recoding by how high the pendulum swings after the bar breaks [1].

Seenaa Ibrahim Hussein

Polymer materials available today, especially the plastics, are the result of decades of evolution. Their production is extremely efficient in terms of utilization of raw materials and energy, as well as of waste release. The products present a series of excellent properties such as impermeability to water and microorganisms, high mechanical strength, low density, and low cost due to manufacturing scale and process optimization [3].Organic–Inorganic hybrids materials are important in a variety of fields as they combine the desirable properties of the organic phase (flexibility, process ability, ductility) with that of the inorganic phase (thermal stability, rigidity) [4]. Polymer–silica hybrids with enhanced thermal and mechanical properties (because of the silica component), better flexibility (due to the polymer content), and various tailored properties have attracted a lot of attention for a variety of applications including catalysis. [5]

The sol-gel process is of interest in preparing these materials due to its mild conditions such as low temperature and pressure [6].This process provides a convenient route to combine inorganic and organic components as a homogeneous hybrid material. Many researchers have demonstrated that, monolithic transparent hybrid materials without macroscopic phase separations can be prepared by controlling properly the conditions of hydrolysis and condensation of sol-gel materials such as tetraethoxysilane (TEOS)[6]. Silica-polymer hybrid materials have received much attention, due to their wide application in adhesion, biomaterials, protective coatings, composites, microelectronics, thin-films [7]. Polymers can be divided into type thermoplastics and thermosets, according to their behaviour under temperature rise. Thermoplastics become softer when they are heated and harden back when they are cooled. This is a reversible process. When thermosets are heated, they become permanently hard[8].

Bandyopadhyay et al. (2005) described effect of polymer–filler interaction on solvent swelling and dynamic mechanical properties of the sol–gel derived acrylic rubber (ACM)/silica, epoxidized natural rubber (ENR)/silica, and poly (vinyl alcohol) (PVA)/silica hybrid nano composites.Tetraethoxysilane (TEOS) at three different concentrations (10, 30, and 50 wt %) was used as the precursor for *in situ* silica generation. Equilibrium swelling of the hybrid nano composites in respective solvents at ambient condition showed highest volume fraction of the polymer in the swollen gel in PVA/silica system and least in ACM/silica, with ENR/silica recording an intermediate value [9]

Experimental part

MATERIALS AND METHODS

TEOS :was a silica precursor was produce from sigma-aldrich (Germany company) . Ethonal England company

Ethonal : Ethanol (EtOH) ,GCC/Gainland chemical company ,C_2H_5OH Molecular Weight (g/mol) =46.07 ,density= 0.785, purity= 99.9% LiquidH_2O: Deionized Water (H_2O) ,University of Baghdad/ College of Science/Laboratory of service , Molecular Weight (g/mol) = 18, density = 1 ,high degree of purity/empty of additional ions ,Liquid

Epoxy : epoxy (105) Don construction products (DCP) , Amman –Jordan The ratio of hardener to epoxy used in this study was approximately 3:1.

Hydrochloric Acid (HCl) ,BDH , Molecular Weight (g/mol) =36.46, density= 1.19 ,purity= 37% , Liquid.

Preparation of alkoxysilane solution and epoxy matrix materials solution

To prepared silica using sol-gel method to use TEOS and ethonal and H_2o with HCL (PH=1) the value in table (1) was stirred at 50 °c unit it become homogenous solution The composition of the matrix materials epoxy and hardener the ratio (3:1) the liquid hardener was slowly added epoxy resin at room temp. , this mixture was stirred at 10 min.

Preparation of epoxy –silica hybrid materials

To added the alkoxysilane solution to the epoxy and stirred solution in a glass tube was prepared using different concentration alkoxysilane -epoxy -hardener solution composition of which in table (2)

Preparation of Tensile and Impact Test Samples

Epoxy –silica hybrid solutions casting into molds at 24 hours to dry out and cutting the ASTM of tensile (D638) and impact test (Iso-179).

Thermogravimetric analysis (TGA)

TGA use helium as inert gas in rate 20ml/min ,at temperature range of 0 to 1000□ c at a heating rate of 10˚ c/min. the samples were ground into fine powder .The measurements were taken using 3-5mg samples.

RESULTS AND DISCUSSION

Tensile strength

Tensile strength of the hybrids are mostly affected by the materials, specimen condition, method, preparation of sample, and percentage of the reinforcement [10]. The results of the tensile strength of the hybrids at different percentages of TEOS figure (3) shows that the strength high in ratio 4% TEOS this is due to good bonding between Epoxy and TEOS and that strongly affect the properties of the hybrid materials. but the ratio (6,8,10%) less the values of tensile strength which agree with [11].If the epoxy and TEOS not properly mixed ,cause voids and increase level of porosity in the system .this is the reason the one ratio (4%) exhibited highest value compared with other ratio.

Impact strength

TEOS –polymer hybrid materials can be obtained by simply mixing organic –inorganic components, however, it is usually difficult to obtained homogenous mixture of TEOS and organic polymer, due to the formation interpenetrating network of the organic-inorganic hybrid system. This is duo to formation of hydrogen bonds between epoxy and TEOS [12].

Figure (2) shows the values of impact strength the highest value the ratio 4% due to the good bonding between epoxy and TEOS and the good bonding between them duo to the good impact strength .As the amount of TEOS in the hybrids increased the impact strength decreased which agree with [11].

Thermogravimetric analysis (TGA)

Figure (3) and figure (4) shows the TGA curve of epoxy pure and hybrid epoxy +4% TEOS .it can be observed that the thermal degradation of hybrid take place at higher temperatures than the epoxy pure. The initial degradation temperature of epoxy pure that occurred around (~106.60 ºC) and hybrid epoxy +4%TEOS (~104.99 ºC),due to elimination of water from the systems . for the temperature around (~366.23ºC) ,the decomposition of the polymer matrix occurred which degraded the chemical bonds of the epoxy and hybrid (~559.71 ºC).The slightly improved thermal stability for the hybrids compared to the pure epoxy may be caused by the interactions between the polymer chains and SiO_2, and hydrogen bonding should be the main source of such interactions. Figure (4) has been observed that the area of the curve smaller than the area of curve figure (3) because for this to occur dehydration from the sample.

CONCLUSION

The tensile strength and impact strength high values of only ratio 4% because the good bonding between epoxy and TEOS, this is duo to formation of hydrogen bonds between epoxy and TEOS. The slightly improved thermal stability for the hybrids compared to the pure epoxy may be caused by the interactions between the polymer chains and SiO_2,

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Table 1: The Reagent volumes used in silica preparation

TEOS(ml)	H_2O(PH=1)(ml)	Ethonal (ml)
5	20	5

Table 2: Composition of epoxy –silica hybrid materials

Sample	S1	S2	S3	S4	S5
By weight TEOS %	0	4	6	8	10

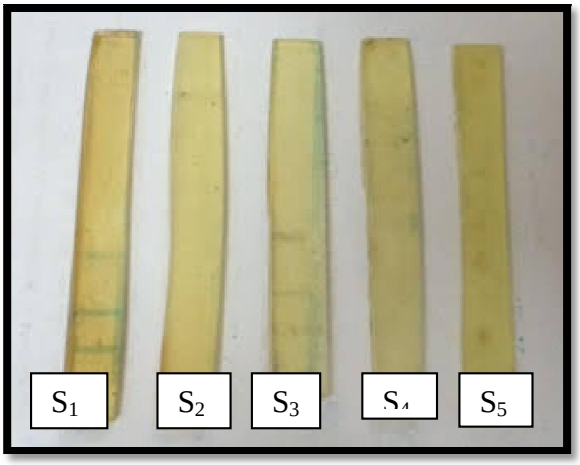

Figure 1. Sample of tensile

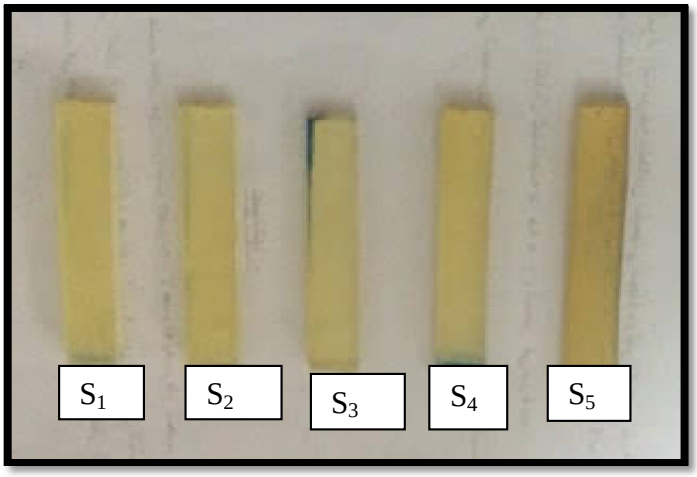

Figure 2. Sample of impact test

Table 3. Values of tensile strength

Sample	(TEOS- Epoxy)%	Max. forces(N)	Tensile strength(Mpa)
S_0	0%	575.45	11.3
S_1	4%	1819.4	25.5
S_2	6%	664	10.3
S_3	8%	443	7.8
S_4	10%	302	4.8

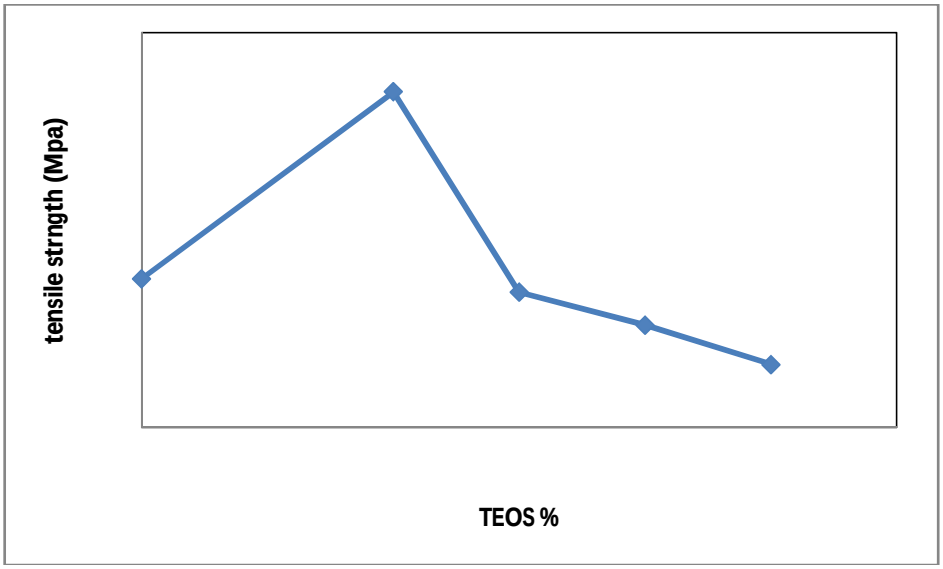

Figure 3.Tensile strength of hybrids materials

Table 4: Values of Impact Strength

Sample	(TEOS- Epoxy)%	Impact strength (KJ/m²)
S_0	0%	30.3
S_1	4%	38.85
S_2	6%	29.09
S_3	8%	18.79
S_4	10%	7.88

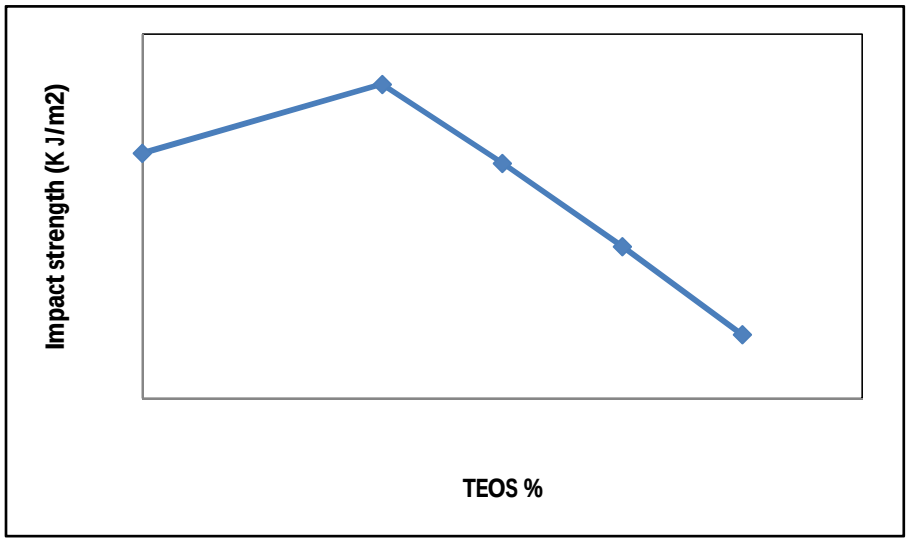

Figure 4. Impact strength of hybrids materials

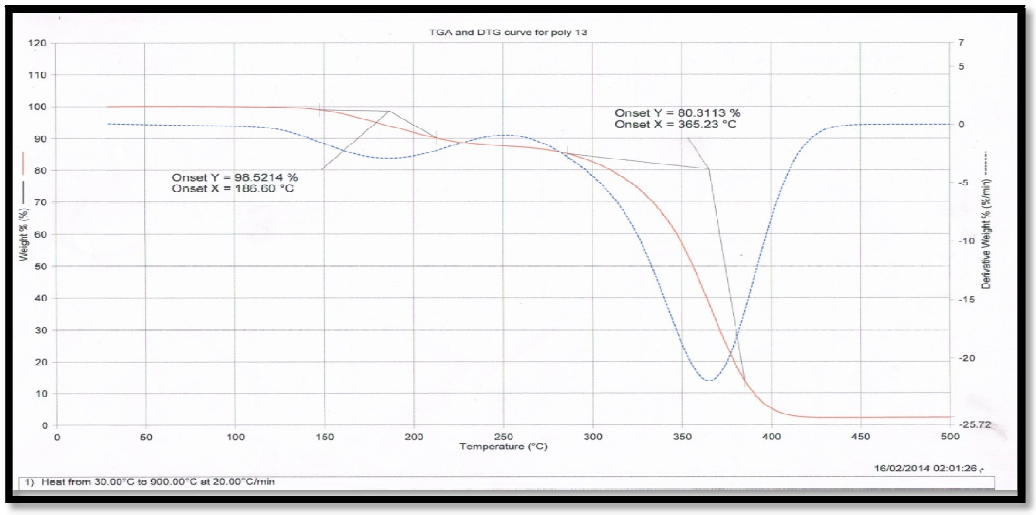

Figure 5.TGA & DTG of epoxy pure

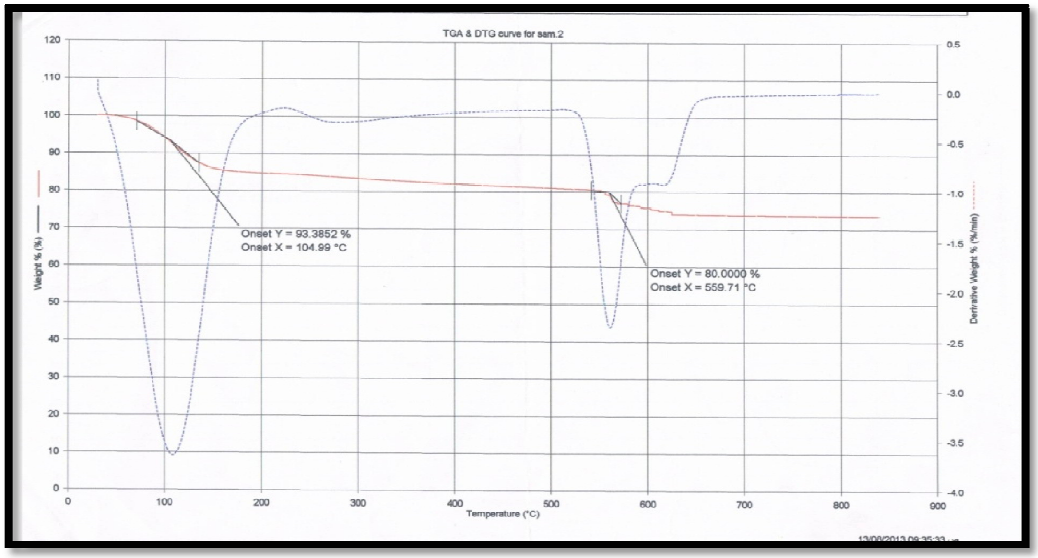

Figure 6.TGA & DTG of epoxy + 4% TEOS

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RESEARCH ARTICLE

An Assay of Kapalbhati and Anuloma-Viloma's Corollary on Vital Capacity and Concentration of High School Students

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ABSTRACT

Yoga is capable to bring natural changes in every single individual of this world and that would be a great revolution indeed. It offers us a conscious process to solve problems such as depression, unhappiness, restlessness, emotional conflicts, hyper activity etc. It helps to evoke the hidden potentialities of human beings in a systematic and scientific way so that human beings can rise intellectually and can prepare themselves to face the challenges of the modern technological era with its hectic speed and live happily without frustration.This study is designed to evaluate the effect of a 12 weeks (except Sunday) daily practice of Anuloma-viloma Pranayama and Kapalbhati on vital capacity (vc) and concentration in male students. Students aged 15-18 years have taken from 150 schools situated in Jaipur. Healthy students who were not suffering from any illness selected for this study. Participants were trained to perform Anuloma –viloma Pranayama and Kapalbhati and the study was scheduled for 12 weeks. The vital capacity and concentration were measured before and after the practice of Anuloma – viloma Pranayama and kapalbhati.The analysis of data reveals the following outcomes:In Pranayama group pre vital capacities mean shows that 3.979 and post vital capacity exemplifies that 4.024 and as far as the pre mean of concentration level is concerned that is 8.4 and post mean is 10.2. So that researcher finds the significant of both variables and gets a positive result. In Kapalbhati group pre vital capacity mean demonstrates that 3.952 and post vital capacity shows that 4.097. But on the other hand as far as pre mean and post mean of concentration is concerned that is 8.54 and 9.28.So that researcher has found the significant of both variables and got the positive result.

After a successful exercise of anuloma- viloma pranayama and kapalbhati on high school students it has been observed that they have significant effect and shown good performance on vital capacity and concentration level from pre to post test.

Keywords: Anuloma-Viloma Pranayama, Kapalbhati, Vital capacity (vc), Concentration.

INTRODUCTION

Rapid growth of industrialization and technologies has brought revolutionary changes by affecting every walk of human life and making them addicted of scientific tools. On one hand these changes have completely changed human life and given them a new insight to draw a caricature based on their opinions and perception but on the other hand it has also cultivated a feeling of competition to which each and every person wants to win without thinking the result. Feeling to win the life race and showing themselves more superior than others has sown the seeds of jealousy and frustration among people. Now in most of the people it can be seen that they are suffering from the diseases called stress, tension and some other similar to them which are unbeatable by using any medicine except . Today life is full of stress and strain, of tension and nervousness, of irritability, hurry and excitement. There are so many ways and means though which anyone can keep himself healthy and physically fit in this scientific era by implementing such practices in life like games and sports, aerobic exercises, weight training, dancing, gymnastic exercise, slimming exercises, yoga etc. In this competitive epoch nobody has spare time for other activities but from the busy schedule an individual should steal little bit time for these physical means in order to prepare himself for long term life race. Yoga is a science and its practice must be approached with the dedication of an alert, aware, conscious scientist. The aim of yoga is the development of integrated balance between body and mind and that which possible by attaining the highest state of consciousness through various methods and techniques. Yogic discipline aims to increase the internal awareness from the gross body to the level of pure consciousness[1].In short, it ultimately results in making the personality totally integrated and balanced. This concept is beautifully stated in Bhagavat Gita, "Samatvam Yoga Uchyate (evenness of mind is called YOGA)"[2, 3].

2^{1st} century people are the victim of stress and now a days yoga is widely used among the adult population to alleviate stress but lastly concentrated on children in spite of its vast effects (Sivapriya, 2010)[4].

Research Design

Current study is proposed to create awareness of health benefits by pranayama and to inculcate to do yoga in school students so that they can get a healthy life in future. The essence of the pranayama practice is slow and deep breathing which is economical as it reduces dead space ventilation. During the process of inhale and exhale it also refreshes air throughout the lungs; in contrast in shallow breathing it refreshes air only at the base of the lungs. Thus, a yoga practitioner, through pranayama, can at some stage control other physiological functions and finally control manifestations of prana even outside the body (Kinabalu, 2005)[5].Anulom pranayama is a type of pranayama. Anulom in sanskrit it means "alternate". It is one of the easiest types of pranayama, and can be practiced without expert guidance (Joshi, 1982). In Anulom pranayama one breathes through alternate nostrils of the nose. It is otherwise known as nadi shuddhi pranayama or nadi shodhona pranayama. It is practiced by sitting in any asana, such as sukhasana, vajrasana or padmasana. Alternate nostrils are kept close generally by right hand's thumb, ring finger, and little finger. The thumb is used for closing the right nostril and the ring and little fingers are used to close the left nostril. During the process mouth is not used for breathing. No sound should be produced while inhaling or exhaling. The cycle of practice is: The right nostril is closed with the thumb. Air is exhaled through the left nostril, and inhaled back through the same nostril. The left nostril is closed with the ring finger. Air is exhaled through the right nostril, and inhaled back through the same nostril (Khanam, 1996)[6]. Breath is a dynamic bridge between the body and mind. Pranayama is the art of prolongation and control of breath that helps in bringing conscious

awareness about breathe and reshape breathing habits and patterns[7]. Meditation is a yogic process to provide deep rest to the system by allowing the mind to calm down to its basal states. It is famous as a relaxation technique to be used for treating stress and stress- related illnesses[8].

Objectives of the Study

1. To measure the breathtaking effect of anuloma-viloma pranayam and kapalbhati on vital capacity of school going children residing in Jaipur.
2. To analyze the effect of anuloma-viloma pranayam and kapalbhati on concentration level which is now getting decrease in school going children due to some macro reasons which are avoided by us calling them micro.

MATERIALS AND MATHODS

150 male healthy school students of 15 to 18 years were selected for the study. Random sampling method was used for the selection. After getting the permission of parents or guardian students were selected by using random sampling. Before starting the study a health check up was conducted for all the participants before the start of the study.

Included Criteria

Healthy participants aged 15 to 18 years.

Excluded Criteria

Participants suffering from respiratory illness, respiratory disorders, respiratory medication, congenital heart disease, epileptic, recent injury or immobilization, physically challenged and spinal deformities were excluded from the study.
Experimental research method was applied by the researcher in the study.
There were three groups one acted as a kapalbhati group and second acted as a pranayama group and no act control group.
I. Group “A”-Kapalbhati
II. Group “B”- Anuloma-Viloma Pranayama
III. Group “C” – Control
The duration of experimental period was twelve weeks.
The pre tests were conducted before the practice.
The post tests were conducted after the practice.

Techniques of Anuloma Viloma Pranayama-

1. Sit in a comfortable balanced meditative pose.
2. Use the right hand thumb to close your right nostril.
3. Inhale from the left nostril.
4. Close your left nostril with your right hand’s index and middle fingers
5. Exhale from the right nostril.
6. Do the reverse: inhale with the right nostril.
7. Close your right nostril with your right hand thumb.
8. Exhale with the left nostril.

Above mentioned activities completes one round of anulom-vilom pranayama.

Techniques of Kapalbhati

1. Keep breathing gradually while sitting in Padmasana.
2. Inhale and start doing Kapalbhati as stated above. That means a strong Rechaka (exhalation), natural Pooraka (inhalation) and again strong Rechaka and natural pooraka .
3. Keep on doing this rotation swiftly in rhythmic manner.

Perform as many cycles as possible and then keep breathing gradually. All these processes are to be completed in one cycle of Kapalbhati.

Descriptive Statistics of Vital Capacity

Above drawn table indicates the value of descriptive statistics come out after the experiment done on groups **(**Pranayama Group, Kapalbhati Group) & Control Group for physiological variable of vital capacity which shows the mean and S.D. values of Pranayama Group, Kapalbhati Group and the Control Group are 4.024±0.099, 4.100±0.108, and 3.928±0.182 respectively and the total is 4.017±0.151.

Table no. 2 signifies the values which has come out after the test that shows difference among the subject there is significant difference in pre test values of vital capacity and 383.962 the given value has found which proves the base of Analysis of Co-Variance. Also, a significant difference is found between the post test values of the experimental and Control Group and as an outcome 58.539 the given value has found which is significant at 0.05 level.
Based on estimated marginal means

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).The mean difference is significant at the 0.05 level.
Table no. 3 indicates the values of post hoc test of the Groups selected for physiological variable of vital capacity shows a noteworthy difference between the post test values of Pranayama Group and the Kapalbhati Group and the value is .095* which is significant at 0.05 level and the post test value of Pranayama Group and the Control Group is .057* which is significant at 0.05 level. 152* is the value of kapalbhati Group and the Control Group which is significant at 0.05 level.

Table no.4 indicates the values of descriptive statistics of the experimental Groups (Pranayama Group, Kapalbhati Group) & Control Group for psychological variable of concentration which shows that the mean and S.D. values of Pranayama Group, Kapalbhati Group and the Control Group are 10.200±3.091, 9.280±2.726, and 6.520±1.854 respectively. Total the same is 8.667±3.029

Table no. 5 shows the values test of difference between the subject effects, which shows that there are a significant difference in pre test values of psychological variable of concentration for the three selected Groups, as the value has founded to be 437.596, which proves to be the base of Analysis of Co-Variance. Also, a significant difference is found between the post test values of the experimental and Control Group as the value has founded to be 79.638, which is significant at 0.05 level.Based on estimated marginal means

a. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).
***** The mean difference is significant at the 0.05 level.
Table no. 6 indicates the values of post hoc test for the selected Groups for psychological variable of concentration, which shows that a significant difference has founded between the post test values of Pranayama Group and the

Kapalbhati Group as the value has founded to be 1.032* which is significant at 0.05 level, the post test values of Pranayama Group and the Control Group as the value has founded to be 3.248* which is significant at 0.05 level, kapalbhati Group and the Control Group as the value has founded to be 2.216* which is significant at 0.05 level.

DISCUSSION

In the present study a significant effect was appeared on vital capacity and concentration after conducting 12 weeks regular practice of anuloma–viloma pranayama and kapalbhati. The output of other studies shows a eye-catching declination in vital capacity but in contrast of this the present study has shown positive effects on human physiology.9Kapalbhati practice is more effective as compare to anuloma-viloma pramayama practice in the matter of vital capacity and on the other hand anuloma-viloma practice is more beneficial rather than to kapalbhati practice in the case of concentration. This present study has given above stated outcome. In order to improve vital capacity and concentration in students both practices can be applied.10

Thus in a nutshell in this study it is proved beyond doubt that the regular practice of anuloma-viloma and kapalbhati for 12 weeks is advantageous to ameliorate the vital capacity in order to overcome other lung diseases .The results of this study and their explanations justifies the incorporation of yoga as a part of our lifestyle is necessary to be healthy and also will help to human beings preventing from age related respiratory diseases.11

CONCLUSION

This research paper has achieved the expected and fruitful result which has been assumed by the researcher a positive effect of anuloma- viloma pranayama and kapalbhati kriyas on vital capacity and concentration level. So, if such yogas start in school would be helpful to increase the health graph of school students.

Recommendation: The positive result has found in the present study so that these yogas can be implemented to all schools to improve the respiratory functions and to increase the concentration level of the students. A few minutes practice daily may help to achieve the expected focus level of mind which is required for better works and studies. Through daily practice one can maintain good physical and mental health for a long period.

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Table.1: Descriptive Statistics Of The Data Measured In The Post Testing Vital Capacity

Different Groups	Mean	Std. Deviation	N
Pranayama	4.024	0.099	50
Kapalbhati	4.100	0.108	50
Control	3.928	0.182	50
Total	4.017	0.151	150

Table.2:Ancova Table For The Post-Test Data Of Vital Capacity

Source	Sum of Squares	Df	Mean Square	F	Sig.(p-value)
Pre-Respiratory rate	1.940	1	1.940	383.962	.000
Treatment Group	.591	2	.296	58.539	.000
Error	.738	146	.005		
Corrected Total	3.413	149			

Table.3: Post Hoc Comparison For The Group Means In Post-Measurement Adjusted With The Initial Differences Respiratory Rate

(I) Different Groups	(J) Different Groups	MEAN DIFFERENCE (I-J)	SIG.a (p-value
Pranyama	Kapalbhati	-.095*	.000
	Control	.057*	.000
Kapalbhati	Pranayama	.095*	.000
	Control	.152*	.000
Control	Pranayama	-.057*	.000
	Kapalbhati	-.152*	.000

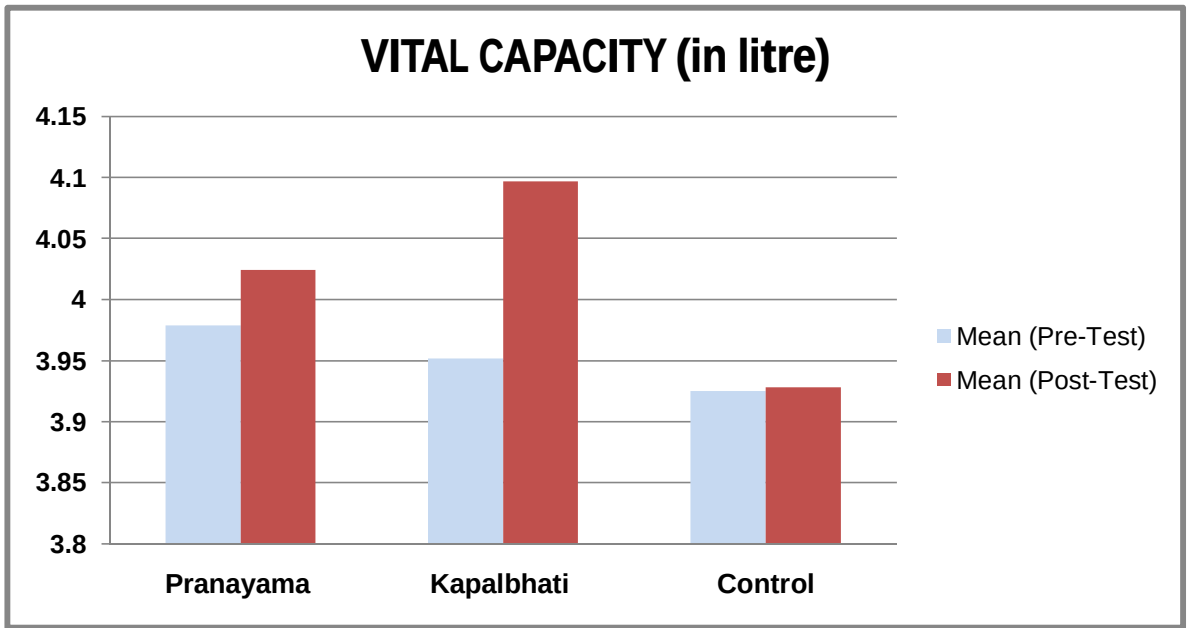

Figure- 1. Bar Diagram Showing the mean Value of Vital Capacity among Pranayama Group, Kapalbhati Group and Control Group

Table-4: Descriptive Statistics Of The Data Measured In The Post Testing Concentration

Different Groups	Mean	Std. Deviation	N
Pranayama	10.200	3.091	50
Kapalbhati	9.280	2.726	50
Control	6.520	1.854	50
Total	8.667	3.029	150

Table-5:Ancova Table For The Post-Test Data Of Concentration

Source	Sum of Squares	Df	Mean Square	F	Sig.(p-value)
Pre-Anxiety	750.247	1	750.247	437.596	.000
Treatment Group	273.076	2	136.538	79.638	.000
Error	250.313	146	1.714		
Corrected Total	1367.333	149			

Table-6:Post Hoc Comparison For The Group Means In Post-Measurement Adjusted With The Initial Differences Concentration

(I) Different Groups	(J) Different Groups	MEAN DIFFERENCE (I-J)	SIG. (p-value
Pranyama	Kapalbhati	1.032*	.000
	Control	3.248*	.000
Kapalbhati	Pranayama	-1.032*	.000
	Control	2.216*	.000
Control	Pranayama	-3.248*	.000
	Kapalbhati	-2.216*	.000

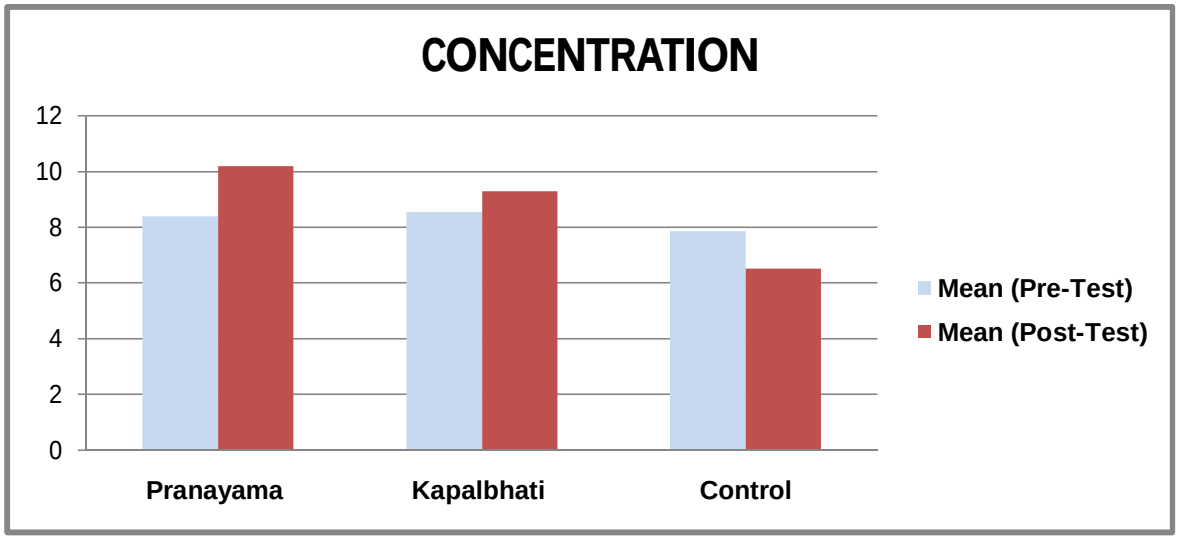

Figure- 2:Bar Diagram Showing the mean Value of Anxiety among Pranayama Group, Kapalbhati Group and Control Group

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RESEARCH ARTICLE

Growth Scenario of Major Agricultural Crops in Tamil Nadu

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ABSTRACT

Agriculture plays an important role in economic development, such as provision of food to the nation, enlarging exports, transfer of manpower, capital formation and securing markets for industrialization. With around 24 per cent contribution to GDP, agriculture still provides livelihood support to about two-thirds of country's population. Tamil Nadu has about six per cent of nation's population, occupies four per cent of the land area and has three per cent of the water resources of the nation. In the state, agriculture provides livelihood to about 65-70 per cent of the population. Hence, the state's economy swirls around agriculture and allied sectors. At this juncture, it is appropriate to analyse the performance of agriculture by analyzing the growth rate in area, production and productivity of crops over years. Obviously, it would pave way for re-orienting the programmes of agricultural development so as to achieve higher growth. Therefore, an attempt was made in the present study to estimate the growth rate of major crops by incorporating the secondary data on area, production and productivity of agricultural crops *viz.,* rice, maize, sorghum, total pulses, groundnut, cotton and sugarcane from the year 1970-71 to 2011-12. Over the period of 36 years, maize crop alone recorded positive growth rates with reference to area (5.10), production (5.38) as well as productivity (1.46) among all other major agricultural crops. Rice crop witnessed a negative growth rate in area during 1980-81 to 1989-1990, while total pulses exhibited a negative growth in area in all the period with an exception of 1980-81 to 1980-90. Cotton (-2.89) and groundnut (-5.61) exhibited a negative growth rate in area during 1970-71 to 1979-80. Considering the production, positive growth was observed over the period in rice, maize, cotton and groundnut crops. Regarding the productivity, the crops *viz.,* rice, maize and total pulses possessed positive growth rate in

all the period under consideration. Similar phenomenon was recorded in groundnut (-2.27), cotton (-0.35) and sugarcane (-3.59) where the productivity was negative during 2000-01 to 2011-12. It is concluded from this study that the declining trend in productivity in recent period in crops like groundnut, cotton and sugarcane is causing a great concern and this has to be reversed. Similarly, the declining trend in the production of sugarcane has to be arrested. Moreover, special attentions should be given to the districts which are showing negative trend in area, production and productivity. Hence, an appropriate strategy must be adopted for increasing the area, production and productivity of agricultural crops to achieve greater significance with the increasing population and accelerating economic growth in down trending districts of Tamil Nadu.

Keywords: Growth rates, Trend analysis, Area, Production, Productivity, Agricultural crops.

INTRODUCTION

Agriculture is the mainstay of the Indian economy. Agriculture and allied sectors, contribute nearly 24 per cent of Gross Domestic Product (GDP) of India. About 65-70 per cent of the population is dependent on agriculture for their livelihood (Saraswati et al., 2012). The agricultural development is a precondition not only to provide food and nutrition security for the growing population but also inevitable for overall economic development of the State (Pandit, 2012). It is essential, not only to achieve self reliance at State level but also to get household food security and to bring equity in distribution of income and wealth thereby reducing the poverty and no parity in living standard (Fasih et al., 2011). The government is keen to transform agriculture into a viable avocation in order to improve the standard of living of the farming community. Over the years, the government had invested significant amount of its resources in building a strong human and infrastructure base for a sustained development in agricultural sector in the State. Agricultural research, education and extension activities, along with supply of critical inputs such as water, fertilizers, seeds, implements and machinery, and pesticides was given adequate importance by the government so as to ensure steady growth in agriculture (Safiquallah, 2013).

Agriculture in Tamil Nadu – The Present Scenario

Tamil Nadu state has performed extremely well in irrigated agriculture when compared to rainfed condition, particularly in crops like rice, cholam, cumbu, maize, sugarcane, ragi and groundnut, which are the major crops of the state (Dharmasiri, 2010). Over the past decades, the agricultural production in districts of Tamil Nadu has faced increased yields in almost all crops, especially in cereals (Kalaivani and Saravanadurai, 2010). The State government has taken several efforts in order to increase the yield and production in major crops by implementing mechanization of production by wide utilization of farm machinery in agriculture at subsidized prices and granted loans investment in agricultural infrastructure, supplying inputs such as fertilizers, pesticides, seed and pricing policy for several main crops, in particular cereal crops (Muthumurugan et al., 2012). This makes Tamil Nadu as one among the leading state that records the huge agricultural productions in cereals every year. Moreover, the State has provided various farm related assistance to the farmers that have created hope and confidence among them. In the present analysis, the growth performance of area, production and productivity with special reference to selected major agricultural crops was examined in the context of Tamil Nadu state.

Objectives

The objectives of the study are as follows:

1. To study the average area, production and productivity of major agricultural crops in Tamil Nadu.

2. To analyze the growth rate in area, production and productivity of major agricultural crops over years in Tamil Nadu.
3. To examine the district-wise growth performance of major agricultural crops of Tamil Nadu state.
4. To give suggestions for improving the growth performance of major agricultural crops in the study region.

MATERIALS AND METHODS

In present investigation, Compound Growth Rate (CGR) of area, production and productivity for the selected major agricultural crops in the Tamil Nadu state were estimated for each period to study the growth in area, production and productivity of these crops. The Compound Growth Rates are found very convenient for any comparison of growth between two period and two crops. It seems more appreciable to analyse the movement of agricultural crops in terms of compound rather than linear growth rate (Dandekar, 1980, Shadmehri, 2008). Hence, the compound growth rates are computed for the selected major agricultural crops in Tamil Nadu state. The Compound Growth Rate (CGR), are usually estimated by fitting a semi-log trend equation of the form:

$\log Yt = \alpha + t\beta t + \varepsilon t$ ..... (1)
where,

Yt: Area, production and yield of selected major agricultural crops in years 't' respectively.
t : Year which takes value 1, 2.....n
α & β are the parameters to be estimated, and ε = random error term.

Equation (1) was estimated using Ordinary Least Squares (OLS) technique. The t-test was applied to test the significance of β. This equation is generally used on the consideration that change in agricultural output in a given year would depend upon the output in the preceding year (Chandrahekhar, 2004 and Shadmehri, 2008).
Compound Growth Rate was then estimated by using the following equation:
CGR = [(Antilog of β - 1)*100] ..... (2)

The study considers the yearly database for the major agricultural crops to examine the growth performance of area of cultivation, production and productivity in Tamil Nadu. The seven agricultural crops under examination included Rice, Maize, Cholam, Total Pulses, Groundnut, Cotton and Sugarcane for analyzing the growth rates of area, production and yield over the periods; instead of analyzing the total pulses, Red Gram, Black Gram and Green Gram were analyzed to predict the better growth performance in district wise. In the present study, the necessary data was collected from 1970 to 2012 was purely based on secondary sources and it was collected from various issues of Statistical Hand Books, Seasonal and Crop Reports of Tamil Nadu. Also, the data was gathered from the unpublished sources of Department of Economics and Statistics, Chennai.

RESULTS AND DISCUSSION

Average Area, Production and Productivity of Major Agricultural Crops

The total area, production and productivity of selected agricultural crops and their average value for the period of 2007- 08 to 2011-12 is presented in Table 1. The average area of rice for the period 2007-08 to 2011-12 was 18.75 lakh ha followed by groundnut (4.4 lakh ha) and sugarcane (3.2 lakh ha). Similarly, the average production of sugarcane was 340.70 lakh tonnes followed by rice (58.27 lakh tonnes) and maize (11.85 lakh tonnes) crops. High productivity was observed in maize (4635 kg/ha) among all other agricultural crops. It revealed that the trend of area under cultivation of rice was seems to be volatile over the study period. During 2007-08, land under rice cultivation was 17.89 lakh ha; further increased to 19.32 lakh ha in 2008-09; later decreased in 2009-10 and continuously increased

upto the period 2011-12. Likewise, the production and yield of rice had shown upward and downward trend in the same period. The period, i.e. 2007-08 to 2008-09, the area cultivated for cholam, red gram, black gram, green gram, groundnut, sugarcane were seems to have declining trend. While in case of maize and cotton, the crop data had shown a considerable positive trend among other crops over the study period. During the period 2008-09, the production of major crops like cholam, red gram, green gram, groundnut, cotton and sugarcane were showing declining trend, later it has increased in 2011-12 period. In case of maize and black gram it was decreasing in 2009-10 and 2008-09 respectively, and shown a increasing trend in 2011-12. Similarly, the productivity of agriculture crops had shown upswings and downswings over the period under study.

Growth Rates of Area, Production and Productivity of Major Agricultural Crops

The growth rates of area, production and productivity of major crops of Tamil Nadu state are furnished in Table 2. To have a better perception of the performance of area, production and productivity, the total period considered were classified into four periods viz., 1970-71 to 1979-80, 1980-81 to 1989-90, 1990-91 to 1999-2000 and 2000-01 to 2011-12. It is evident from the table 2 that, during the period from 1970-71 to 1979-80, the area under sorghum, total pulses, cotton and groundnut exhibited a negative growth rates. Keeping the other crops under question, the growth rate of area ranged from 0.17 per cent per annum in rice to 5.10 per cent per annum in maize. With regards to the production, all the chosen crops showed a positive growth rate. The growth rates in the productivity of the remaining chosen crops varied from 0.60 per cent (rice) to 11.5 per cent per annum (sorghum).

During the period from 1980-81 to 1989-90, the area under rice and sorghum witnessed a negative growth rate among the other chosen crops. The growth rate in area ranged from 2.33 per cent per annum in groundnut to 5.89 percent per annum in maize. As regards to the growth rate in production, the rate of growth was found to be maximum (7.87 per cent per annum) in cotton and minimum (4.14 per cent per annum) in groundnut. Sugarcane exhibited a negative growth rate in production and the same varied from -0.98 to -2.69 per cent per annum respectively. All the chosen crops with the exception of sugarcane exhibited a positive growth rate in productivity. The rate of growth of productivity was found be the highest in rice (7.52 per cent) followed by sorghum (5.86 per cent), cotton (3.10 per cent) and total pulses (2.90 per cent) in that order.

A different phenomenon was observed in the growth rates of area, production and productivity of the crops under question during the period from 1990-91 to 1999-2000. It could be seen that sorghum and total pulses exhibited a negative growth rate in area. With regard to the production, sorghum and total pulses exhibited negative growth rate. Considering the growth rates of productivity it was found to be highest in sugarcane (6.11 per cent per annum) and lowest in cotton (0.77 per cent per annum). Sorghum witnessed a negative growth rate in productivity. In maize and cotton, area contributed more for production while in sugarcane and groundnut productivity contributed much. During the period 2000-01 to 2011-12 sorghum, total pulses and sugarcane exhibited negative growth rate in area. Among the remaining crops under question, the growth rate in area ranged from 0.39 per cent per annum in rice to 11.93 per cent per annum in maize. The growth rate in production with the exception of sorghum and sugarcane, all other crops under question exhibited positive growth rate. The growth rate in production was found to be maximum in maize (28.44 per cent per annum) and minimum in total pulses (0.06 per cent per annum). Sugarcane, cotton and groundnut witnessed a negative growth rate in productivity whereas, the remaining crops showed a positive growth rate in productivity.

In sum, it could be inferred that over the period of 36 years (from 1970-71 to 2011-12) among the seven major crops for which the growth rates were estimated, the growth rates were found to be positive with reference to area, productivity as well as production in maize alone. Rice witnessed a negative growth rate during 1980-81 to 1989-1990. Total pulses exhibited a negative growth rate in area in all the periods under consideration with the exception of 1980-81 to 1980-90. Cotton and groundnut exhibited a negative growth rate in area during the period 1970-71 to 1979-80 only. Positive growth in production was observed in all the periods under consideration in rice, maize, cotton

and groundnut only. Similarly, positive growth rate in productivity was observed in all the periods under consideration in rice, maize and total pulses. Sugarcane, cotton and groundnut exhibited a negative growth rate in productivity during the period 2000-01 to 2011-12.

The declining trend in productivity in recent period particularly in sugarcane, groundnut and cotton is causing a great concern and this has to be reversed. Similarly, the declining trend of production in sugarcane has to be arrested. Hence, appropriate strategy must be adopted for increasing the area under sugarcane and productivity in sugarcane, cotton and groundnut.

District-wise Growth Performance of Major Agricultural Crops

District-wise compound growth rate of area, production and productivity of major agricultural crops during 2000-01 to 2011-12 (% per year) is presented in Table 3 to Table 5.

Rice

During 11th five year plan, the area under rice was on an average of 18.75 lakh ha with an production of 58.27 lakh tonnes and the productivity was 3105 kg/ha, covering almost all the districts of Tamil Nadu state. The growth rate of area was positive in 18 districts namely Cuddalore, Dindigul, Erode, Karur, Krishnagiri, Madurai, Nagapattinam, Pudukottai, Ramanthapuram, Sivagangai, Thanjavur, Thiruvannamalai, Thiruvarur, Thoothukudi, Tirunelveli, Trichy, Villupuram and Virudhunagar. In all the other 13 districts, the trend was negative. The growth rate of production was found to be positive in 21 districts and negative in other 10 districts viz., Coimbatore, Cuddalore, Dharmapuri, Kancheepuram, Kanyakumari, Namakkal, Perambalur, The Nilgiris, Tiruppur and Vellore. Similarly, the productivity trend was positive in 27 districts of Ariyalur, Coimbatore, Dharmapuri, Dindigul, Kancheepuram, Kanyakumari, Karur, Krishnagiri, Madurai, Nagapattinam, Namakkal, Perambalur, Ramanthapuram, Salem, Sivagangai, Thanjavur, The Nilgiris, Theni, Thiruvannamalai, Thiruvarur, Thoothukudi, Tirunelveli, Tiruppur, Tiruvallur, Trichy, Villupuram and Virudhunagar, while it was negative in all other four districts. This implied that the downward trend of growth rate in certain districts like Vellore must be arrested and stepped up in order to increase area, production and productivity under rice. In sum, the strategy must be to increase production through productivity increase in all the districts of Tamil Nadu by adopting modern technologies like System of Rice Intensification (SRI) and also by the distribution of quality seeds, farm machineries and other management practices.

Maize

Maize crop is cultivated in 19 districts during the 11th year plan period with an average area of 2,53,069 ha with a production of 1185814 tonnes and productivity of 4635 kg/ha. With reference to area, 16 districts of the State experienced positive growth, while three districts viz., Coimbatore, Tiruppur and Vellore have shown negative trend. Similarly, in production, all the 19 districts witnessed positive growth and none of the district had negative trend. The productivity witnessed uptrend in 18 districts and downtrend in Cuddalore district alone. The growth rates in area, production and productivity are quite perceptible in majority of the districts where maize is cultivated. Maize in one of the important crops introduced for crop diversification in Tamil Nadu State. Moreover, the growing poultry feed industry keeps demanding maize, as it is an important ingredient in feed mix. So, the maize crop improvement should be concentrated mainly on interventions like quality seed supply, soil health enhancement, integrated pest and disease management, irrigation management, farm mechanization, infrastructure, extension and special programmes like millet mission.

Cholam

Cholam is grown in around 22 districts in the State with an area of 2,44,408 ha, production of 2,36,547 tonnes and productivity of 984 kg/ha, during 11th five year plan. The growth rate of area was positive only in two districts namely Thoothukudi and Virudhunagar. Similarly, the production was positive in seven districts viz., Karur, Krishnagiri, Ramanthapuram, Theni, Thoothukudi, Tiruppur and Virudhunagar, whereas the productivity recorded positive in almost all the districts except Ariyalur, Madurai, Namakkal, Salem, Tiruvallur, Trichy and Vellore. Thus, the negative trend in majority of the districts is a common phenomenon. Due to changing purchasing power and food habits, the consumption of cholam has drastically come down in majority of small farmer / labour households. One of the important crops that replaced cholam is maize. This indicates the need for development strategy for cholam to give full thrust on productivity through increased concentration on major interventions as discussed in maize crop.

Red gram

Pulses are the major sources of cheap protein particularly for the vegetarians and poor. Therefore, there is a need to keep producing more and more pulses. Pulses are more sensitive to excessive moisture and the un-usual continuous rain and flooding also devastate the entire rice-fallow pulses once in 3 or 4 years thus reducing production drastically in the State. Therefore, the development strategy must focus not only on productivity increase, but also on the water management / flood management tactics.

Red gram is majorly grown in 4 districts of the Tamil Nadu state covering about 31,163 ha of area, 22,603 tonnes of production and 601 kg/ha of productivity during 11th five year plan. The growth rate of area was positive only in one district namely Krishnagiri. Similarly, the production was positive in three districts viz., Karur, Krishnagiri and Theni, whereas the productivity recorded positive in all the four districts viz., Karur, Krishnagiri, Theni and Vellore. Thus, there is a need to increase area, production and productivity of red gram to meet the growing demand through the development of strategic plans like adoption of Red gram Transplantation Technology and programmes like Pulses Mission, Expansion of Area under Rainfed Pulses etc.

Black gram

Black gram is cultivated invariably in almost 19 districts viz., Cuddalore, Dharmapuri, Erode, Kanyakumari, Karur, Nagapattinam, Pudukkottai, Ramanthapuram, Sivagangai, Thanjavur, Thiruvannamalai, Thiruvarur, Thoothukudi, Tirunelveli, Tiruppur, Tiruvallur, Trichy, Vellore and Villupuram of Tamil Nadu State with an area of 2,88,721 ha, production of 1,12,700 tonnes and productivity of 388 kg/ha during 11th five year plan. Majority of the districts experienced positive growth trends regarding area and production except Dharmapuri, Erode, Kanyakumari, Thiruvannamalai, Tiruvallur and Vellore, in addition to Tirunelveli in area which recorded negative growth rate. More than half of the districts in Tamil Nadu had positive productivity growth. However the productivity of black gram is low in Kanyakumari, Nagapattinam, Ramanthapuram and Thiruvarur districts. Therefore, the development strategy must focus not only on productivity increase, but also on the management tactics like distribution of integrated nutrient management kit etc.

Green gram

With an area of 1,54,232 ha, production of 53,605 tonnes and productivity of 343 kg/ha, green gram is grown majorly in 12 districts of the Tamil Nadu state. The growth rate of area was positive only in eight districts namely Nagapattinam, Thanjavur, Theni, Thiruvarur, Thoothukudi, Tirunelveli, Tiruppur and Virudhunagar. Similarly, the production was positive in all the above districts except Thanjavur, whereas the productivity recorded positive in half of the districts viz., Coimbatore, Theni, Thoothukudi, Tirunelveli, Tiruppur and Virudhunagar. The remaining districts recorded

negative growth trend in area, production and productivity, and there exists a necessity to increase growth rate of green gram to meet the growing needs of the population through special programmes like Accelerated Pulses Production programmes.

Groundnut

During 11th five year plan, the average area under groundnut was 4,41,837 ha with a production of 9,74,873 tonnes and 2238 kg/ha of productivity, covering almost 28 districts in Tamil Nadu State. Groundnut is yet another important food/oilseeds crop, and its area and production performance had shown negative growth in majority of the districts. A positive trend in growth of productivity in groundnut was observed in all the 28 districts except Thiruvannamalai. This indicates the need for strategy to be formulated for groundnut to give more thrust on area and increasing productivity in all districts by implementing groundnut mission, integrated production improvement programme for oilseeds etc.

Cotton

During 11th five year plan, the area under cotton was on an average of 1,14,903 ha with a production of 2,48,699 tonnes and the productivity was 364 kg/ha, covering almost 16 districts of Tamil Nadu state. Cotton is the raw material for the textile industry, which is the largest manufacturing industry in the country. Traditionally cotton is cultivated more in the districts of Salem, Coimbatore, Erode, Madurai, Virudhunagar, Theni, Tirunelveli, etc. However, recently the area and production of cotton has been dwindling to the alarming level especially in Ramanthapuram, Theni, Thiruvannamalai, Thiruvarur, Thoothukudi and Virudhunagar districts. The crop development strategy must aim at reversing the recent trend to that of the past, so as to keep increasing cotton production and feeding the cotton textile mills in the State. The pricing is an important factor that merit consideration in addition to assured market demand through contract farming. Promotion of precision farming along with drip irrigation, advocacy of integrated pest management practices may be followed to increase area, production and productivity of cotton crop.

Sugarcane

Sugarcane is an important industrial crop grown in more than 25 districts in Tamil Nadu state covering an area of 3,23,692 ha with a production of 347,70,940 tonnes and productivity of 107 tonnes/ha. It forms the raw material for the sugar industry, which is the second largest manufacturing industry in the country. The growth in area, production and productivity of sugarcane was quite convincing with positive trend in more than 12 districts. The growth trend must be maintained to meet the growing demand for sugar. Therefore, the development strategy must focus on increasing sugarcane productivity as well as area increase in the years to come, so as to keep increasing production in almost all the districts especially in Madurai and Thiruvarur districts of Tamil Nadu state. However, the negative trend in area, production and productivity need to be reversed through proper strategy planning including the adoption of Sustainable Sugarcane Initiative (SSI), precision farming and production of other by-products like ethanol production etc.

CONCLUSION

The present study examines the growth performance of area of cultivation, production and yield of selected major agricultural crops in Tamil Nadu state. The average area under rice was higher in Tamil Nadu followed by groundnut and sugarcane, for the period 2007-08 to 2011-12. Similarly, the average production was higher in sugarcane followed by rice and maize. Higher the average productivity was observed in maize among the other agricultural crops under consideration. Moreover, the trend of area under cultivation of rice was seems to be volatile

over the study period. Likewise, the production and productivity of rice had shown upward and downward trend in the same period. During the period 2007-08 to 2008-09, the area cultivated for cholam, red gram, black gram, green gram, groundnut, sugarcane were seems to have declining trend. While in case of maize and cotton, the average data had shown the considerable positive trend among other crops over the study period. Similarly, the productivity of agriculture crops had shown upswing and downswings over the period.

Over the period of 36 years, maize crop alone recorded positive growth rates with reference to area (5.10), production (5.38) as well as productivity (1.46) among all other major agricultural crops. Rice (1980-81 to 1989-1990), Cotton (1970-71 to 1979-80) and groundnut (1970-71 to 1979-80) crops witnessed a negative growth rate in area during the study period while, total pulses exhibited a positive growth in area in during 1980-81 to 1980-90. Considering the production, positive growth was observed over the period in rice, maize, cotton and groundnut crops. Whereas, the productivity possessed positive growth rate in crops under consideration like rice, maize and total pulses, over the period. During 2000-01 to 2011-12, the growth rate for productivity were negative in groundnut (-2.27), cotton (-0.35) and sugarcane (-3.59).

It is concluded from this study that the declining trend in productivity in recent period in crops like groundnut, cotton and sugarcane is causing a great concern and this has to be reversed. Similarly, the declining trend in the production of sugarcane has to be arrested. Moreover, special attentions should be given to the districts which are showing negative trend in area, production and productivity. Hence, an appropriate strategy must be adopted for increasing the area, production and productivity of agricultural crops to achieve greater significance with the increasing population and accelerating economic growth in down trending districts of Tamil Nadu.

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Table 1. Average Area, Production and Productivity of Major Agricultural Crops (2007-08 to 2011-12)

Crops	Category	2007-08	2008-09	2009-10	2010-11	2011-12	Average
Rice	Area	17.89	19.32	18.46	19.06	19.04	**18.75**
	Production	50.40	51.83	56.65	57.92	74.59	**58.28**
	Productivity	2.82	2.68	3.07	3.04	3.92	**3.11**
Maize	Area	2.23	2.87	2.44	2.30	2.81	**2.53**
	Production	8.10	12.58	11.38	10.28	16.95	**11.86**
	Productivity	3.63	4.39	4.66	4.46	6.04	**4.64**
Sorghum	Area	2.84	2.59	2.38	2.43	1.98	**2.44**
	Production	2.48	2.13	2.22	2.47	2.53	**2.37**
	Productivity	0.87	0.82	0.93	1.01	1.28	**0.98**
Red gram	Area	0.30	0.27	0.27	0.36	0.36	**0.31**
	Production	0.21	0.17	0.20	0.24	0.31	**0.23**
	Productivity	0.70	0.61	0.76	0.66	0.27	**0.60**
Black gram	Area	3.08	2.64	2.60	3.04	3.08	**2.89**
	Production	0.80	0.83	0.99	1.23	1.79	**1.13**
	Productivity	0.26	0.32	0.38	0.40	0.58	**0.39**
Green gram	Area	1.59	1.39	1.38	1.72	1.64	**1.54**
	Production	0.46	0.31	0.48	0.58	0.85	**0.54**
	Productivity	0.29	0.23	0.35	0.34	0.52	**0.34**
Groundnut	Area	5.35	4.90	4.13	3.86	3.86	**4.42**
	Production	10.48	9.75	8.96	8.96	10.61	**9.75**
	Productivity	1.96	1.99	2.17	2.32	2.75	**2.24**
Cotton	Area	0.99	1.15	1.04	1.21	1.36	**1.15**
	Production	2.01	1.88	2.25	2.48	3.82	**2.49**
	Productivity	0.34	0.28	0.37	0.35	0.48	**0.36**
Sugarcane	Area	3.54	3.09	2.93	3.16	3.46	**3.24**
	Production	380.71	327.99	297.58	342.52	389.75	**347.71**
	Productivity	0.11	0.10	0.11	0.11	0.11	**0.11**

Source: Season and Crop Report, 2007-08 to 2011-12.

Note: Area - Lakh hectares, Production - Lakh tonnes, Productivity - Tonnes/hectare.

Table 2. Growth Rates of Area, Production and Productivity of Major Crops in Tamil Nadu (per cent per annum)

Crop	1970-1971 to 1979-1980			1980-1981 to 1989-1990			1990-1991 to 1999-2000			2000-2001 to 2011-2012		
	A	P	Y	A	P	Y	A	P	Y	A	P	Y
Rice	0.17	0.77	0.60	-2.53	4.80	7.52	0.80	1.75	0.95	0.39	1.69	1.29
Maize	5.10	5.38	1.46	5.89	6.95	1.00	8.60	9.79	1.10	11.93	28.44	14.75
Cholam	-4.16	6.52	11.15	-0.99	4.81	5.86	-5.16	-6.34	-1.24	-4.52	-1.13	3.56
Total Pulses	-4.91	0.64	5.83	3.76	6.77	2.90	-4.02	-2.33	1.77	-0.43	0.06	0.50
Groundnut	-5.61	0.10	6.05	2.33	4.14	1.77	1.38	6.10	4.66	3.86	1.51	-2.27
Cotton	-2.89	2.79	5.85	4.62	7.87	3.10	3.72	4.52	0.77	1.98	1.63	-0.35
Sugarcane	2.46	8.11	5.51	2.82	-0.98	-3.70	1.42	7.62	6.11	-2.63	-6.12	-3.59

Source: Season and Crop Report, 2007-08 to 2011-12. *Note: A - Area, P - Production, Y - Yield.*

Table 3. District-wise Compound Growth Rate (CGR) of Area under Major Agricultural Crops during 2000-01 to 2011-12 (per cent per annum)

S.No.	Districts	Rice	Maize	Cholam	Red gram	Black gram	Green gram	Groundnut	Cotton	Sugarcane
1	Ariyalur	-0.88	6.65	-36.19				-4.57	30.80	-1.58
2	Coimbatore	-12.77	-14.22	-11.16			-9.75	-10.38		-18.64
3	Cuddalore	0.56	79.32			4.63		-10.10	18.67	-2.24
4	Dharmapuri	-3.57		-2.82		-6.56		-10.44	0.53	2.12
5	Dindigul	0.57	5.69	-5.41				-2.18		
6	Erode	3.77	10.22	-55.33		-9.14	-20.67	-7.51		3.03
7	Kancheepuram	-2.95						-5.09		-16.08
8	Kanyakumari	-4.58				-17.04		-15.36		
9	Karur	0.44		-4.49	-1.33	19.31		1.66		-0.12
10	Krishnagiri	2.07		-6.32	19.18			-0.53		
11	Madurai	1.51	33.36	-1.33				-7.54	-5.34	-3.15
12	Nagapattinam	0.58				3.93	8.64	0.85		1.48
13	Namakkal	-3.36	36.02	-1.14			-9.58	-7.84	-5.52	11.37
14	Perambalur	-13.19	23.11	-22.96				-27.67	15.41	-8.33
15	Pudukkottai	1.05	39.04	-7.80		1.10		-3.58		4.88

16	Ramanathapuram	0.55		-0.68		6.20		-8.31	-12.62	
17	Salem	-0.01	16.98	-1.64				-7.25	3.72	6.41
18	Sivagangai	0.24				4.08		-3.03		3.15
19	Thanjavur	0.29	21.92			10.29	0.53	4.25		-0.96
20	The Nilgiris	-15.13								
21	Theni	-0.95	6.97	-0.92	-6.44		16.32	-9.69	-13.35	-5.57
22	Thiruvannamalai	3.71		-14.27		-6.57		1.34	-9.46	7.21
23	Thiruvarur	1.31				4.38	9.52	12.14	-11.18	-6.98
24	Thoothukudi	4.39	16.77	1.53		13.50	14.65		-10.22	
25	Tirunelveli	2.44	4.93	-1.85		-0.74	0.32			3.88
26	Tiruppur	-1.37	-2.60	-22.29		17.95	7.48	-4.84	5.00	5.81
27	Tiruvallur	-0.90		-5.39		-11.49	-3.60	-6.75		1.24
28	Trichy	0.25	39.58	-3.99		9.66		-1.43	11.31	1.75
29	Vellore	-0.19	-5.30	-4.79	-4.93	-15.60		-2.36		-3.35
30	Villupuram	1.74	43.25			6.17		-4.34	4.39	7.35
31	Virudhunagar	0.70	8.45	2.49			1.60	-1.57	-9.32	0.14

Source: Statistical Handbook of Agriculture, 2000-01 to 2011-12.

Note: Shaded area implies that the specified crop is not a major crop in that particular district.

Table 4. District-wise Compound Growth Rate (CGR) of Production under Major Agricultural Crops during 2000-01 to 2011-12 (per cent per annum)

S.No.	Districts	Rice	Maize	Cholam	Red gram	Black gram	Green gram	Groundnut	Cotton	Sugarcane
1	Ariyalur	9.94	62.72	-44.93				41.23	82.12	4.25
2	Coimbatore	-9.89	9.03	-3.85			-5.47	-3.68		-16.69
3	Cuddalore	-3.17	77.25			6.49		-8.12	13.81	-4.81
4	Dharmapuri	-1.88		-0.58		-6.10		-7.19	8.77	2.93
5	Dindigul	3.00	21.30	-4.02				0.47		
6	Erode	3.60	25.64	-47.65		-6.26	-20.78	-6.68		1.52
7	Kancheepuram	-0.27						-0.98		-15.77
8	Kanyakumari	-2.55				-17.09		-12.44		
9	Karur	1.86		1.83	5.53	21.72		4.89		1.68
10	Krishnagiri	8.31		22.95	19.78			7.86		
11	Madurai	3.75	43.85	-1.73				-4.67	4.74	-3.54

12	Nagapattinam	2.82				1.03	3.31	9.48		-1.65
13	Namakkal	-2.67	57.90	-7.06			-12.12	-5.24	4.17	5.78
14	Perambalur	-6.87	37.72	-21.19				-35.08	19.97	-5.25
15	Pudukkottai	0.23	62.97	-6.35		2.70		-1.41		5.04
16	Ramanathapuram	6.47		4.68		3.22		-5.41	-9.40	
17	Salem	0.94	31.57	-2.55				-3.77	8.84	5.00
18	Sivagangai	2.53				5.70		-2.46		3.09
19	Thanjavur	0.47	33.79			11.77	-3.64	10.16		-0.83
20	The Nilgiris	-14.9								
21	Theni	1.17	23.46	11.32	0.83		15.66	-3.66	-3.14	-4.00
22	Thiruvannamalai	4.90		-11.92		-1.87		0.21	-0.60	9.87
23	Thiruvarur	3.77				3.97	6.07	23.00	-8.94	-7.30
24	Thoothukudi	5.47	42.96	13.20		18.24	19.06		1.80	
25	Tirunelveli	3.63	26.07	-1.03		2.84	3.77			1.75
26	Tiruppur	-1.25	10.59	30.24		48.06	36.73	3.90	87.07	11.55
27	Tiruvallur	0.07		-8.67		-9.99	-4.95	-4.67		1.68
28	Trichy	2.31	61.34	-6.02		14.47		-0.24	14.85	1.70
29	Vellore	-0.38	10.16	-7.17	-4.28	-13.76		1.29		-2.56
30	Villupuram	2.08	68.69			10.28		-0.30	3.94	6.67
31	Virudhunagar	3.02	22.12	8.48			3.02	3.45	-4.07	-0.84

Source: Statistical Handbook of Agriculture, 2000-01 to 2011-12.
Note: Shaded area implies that the specified crop is not a major crop in that particular district.

Table 5. District-wise Compound Growth Rate (CGR) of Production under Major Agricultural Crops during 2000-01 to 2011-12 (per cent per annum)

S.No.	Districts	Rice	Maize	Cholam	Red gram	Black gram	Green gram	Groundnut	Cotton	Sugarcane
1	Ariyalur	14.16	47.56	-5.13				45.75	31.55	-2.62
2	Coimbatore	1.61	23.92	7.16			5.54	6.67		0.60
3	Cuddalore	-3.30	-1.36			2.02		2.68	-5.45	-2.59
4	Dharmapuri	3.94		3.16		1.10		5.32	9.07	1.25
5	Dindigul	2.42	14.77	1.47				2.79		
6	Erode	-0.17	13.99	17.18		3.17	-0.13	0.91		-1.47

7	Kancheepuram	2.77						4.34		0.37
8	Kanyakumari	2.12				-0.07		3.44		
9	Karur	1.41		6.62	6.95	2.02		3.18		1.80
10	Krishnagiri	9.38		23.46	6.64			11.37		
11	Madurai	2.20	7.87	-0.41				3.10	10.64	-0.40
12	Nagapattinam	2.23				-2.79	-4.90	8.56		-3.09
13	Namakkal	0.72	16.08	-5.98			-2.81	2.82	10.25	-5.02
14	Perambalur	7.28	11.87	2.29				7.61	3.95	3.36
15	Pudukkottai	-0.81	17.21	1.57		1.59		2.25		0.15
16	Ramanathapuram	5.89		5.39		-2.81		3.17	3.68	
17	Salem	0.95	12.47	-0.92				3.75	4.94	-1.33
18	Sivagangai	2.28				1.55		0.59		-0.05
19	Thanjavur	0.98	9.77			1.23	-4.17	5.67		0.15
20	The Nilgiris	0.28								
21	Theni	2.15	15.42	12.35	7.78		0.57	6.67	11.78	1.66
22	Thiruvannamalai	1.15		2.74		5.03		-1.12	9.79	2.48
23	Thiruvarur	2.41				-0.40	-2.86	9.69	2.00	-0.16
24	Thoothukudi	1.04	22.43	11.5		4.18	3.85		13.39	
25	Tirunelveli	1.17	20.15	0.82		3.62	3.44			-2.00
26	Tiruppur	4.83	9.42	80.59		29.63	23.07	2.52	80.31	4.54
27	Tiruvallur	0.99		-0.64		1.72	-1.40	2.23		0.46
28	Trichy	2.06	15.58	-2.11		4.40		1.21	3.13	-0.04
29	Vellore	-0.18	16.33	-2.49	0.72	2.10		3.74		0.83
30	Villupuram	0.34	17.81			3.87		4.23	-0.37	-0.61
31	Virudhunagar	2.30	12.61	5.84			1.40	5.10	5.79	-0.98

Source: Statistical Handbook of Agriculture, 2000-01 to 2011-12.

Note: Shaded area implies that the specified crop is not a major crop in that particular district.

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RESEARCH ARTICLE

Development and Quality Evaluation of Chicken Nuggets in Corporated with *Maranta arundinacea* (Arrowroot)

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ABSTRACT

Nutritious diet with additional health promoting functions is driving the consumers' interest towards functional foods. Current study involves the development of chicken nugget with replacement of the binder, refined wheat flour with flour of arrowroot, a health promoting rhizome with anti-diarrhoeal, diuretic and antioxidant properties. Aerobically packed treatment nuggets, AR (replacement of refined wheat flour at 100 percent level) and MAR (replacement at 50 percent level) are compared with aerobically packed control nugget, M (refined wheat flour alone as binder) for physico-chemical, microbiological and sensory attributes on days 0, 30, 60, 90 and 120 of freezer (-18±2^{0}C) storage. No

spoilage was seen till 120 days in control as well as treatment nuggets. M had significantly lower cooking loss and dimension shrinkage compared to MAR and AR. MAR and AR had significantly lower moisture, protein, fat, dietary fibre, but higher ash, carbohydrate and energy levels compared to M. pH significantly increased for all nuggets on storage. TBARS numbers reduced significantly on storage for all, with lower values for AR and MAR and could be attributed to antioxidant effects of arrowroot. Tyrosine values were significantly higher for M and lower for AR and MAR on storage. AR and MAR showed significantly higher hardness, cohesiveness, springiness, colour values and lower adhesiveness values compared to M. Microbiological parameters did not show any significant difference between the treatments and sensory scores were higher for AR on all storage periods. Arrowroot could be successfully incorporated in chicken nuggets as a binder for improved taste and antioxidant effects.

Key words: Chicken nuggets, arrowroot, refined wheat flour, quality characteristics

INTRODUCTION

Researches in understanding the relationship between diets, importance of meat, specific food ingredients and health have led to new insights into the effect of food components on physiological function and health. This awareness has bought more health consciousness in consumers driving them to a new trend towards healthy and nutritious diet with additional health promoting functions, such as functional foods. Functional food is defined as "A food that beneficially affects one or more target functions in the body beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well-being and /or reduction of risk of disease" (Yadav, 2013).Meat is an essential and nutritious food which can fulfil most of the physiological and functional requirements of body mechanisms. Among all the meats, chicken is preferred by majority of the people in our country due to lower cost, greater availability and the fact that chicken meat does not have any religious taboos. Broiler industry is one of the fastest growing segments of the agricultural sector with an increased rate of production of 8 to 10 percent per annum. India has exported 5,56,698.80 MT of poultry products to the world for the worth of Rs. 651.21 crores during the year 2014-15 (APEDA, 2016). Chicken meat has high protein content, low fat content coupled with a balanced n-6 to n-3 polyunsaturated fatty acids ratio and low cholesterol level. These with their suitability for processing enable the meat producers to launch highly attractive, convenient, easy to use products with chicken meat.

Chicken nugget is a popular meat product which can be used as a snack item. It is a comminuted product prepared from minced chicken, beef or other meat with salt, spices, condiments and usually refined wheat flour as binder, breaded or battered and then usually deep-fried. Not only in nuggets but also in most of the processed meat products refined wheat flour is commonly used as a binder. Arrowroot (*Marantaarundinacea*) is a herbaceous rhizomatous plant cultivated widely in tropical countries for its richness of starch in root. In India, Arrowroot is cultivated widely in south western regions. It is used as a staple food, against rice by many of the tribes. It is commonly used for urinary infections, to ease digestion difficulties, has immuno-stimulatory and antioxidant properties. Because of these health benefits it is incorporated as a source of many functional ingredients in soup, noodle, biscuits, cakes, etc. Hence in the current study chicken nuggets incorporated with arrowroot flour at different levels have been developed so as to provide better nutrition, novelty, health benefits and good sensory appeal. Also, quality characteristics of the developed products are compared with control nuggets containing refined wheat flour as binder during freezer (-18 $\pm 2^0$C) storage for 120 days.

MATERIALS AND METHODS

Broiler chicken of the same age group was procured from the local markets in Vythiri, Wayanad district and was brought to the Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Pookode. The birds were provided *adlibitum*water and proper rest. They were slaughtered, dressed under hygienic conditions and the carcasses were washed and chilled overnight (4±1^{0}C). On the next day chilled carcasses were deboned and meat was harvested for the preparation of chicken nuggets.

Preparation of chicken nuggets

Control and treatment chicken nuggets were prepared using ingredients as shown in Table 1. Meat was minced once through 6 mm diameter grinder plate in a meat mincer (Sirman, Italy). Minced meat was chopped with salt, ice, refined sunflower oil, spices and condiments for 8 min in a bowl chopper (Talsa -TC12E, Spain). The batter was collected after chopping and separated into three equal batches and each batch was mixed with binders, at the level of 20 percent. In control nugget (M), refined wheat flour alone was added, in treatment nugget, AR, refined wheat flour was replaced with arrowroot flour at 100 percent level and in treatment nugget, MAR, refined wheat flour was replaced with arrowroot flour at 50 percent level. The batters were separately filled in rectangular moulds and steam cooked for 45 minutes. The cooked meat blocks, after cooling were cut into nuggets of size, 3.5 × 3.5× 1cm and subjected to aerobic packaging in high density polyethylene (HDPE) pouches and sealed using a continuous sealer (Sevanasepack – CS3H, Kochi). The packed nuggets were stored under freezer conditions (-18±2^{0}C) for further studies. Analyses was conducted on days 0, 30, 60, 90 and 120 or until spoilage, whichever was earlier. The spoilage of samples were assessed by physical examination like odour, colour and slime formation on the samples. The samples were analysed for cooking loss (Boccard *et al*., 1981), dimension shrinkage and physico-chemical characteristics like pH (AOAC, 1990), Thio Barbituric Acid Reacting Substances (TBARS) number (Witte *et al*., 1970), tyrosine value (Pearson, 1968), Hunter L, a, b colour values, instrumental texture profile analysis (Bourne, 1978), total phenolics level (Escarpa and Gonzalez, 2001) and DPPH (1, 1-diphenyl-2-picryl hydrazyl) assay (Singh *et al*, 2002). The proximate composition, dietary fibre and energy value of the nuggets were assessed on the day of preparation as per the procedure of AOAC (1990). Microbiological parameters like aerobic plate count (APC) (Morton, 2001), psychrotrophic count (Cousin *et al.* 2001) andyeast and mould count (Beuchat and Cousin, 2001) were also evaluated. Sensory attributes of the nuggets were also assessed by semi-trained panellists(Badr, 2004).The data collected will be analysed statistically according to the procedures of Snedecor and Cochran, (1994) using SPSS version 21.

RESULTS AND DISCUSSION

Cooking loss and dimension shrinkage

There was significant (p□0.001) difference between treatments with regard to cooking loss with the highest loss for AR (2.1250 ± 0.031%) and MAR (1.4900 ± 0.025%) nuggets incorporated with arrowroot and the lowest loss for the control, M nuggets (1.67±0.022 %). In contrast, Verma *et al*. (2012) noticed that salt replacement and chick pea hull flour addition in low fat chicken nuggets resulted in significant decrease (p□0.001) in the cooking yield of treatment products. Santhi and Kalaikannan (2014) reported that nuggets with reduced fat levels (25%) and added oat flour (10 – 20%) had significantly lower values of cooking loss compared to the control. Dimension shrinkage was significantly ($p<0.001$) lower for M when compared to MAR and AR.

PH

pH showed similar values for control and treatment nuggets and the values increased significantly ($p<0.001$) on storage for all.Naveena*et al*. (2005) also found an increase in pH during 21 day of storage of control chicken patties and treatment chicken patties with added finger millet.

TBARS numbers

On storage, TBARS numbers, which is an indicator of secondary lipid peroxidation, decreased significantly ($p<0.001$) in control as well as treatment nuggets. However, the value was significantly ($p<0.001$) higher for M than AR and is shown in figure 1. The decrease in TBARS numbers on storage might be due to the antioxidant activities of the binders or due to reaction of malondialdehyde with proteins (Melton, 1983). Mishra *et al.* (2006) observed significantly lower TBARS values during refrigeration storage of chicken nuggets fortified with extract of garcinia and concluded that the lowering of the values is due to the antioxidant activity of garcinia.

Tyrosine value

On storage, tyrosine values decreased in AR and MAR but increased in M. The insignificant variation of tyrosine value might be due to reduced protein degradation during frozen storage. Ahamed*et al*. (2007) analyzed enrobed frozen buffalo meat cutlets and found no significant difference in tyrosine value during storage and is shown in figure 2. Contrary to this result, Muthulakshmi (2007) reported increase in tyrosine value of buffalo meat sausages stored for 60 days under frozen conditions.

Hunter L, a, b values

Hunter L, a, b values reflects the lightness, redness and yellowness values of the product. The treatment AR and MAR had higher L and b values than M. The 'a" values significantly ($p<0.001$) increased on day 120 when compared to day 0 for M only, whereas the values were similar for all other treatments. This might be due to the antioxidant effect of the binder flours which might have stabilized the 'a' values. Mirshekar*et al*. (2009) studied the effect of rosemary, echinacea, green tea extract and ascorbic acid on broiler meat qualities and reported that the extracts exhibited greater antioxidant properties compared to ascorbic acid and were most effective antioxidants in stabilizing the a* value.

Texture Profile Analysis

Instrumental texture profile analysis revealed significantly higher values for AR and MARwith regard tohardness, cohesiveness and springiness values compared to M, on storage. The higher values might be because of incorporation of arrowroot as binder into the nuggets. M had higher adhesiveness compared to MAR and AR and might be due to the naturally sticky nature of refined wheat flour. The hardness values increased in all nuggetson storage.Kumar *et al*. (2013) reported increased firmness, chewiness and elasticity values in chicken nuggets formulated with soybean skin flour (3 to 5%) stored under refrigeration conditions.

Total phenolic levels of the nuggets, a measurement of antioxidants, were assessed and expressed as milligrams tannic acid equivalents per gram of the sample. There was significant (p□0.001) difference between total phenolics level of different nuggets with M showing the highest values (7.77 ±0.102 mg tannic acid equivalents per g) AR and MAR having lower values4.05 ± 0.05and 3.73 ± 0.048. However, the free radical scavenging ability of arrowroot in DPPH (1, 1-diphenyl-2-picryl hydrazyl) assay was observed to be significantly higher (95± 0.58%) thanthat of refined wheat flour (84.44± 0.71%).This might be due to the presence of antioxidants other than phenolics in arrowroot which might have contributed to its free radical scavenging ability.

Proximate composition

Moisture percentages did not change between treatments or storage periods. However, significantly higher levels of ash, carbohydrate and energy values and lower levels of fat were observed in AR and MAR when compared to M as shown in figure 3. Treatment nuggets, AR and MAR had significantly ($p<0.001$) higher protein level than M.Dietary fibre level of AR was significantly ($p<0.001$) lower (1.77 %) when compared to those of M (2.68 %) and MAR (2.39 %).

Microbiological parameters

Aerobic plate count and yeast and mould count were assessed on all storage periods and the values are presented in figure 4 and 5 respectively. There was significant difference in aerobic plate counts (APC) between treatments on all storage days except on days 60 and 120. All the treatments showed significant decrease in APC (log cfu/g of sample) from day 0 to day 120. The counts were significantly low for AR compared to MAR and M. Similarly Ahamed*et al.* (2006) observed that there was no significant increase in the mesophilic counts in freezer stored enrobed buffalo meat cutlets up to 45th day of storage and thereafter the counts decreased. No psychrotrophic growth was observed in any of the treatment on any storage period. Yeast and mould counts representthe mycotic growth. There was significant ($p<0.001$) difference between treatments on all storage periods with regard to yeast and mould count, with the lowest count for AR compared to M and MAR on all storage periods. On storage the counts remained similar in control and treatment nuggets.

Sensory attributes

Sensory evaluation of sausages was conducted by using nine point Hedonic scale with the help of semi-trained panellists from the Department of LPT, CVAS, Pookode, and the scores obtained were statistically analyzed. Scores for all sensory attributes like colour, flavour, texture, juiciness and overall acceptability were significantly higher for AR followed by MAR and least scores were for M. The scores were in the range of 'acceptable to more acceptable' for all the treatments. No significant difference was observed in sensory attributes of any treatment on storage upto day 120 and no spoilage was seen till 120th day of freezer storage (18 ± 2^0).

CONCLUSION

Arrowroot flour can be successfully incorporated into chicken nuggets replacing refined wheat flour partially or completely to provide novelty and good sensory appeal, along with higher mineral, carbohydrate and energy values and enhanced antioxidant activity.

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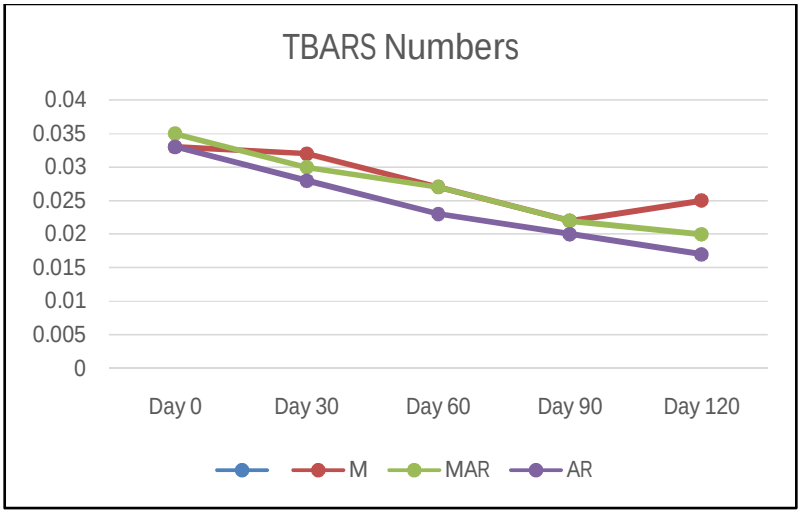
Table1. Formulation for the Preparation of Control and Treatment Nuggets

Ingredients	Control(M) (grams)	AR(grams)	MAR(grams)
Chicken	1000.0	1000.0	1000.0
Binder	200.0 (Refined wheat flour)	200.0 (Arrowroot powder)	200.0 (100g Arrowroot powder + 100 g Refined wheat flour)
Refined oil	100.0	100.0	100.0
Small onion	15.0	15.0	15.0
Ginger	15.0	15.0	15.0
Garlic	10.0	10.0	10.0
Mace	4.0	4.0	4.0
Salt	19.0	19.0	19.0
Pepper	14.0	14.0	14.0
Red chilli powder	5.0	5.0	5.0
Ice water	60.0	60.0	60.0

Table 2. Dimension Shrinkage and Cooking Loss of Nuggets

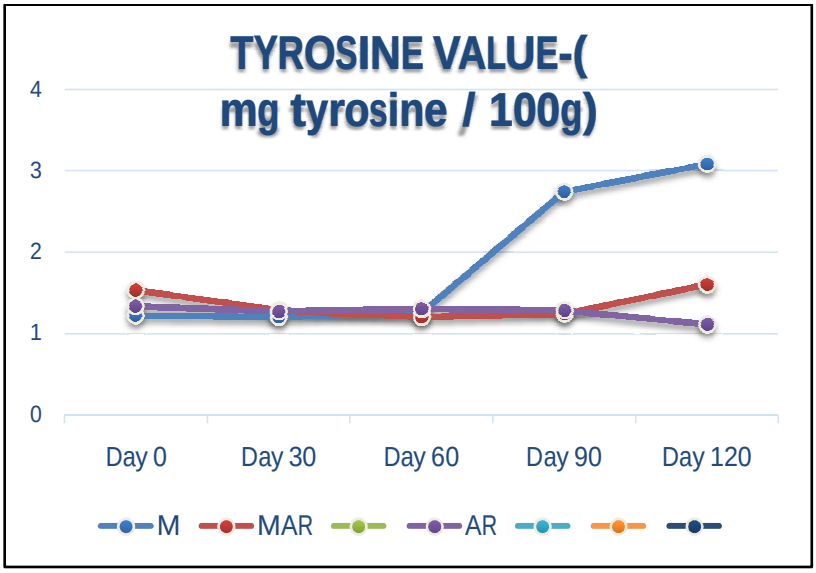
Treatment	Dimension shrinkage	Cooking loss
M	9.3433 ± 0.035^{c}	1.1667 ± 0.022^{c}
MAR	10.5767 ± 0.012^{b}	1.4900 ± 0.025^{b}
AR	14.1883 ± 0.030^{a}	2.1250 ± 0.031^{a}
F-value	12293.642^{**}	640.422^{**}
p-value	<0.001	<0.001

**- significant at 1% level, Means with same letters as superscripts have no significant difference between treatments



X- axis- storage days Y-axis- OD values

Figure 1. Tbars Numbers Of Nuggets On Storage



X- Axis- Storage Days Y-Axis- Mg Tyrosine.

Figure 2. Tyrosine Values of Nuggets On Storage

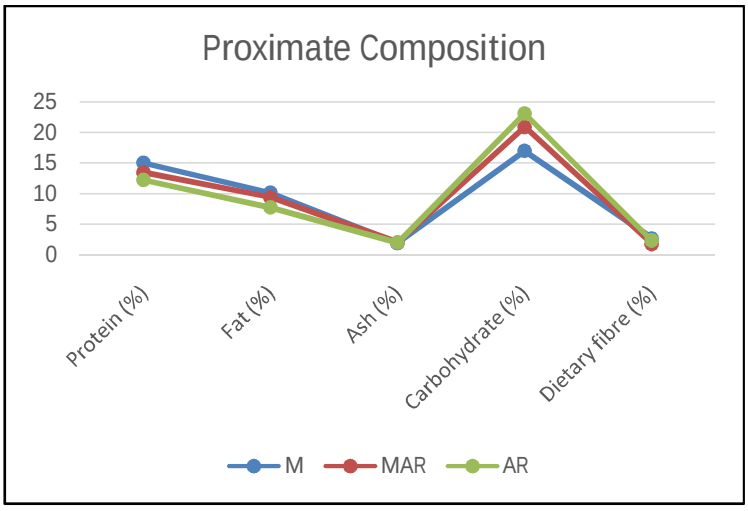
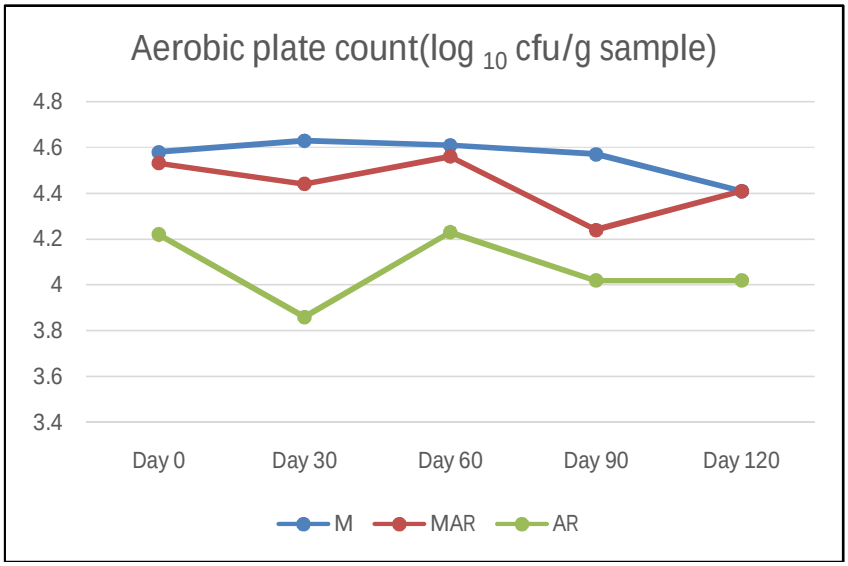


Figure 3. Proximate Composition Of Nuggets

X-axis- proximate principles. Y- axis - % composition



X- axis – Days of storage, Y- axis - log 10 cfu/g sample

Figure 4. Aerobic Plate Counts(Log 10 Cfu/G Sample) Of Nuggets On Different Storage Days

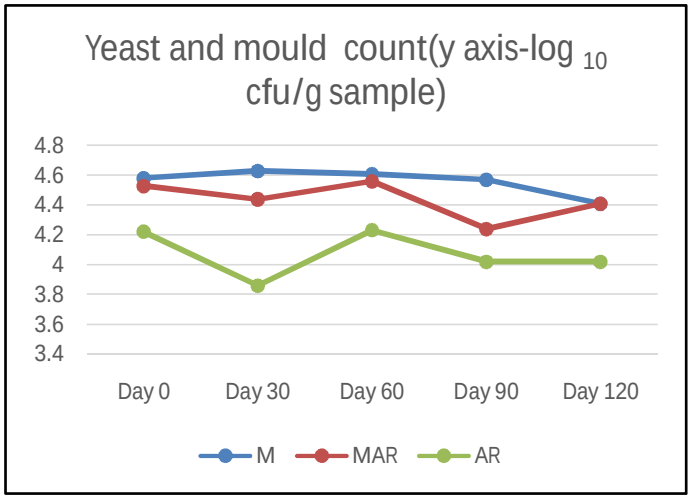


Figure 5. Yeast And Mould Counts (Log 10 Cfu/G Sample) Of Nuggets On Different Storage Days

Table 3. Hardness Values (N/Cm²) of Nuggets on Different Storage Days.

Treatment	Day 0	Day 30	Day 60	Day 90	Day 120	F-value	p-value
M	217.69 ± 7.05^{cB}	248.12 ± 3.488^{cA}	248.59 ± 5.882^{cB}	230.22 ± 8.907^{cA}	237.23 ± 5.324^{cA}	3.840*	0.018
MAR	303.15 ± 1.298^{bD}	305.99 ± 6.317^{bDCB}	316.92 ± 0.583^{bB}	335.74 ± 2.495^{bA}	313.06 ± 0.246^{bC}	18.373**	<0.001
AR	351.89 ± 1.093^{aB}	359.27 ± 0.873^{aA}	360.51 ± 1.664^{aA}	362.29 ± 1.366^{aA}	362.28 ± 0.874^{aA}	10.644**	<0.001
F-value	378.418**	294.405**	466.407**	229.005**	344.382**		
p-value	<0.001	<0.001	<0.001	<0.001	<0.001		

**- significant at 1% level,*- significant at 5% level, ns- not significant. Means with same small letters as superscripts have no significant difference between treatments and with same capital letters as superscripts have no difference between storage periods. ns- Non significant.

Table 4. Cohesiveness Values of Nuggets on Different Storage Days.

Treatment	Day 0	Day 30	Day 60	Day 90	Day 120	F-value	p value
M	0.244 ± 0.005^{e}	0.237 ± 0.003^{e}	0.249 ± 0.001^{d}	0.253 ± 0.013^{d}	0.262 ± 0.002^{b}	2.294^{ns}	0.095
MAR	0.55 ± 0.006^{aB}	0.626 ± 0.007^{aA}	0.56 ± 0.002^{aB}	0.473 ± 0.008^{aC}	0.436 ± 0.003^{bD}	158.061**	<0.001
AR	0.357 ± 0.002^{cA}	0.35 ± 0.001^{cC}	0.341 ± 0.004^{cCB}	0.361 ± 0^{cA}	0.354 ± 0.002^{cBA}	7.419*	0.001
F-value	154.491**	532.647**	247.404**	42.593**	34.513**		
p-value	<0.001	<0.001	<0.001	<0.001	<0.001		

Table 5. Springiness Values of Nuggets on Different Storage Days.

Treatment	Day 0	Day 30	Day 60	Day 90	Day 120	F- value	p value
M	4.34 ± 0.046^{bA}	3.54 ± 0.038^{bCB}	3.48 ± 0.053^{bC}	3.64 ± 0.033^{abB}	3.58 ± 0.031^{BC}	78.852**	<0.001
MAR	4.34 ± 0.009^{bA}	3.43 ± 0.056^{cCD}	3.36 ± 0.034^{D}	3.53 ± 0.016^{cC}	3.64 ± 0.032^{B}	136.779**	<0.001
AR	4.23 ± 0.039^{cA}	3.4 ± 0.021^{dC}	3.54 ± 0.06^{BC}	3.58 ± 0.037^{bcB}	3.54 ± 0.06^{BC}	60.285**	<0.001
F-value	5.222**	7.255**	1.753ns	3.711*	1.876ns		
p-value	<0.001	<0.001	1.38	0.006	0.113		

**- significant at 1% level,*- significant at 5% level, ns- not significant. Means with same small letters as superscripts have no significant difference between treatments and with same capital letters as superscripts have no difference between storage periods.ns- Non significant.

Table 6. Adhesiveness Values of Nuggets on Different Storage Days.

Treatment	Day 0	Day 30	Day 60	Day 90	Day 120	F-value	p value
M	-0.081 ± 0.001^{fAC}	-0.072 ± 0.001^{dC}	-0.006 ± 0.034^{ABC}	-0.08 ± 0.001^{eA}	-0.078 ± 0^{cB}	4.362**	0.011
MAR	-0.03 ± 0^{bB}	-0.033 ± 0.001^{bAC}	-0.037 ± 0.002^{A}	-0.029 ± 0^{cbB}	-0.028 ± 0.002^{bBC}	7.824**	0.001
AR	-0.033 ± 0.001^{cABC}	-0.026 ± 0.002^{aD}	-0.034 ± 0^{B}	-0.032 ± 0^{cC}	-0.039 ± 0.002^{cdA}	14.993**	<0.001
F-value	665.967**	265.973**	0.788ns	286.301**	143.016**		
p-value	<0.001	<0.001	0.585	<0.001	<0.001		

Table 7. Hunter L Values of Nuggets on Different Storage Days.

Treatment	Day 0	Day 30	Day 60	Day 90	Day 120	F-value	p-value
M	48.32 ± 0.565^{cA}	48.13 ± 0.388^{cA}	47.91 ± 0.466^{cAB}	47.05 ± 0.263^{cB}	47.16 ± 0.244^{cB}	5.246**	0.005
MAR	51.91 ± 0.667^{b}	51.63 ± 0.653^{b}	51.07 ± 0.496^{b}	50.56 ± 0.165^{b}	50.76 ± 0.283^{b}	1.958^{ns}	0.140
AR	53.95 ± 0.446^{a}	54.23 ± 0.299^{a}	54.51 ± 0.233^{a}	54.19 ± 0.38^{a}	54.75 ± 0.255^{a}	1.363^{ns}	0.282
F-value	125.716**	170.895**	313.465**	286.790**	394.051**		
p-value	<0.001	<0.001	<0.001	<0.001	<0.001		

**- significant at 1% level,*- significant at 5% level, ns- not significant. Means with same small letters as superscripts have no significant difference between treatments and with same capital letters as superscripts have no difference between storage periods.

ns- Non significant.

Table 8. Hunter 'A' Values of Nuggets on Different Storage Days.

Treatment	Day 0	Day 30	Day 60	Day 90	Day 120	F-value	p value
M	7.49 ± 0.143^{Bb}	7.6 ± 0.104^{bB}	7.74 ± 0.116^{bB}	8.12 ± 0.027^{bA}	8.3 ± 0.069^{bA}	15.235**	<0.001
MAR	8.13 ± 0.068^{aB}	8.15 ± 0.047^{aB}	8.32 ± 0.078^{aA}	8.33 ± 0.097^{aA}	8.06 ± 0.038^{aB}	10.148**	<0.001
AR	7.33 ± 0.058^{b}	7.36 ± 0.069^{d}	7.34 ± 0.054^{c}	7.37 ± 0.05^{c}	7.28 ± 0.071^{c}	0.513^{ns}	0.727
F-value	72.897**	127.178**	117.232**	121.410**	102.794**		
p-value	<0.001	<0.001	<0.001	<0.001	<0.001		

Table 9. Hunter 'B' Values of Nuggets on Different Storage Days.

Treatment	Day 0	Day 30	Day 60	Day 90	Day 120	F-value	p value
M	16.79 ± 0.058^{b}	16.61 ± 0.093^{b}	16.72 ± 0.048^{b}	16.69 ± 0.076^{b}	16.82 ± 0.072^{b}	1.365^{NS}	0.281
MAR	17.68 ± 0.053^{a}	17.63 ± 0.069^{a}	17.67 ± 0.072^{a}	17.6 ± 0.046^{a}	17.58 ± 0.059^{a}	0.927 ^{NS}	0.468
AR	17.71 ± 0.093^{a}	17.63 ± 0.075^{a}	17.77 ± 0.053^{a}	17.66 ± 0.075^{a}	17.76 ± 0.051^{a}	0.940 ^{NS}	0.461
F-value	231.240**	263.223**	325.908**	268.948**	281.661**		
p-value	<0.001	<0.001	<0.001	<0.001	<0.001		

**- significant at 1% level,*- significant at 5% level, ns- not significant. Means with same small letters as superscripts have no significant difference between treatments and with same capital letters as superscripts have no difference between storage periods.

ns- Non significant.

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SPECIAL ARTICLE

The Asiatic Cheetah through the Ages

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ABSTRACT

The Asiatic Cheetah is detected in the desert regions of Iran, which encompasses portions of the Kerman, Khorasan, Semnan, Yazd, Tehran, and Markazi provinces. The cheetah also seems to survive in the broad and dry Baluchistan province where sufficient prey is available. The strategy to confront the cheetah's extinction has even approached the realm of sport. Conservation attempts were given a representative hike when Iran's national football team wore photographs of the cheetah on their outfit at the 2014 World Cup in Brazil. Furthermore, Iran named August 31st as "Iran's National Cheetah Day" since 2006. Ancient references further provide a few slightly inconclusive and distorted clues to the use of trained big cats for hunting purposes. Allsen refers to tame leopards and recites that an Indian king "has tame Panthers. India has many lions, the smaller of which can be trained to the tether and directed to hunt deer. These cats are smart at tracking by scent." Iran also had a native population of wild cats and Persian kings hunted with trained cheetahs. Occasionally, the cheetah was tamed and used as a hound by Egyptian noble sovereigns. Live animals and skins were taken to Egypt as part of the levy from Nubia. Queen Hatshepsut (1473-1458 BC, 18th Dynasty) is reported as bringing cheetahs in from Punt to be part of her royal zoo, and she further kept a pair of Cheetah as her particular pets.Iranian cheetah lives at the lowest density documented anywhere for the species, one to two cats per 1000 square kilometers; an area the same in East Africa can undoubtedly hold 100 cheetahs. The construction of a border fence between Iran and Turkmenistan has made the Asiatic cheetahs natural movement challenging. Iran considers the Cheetah to be an important part of its natural and cultural heritage, and it has now become a symbol of the country's conservation efforts, and the government has now begun to create a broad conservation program.

Key words : Asiatic Cheetah, extinction, Egyptian noble sovereigns, conservation program.

Bahar Hamzehpour

If a person were to search the term 'cheetah' now on the internet, he would be confronted with millions of pages displaying everything from academic articles on the animal, to computer applications and robots, sports teams. Cheetahs remain fascinating too; so that you can see them on the covers of fashion magazines, riding in the sports cars alongside their owner on a jeweled collar (Smith. 2012). Truths about the Cheetah can be cited easily too; every school child knows that the cheetah is the fastest animal on the planet (Cassidy. 2013).Cheetah is a miracle of evolution; it is developed for speed, running its prey down with outbursts of speed that frequently beat 160 km an hour(Cassan. 2012).

Earlier found throughout Africa and Asia; cheetahs can be now detected in parts of central, eastern, and southwestern Africa and some parts of Iran. From a high number of an estimated 100,000 in 1910 the cheetah population has shrunk to an estimated 9000 in Africa and probably 200 in Iran (some specialists even put this number at less than 100) in scattered portions (Scott. 1996). Realizing the cheetah's uncertain state, Iran's Department of Environment has started cooperating with the UN Development Program-Global Environment Facility and Wildlife Conservation Society in New York, since 2001 to save this magnificent animal of the brink of extinction. (Breitenmoser/Alizadeh. 2009)The Asiatic Cheetah is at the present known as the Iranian Cheetah, as the world last few are identified to survive principally in Iran. Once, they were further recorded across southwest Asia but can now just be observed in Iran. Mazandaran Tiger and the Persian lion having already been driven to extinction, the Asiatic Cheetah, the Persian leopard, and the Eurasian lynx remain the only surviving species of big cats in Iran.

The Asiatic Cheetah is detected in the desert regions of Iran, which encompasses portions of the Kerman, Khorasan, Semnan, Yazd, Tehran, and Markazi provinces. The cheetah also seems to survive in the broad and dry Baluchistan province where sufficient prey is available. Various species of birds are likewise to be located in this area such as the partridge, a sort of black-breasted bird, wild pigeon, dull yellow partridge, and birds of prey such as the hawk and falcon. In the hot and cold quarters of Kerman province, wild animals such as leopard, cheetah, panther, wolf, fox, jackal, hyena, black bear, hare, wild sheep, wild goat, ram, and abundant species of snakes, are observed. The wild ass has also been recorded in this region.The strategy to confront the cheetah's extinction has even approached the realm of sport. Conservation attempts were given a representative hike when Iran's national football team wore photographs of the cheetah on their outfit at the 2014 World Cup in Brazil. Furthermore, Iran named August 31st as "Iran's National Cheetah Day" since 2006(Karimi. 2014).

Ancient Origins

Experts have long discussed the origin of these super fast felines, with traces getting from analogously rare fossils. These samples include the North African Acinonyx Aicha, which dates to about 2.5 million years ago and the European Acinonyx Pardinensis with an estimated life of 2.2 million years. Fossils of cheetah-like cats in the category of the Miracinonyx genus known as American cheetah have also been found in North America. However, an almost complete skull of a primitive cheetah that inhabited China over 2 million years ago suggests the fast cats originated in Asia rather than in the America. The skull found in Gansu Province, China, represents a distinct cheetah species, now denominated Acinonyx Kurteni. This animal presumably lived sometime between 2.2 million and 2.5 million years ago. The specimen is one of the most primitive cheetah fossils known to date, which sheds light on cheetahs' original habitat(Bryner.2008).Because this latest skull is much older than both Miracinonyx cats and cheetahs and was discovered in China, it holds debates for an African-Eurasian ancestry of the whole assembly, with the Miracinonyx cats or their ancestors' succeeding migration into America(Bryner. 2008).

The Cheetah in Antiquity

Ancient Egyptians and Assyrians and modern Indians tamed cheetahs for use in hunting. They prized tame cheetahs as hunting animals infinitely superior to dogs (Diamond. 1997).

Scholars have long assumed, that the practice of taming and then hunting with cheetahs began in ancient Egypt, then spread to Mesopotamia in Assyrian times, and from there to Iran, and eventually to Central Asia. These assumptions are not without reason since the Egyptians did, in fact, keep tame cheetahs. The earliest data comes from the famous Punt reliefs in Thebes, which record an expedition returning with tributes from the Land of Punt in the Horn of Africa to the court of the pharaoh queen Hatshepsut. These tributes clearly show a pair of cheetahs with collars and leashes, along with the heavier Leopard (Panthera Pardus) (Allsen. 2011).

Occasionally, the cheetah was tamed and used as a hound by Egyptian noble sovereigns. Live animals and skins were taken to Egypt as part of the levy from Nubia. Queen Hatshepsut (1473-1458 BC, 18th Dynasty) is reported as bringing cheetahs in from Punt to be part of her royal zoo, and she further kept a pair of Cheetah as her particular pets. The ancient Egyptians adopted a similar name for both cheetah and leopard, despite the fact that the two felines are obviously distinguishable from each other in the grave paintings, due to the cheetah's slim form and characteristic "tear drops" below the eyes.

Ancient references further provide a few slightly inconclusive and distorted clues to the use of trained big cats for hunting purposes. Allsen refers to tame leopards and recites that an Indian king “has tame Panthers. India has many lions, the smaller of which can be trained to the tether and directed to hunt deer. These cats are smart at tracking by scent.” Iran also had a native population of wild cats and Persian kings hunted with trained cheetahs. Firdowsi’s Shahnameh composed around 1010 AD tells of the Sassanid emperor Bahram Gour (421-39 AD) to go for hunting with a cheetah. There is also a thirteenth-century chronicle based on an earlier local tradition of the ruler of Tabaristan (Mazandaran Province of Iran), a contemporary of the Sassanid emperor Yazdgerd (632-51 AD), hunting regularly with Cheetahs and Falcons. Hunting with cheetahs grew in popularity and became visible with the Arab invasion of Persia (Iran). This is instantly documented from literary and pictorial references throughout the Abbasid Dynasty time. It was necessary to be captured in the wild since cheetahs refuse to breed in captivity. The preference held for adult female cheetahs because they were believed to be better hunters than the male cheetahs considering they had to feed their cubs. Medieval Arabic manuals do not give a particular time for the training period, but in the Indian tradition, it was thought to take anywhere from three months to a full year. The thirteenth-century manual of Al-Mansur says that ratios of up to 3 kilograms of mutton per day were required to fitly build up a cheetah. Through Mughal India, the Emperor Akbar hold more than one thousand cheetahs that had decorated collars and leashes, and golden brocaded saddlecloths (Allsen. 2011).

The Cheetah in Ancient Iran

Newly unearthed artifacts have led to claims that cheetahs were first domesticated in ancient Persia more than four and a half millennia ago, rather the commonly held assumption that they were first tamed in Egypt. Much of this evidence comes from the artifacts unearthed in Jiroft in Kerman province of southeastern Iran (Mahtoutabad Cemetery). The discovery of this region arose by accident. In 2001, flash floods along the Halil-Rud River swept the topsoil away from the thousands of previously undiscovered graves. Six seasons of excavations followed soon subsequently. Among the magnificent discoveries, are the remains of a town a kilometer and a half in diameter. Furthermore, archaeologists found an exceptional two-story citadel surrounded by a fortress wall of almost 10.5 meters thick, and a ziggurat resembling Mesopotamian ones. This structure is among the biggest in the ancient world, 17 meters high and 350 meters on each side at the foot. The team has likewise uncovered stamp and cylinder seal impressions that depict human figures, bulls, ibex, lions, panthers, cheetahs, snakes, and writing. Likely the most notable findings are significant numbers of carved and decorated cups, vessels, goblets, and boxes made of a soft, fine-grained, durable greenish-grey stone called steatite. Tens of thousands of pieces have been found, but the enormous majority had been looted from the original graves by local people, who were the first to locate the gravesites uncovered by the flood in 2001(Majidzadeh 2003).

Bahar Hamzehpour

The Cheetah in Iran Today

In 2001, a shared project of the Global Environment Facility, the United Nations Development Programme (UNDP) and the Department of the Environment in Iran started a comprehensive program to protect the cheetah of extinction. This ambitious-Conservation of the Asiatic Cheetah Project (CACP)-provided the supplies. Havens dozens of dedicated cheetah guards, motorbikes, new vehicles, and other resources were given to secure them safely. The primary analysis of the social situation was carried out in 2003 by IUCN's Commission on Environmental, Economic and Social Policy (CEESP), supported by the Iranian Centre for Sustainable Development (CENESTA). Unlike the numerous African cheetahs, the Iranian cats are virtually undetectable and on the rim of the extinction. Alongside these activities, the CACP further arose a nationwide campaign to draw attention and awareness to the cheetah's plight. The Team also held a professional from the Government's Organization for Nomadic Peoples Affairs. After lengthy discussions, it was discerned that the NRM section for the Cheetah and its associated prey and habitat was in a shape of a ring around the central deserts in Iran, some 1500 kilometers across. There is now also confirmation of residency and breeding including pictures of young cubs in 10 of the 15 localities.

These surveys corroborate what Iranian biologists have long suspected that there are fewer than 100 Asiatic cheetahs left on Earth. Iranian cheetah lives at the lowest density documented anywhere for the species, one to two cats per 1000 square kilometers; an area the same in East Africa can undoubtedly hold 100 cheetahs. Cheetah, in particular, is a highly mobile species, often going up tens and even hundreds of kilometers in search of prey or mate(Hunter. 2012 & Borrini-Feyerabend. 2004).

The construction of a border fence between Iran and Turkmenistan has made the Asiatic cheetahs natural movement challenging. Iran considers the Cheetah to be an important part of its natural and cultural heritage, and it has now become a symbol of the country's conservation efforts, and the government has now begun to create a broad conservation program. This project seeks to gather data on the cats, such as their habitat, local ecology, prey base as well as human threats facing them.The data collected from camera traps throughout 2009-2013, located an adult female. The cat was identified moving some 150 km multiple times between two reserves in 3 years, covering an estimated 3,629 km. An adult male was also found patrolling three reserves, going up to 40 km across an estimated 807 km. These reports demonstrate that the Asiatic cheetah in Iran covers vast ranges, and signifies the necessity of a large connected space for the long-term conservation of the last Asiatic cheetahs (Farhadinia. 2013).

Protected Zones; Kerman Province of Iran

Efforts have also been made to investigate and analyze the other parts of Iran. One such region is south-eastern Iran, which borders Pakistani Baluchistan. Due to specific factors such as its geographical position, environment and other natural aspects of the province, a variety of wildlife species can be spotted in the mountainous regions and plains of this vicinity; thus bringing forth reserves here, and prohibition as to hunting by the Department of the Environment. The most significant protected areas in the province are Khobr and Arzoiyeh of Baft and RAVAR captive wildlife sanctuary with an area of 1357671 ha through the Act No. 322 dated November 2011 attributed to the areas controlled by the Environmental Protection Agency. RAVAR captive wildlife sanctuary in the Northeast of this city, south of Kalagh-Par Highlands and Tabas mountain, east of Malakh-Khordeh and Markosh Highlands, north of Tighe-Siah and Ghaffar Highlands, and West of Sang-Andaz mounds and Lut desert. RAVAR city is the most northern town in the Kerman Province in the Central Iranian Plateau, on the edge of the central desert. Species located and counted in the Ravar captive wildlife sanctuary include 950 goats and sheep, 300 Jabir, 21 Persian leopards, about 10-15 Asiatic Cheetahs, 14 Caracals, 3 Pallas cats, 186 foxes, 415 jackals, hyena, 57 wolves, 37 porcupines, 1890 Partridges, 875 See-see partridge, 124 grouses, and 86 Eagles. In late 2011, the Iranian Cheetah Society (ICS) began a study of the Darband Ravar Wildlife Refuge fundamentally by making use of camera traps. This area was first reported as a cheetah site when in 2008, within the space of a few months no less than three males were found shot. These unfortunate incidents confirmed that the semi-arid region of the Ravar River Valley was a proper habitat for cheetah,

and where they were once discovered to exist. Investigating the area and training of the game wardens has been carrying on since late 2012. This region is proving hard to study, situated so close to the Iran-Pakistan border with a high risk of confronting drug smugglers. However, eight camera traps have been deployed with a planned rise in their number and area of coverage. The Iranian Cheetah Society is hoping to find evidence of the cheetah as well as Persian leopard(Wildlife Extra News. 2012).

Despite there has been speculation about the cheetah's movement to and from bordering protected zones, there has not been enough observational data about their move. Incidents such the one mentioned above are now approving such speculations. Regrettably several of these cats have been found dead in areas in the vicinity of and in between wildlife refuges. Their deaths have always been due to human activity such as poisoning them and vehicular accidents. This just highlights an urgent need for providing biological corridors for the cats and well as educating and raising the awareness of people living and working in the vicinity of these areas.One hopes now that the efforts to conserve this beautiful animal bring it to a broader public both worldwide and in Iran itself.Now, hopefully, the cheetah may finally outrun extinction.

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Figure 1. A Cheetah stalking a mountain goat. Fragment of a drawing from a painter's sketchbook, Mughal, c 1625. Taken from: Divyabhanusinh. 2002. The end of the trail. Second edition. Oxford University Press, Oxford, New York. (Breitenmoser/Alizadeh. 2009)

Figure 2. Male Asiatic Cheetah in Semi Captive-Breeding and Research Center of Iranian Cheetah in Miandasht protected area. (Breitenmoser/Alizadeh. 2009)

Figure 3. A tribesman brings a cheetah along with ebony as tribute to the King of Thebes (1700 BC)

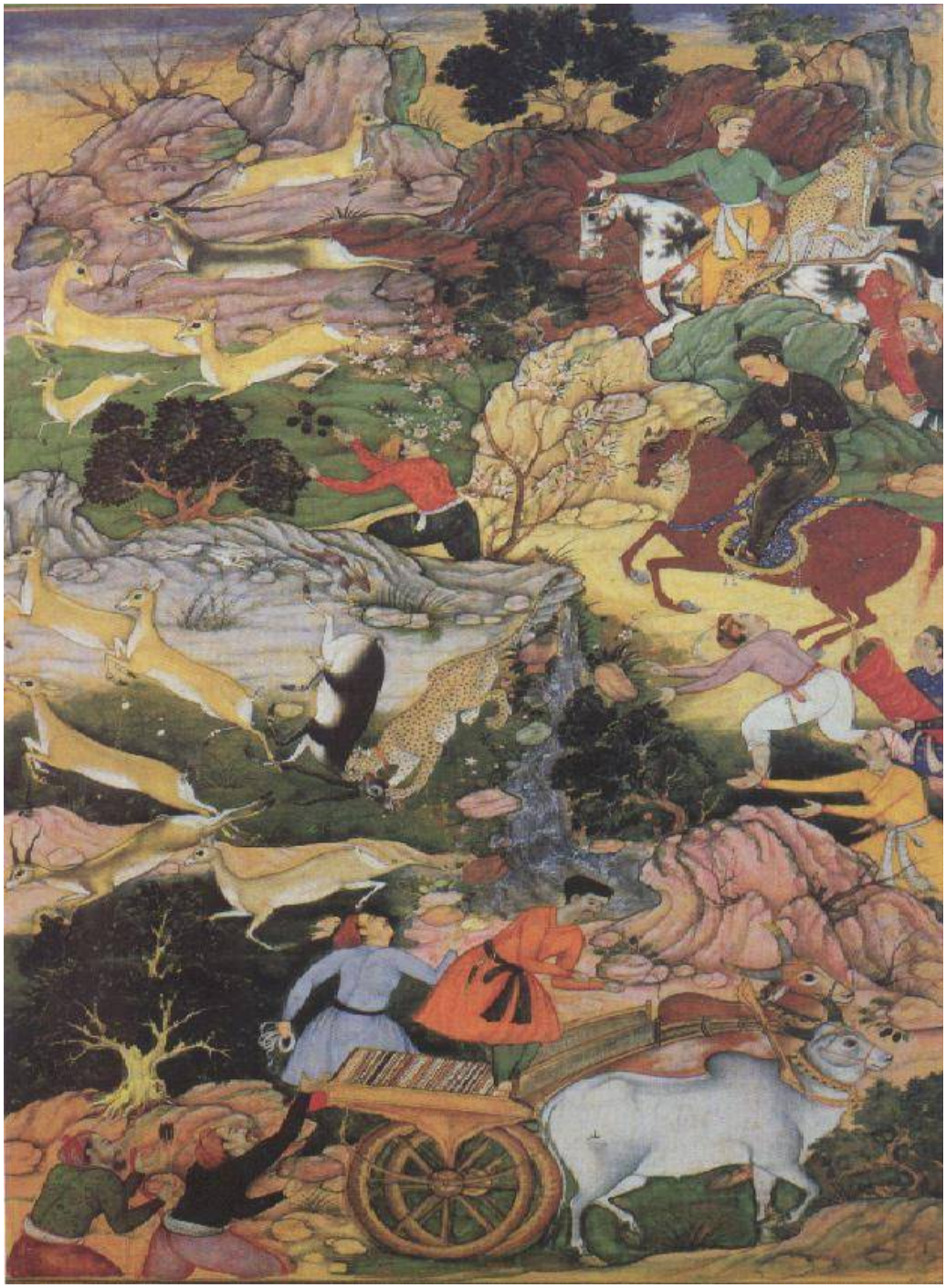


Figure 4. Akbar Hunting with Trained Cheetahs Painting by Sanwala, Outline by Lal Mughal, North India, 1590 AD. From Akbar-nama. Victoria and Albert Museum, London. Life at Court.

Figure 5. Hunting of blackbuck with Asiatic cheetah; drawn by James Forbes in South Gujarat, India. Oriental Memoirs, 1812.

Figure 6. A find from Jiroft, of the Halil-Rud Civilization depicting a priest-like man with two cheetahs. (Majidzadeh. 2003. P. 11)

Figure 7. Persian Leopard (Majidzadeh. 2003. Pp. 71/80)

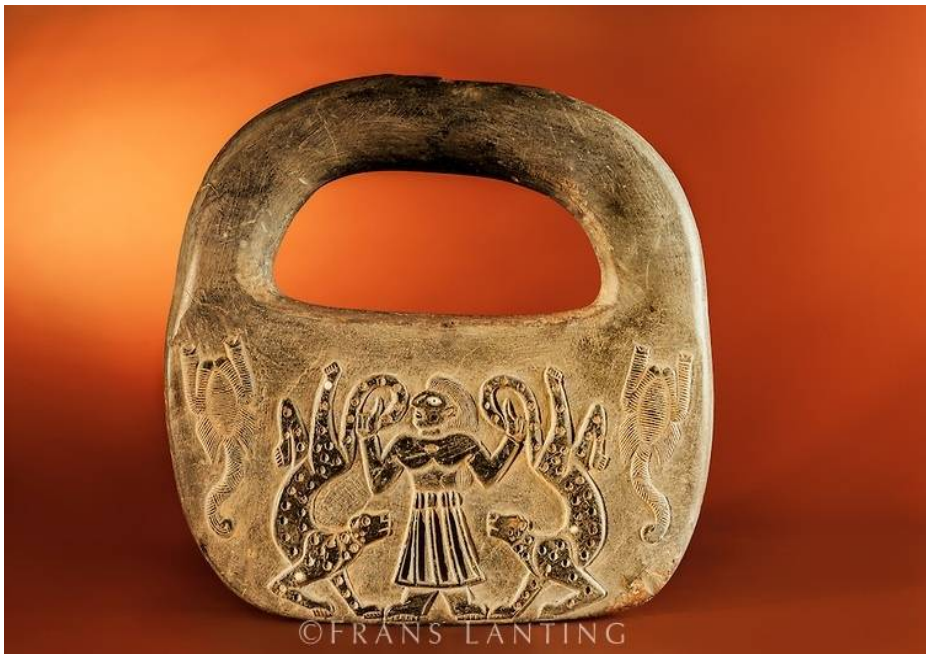
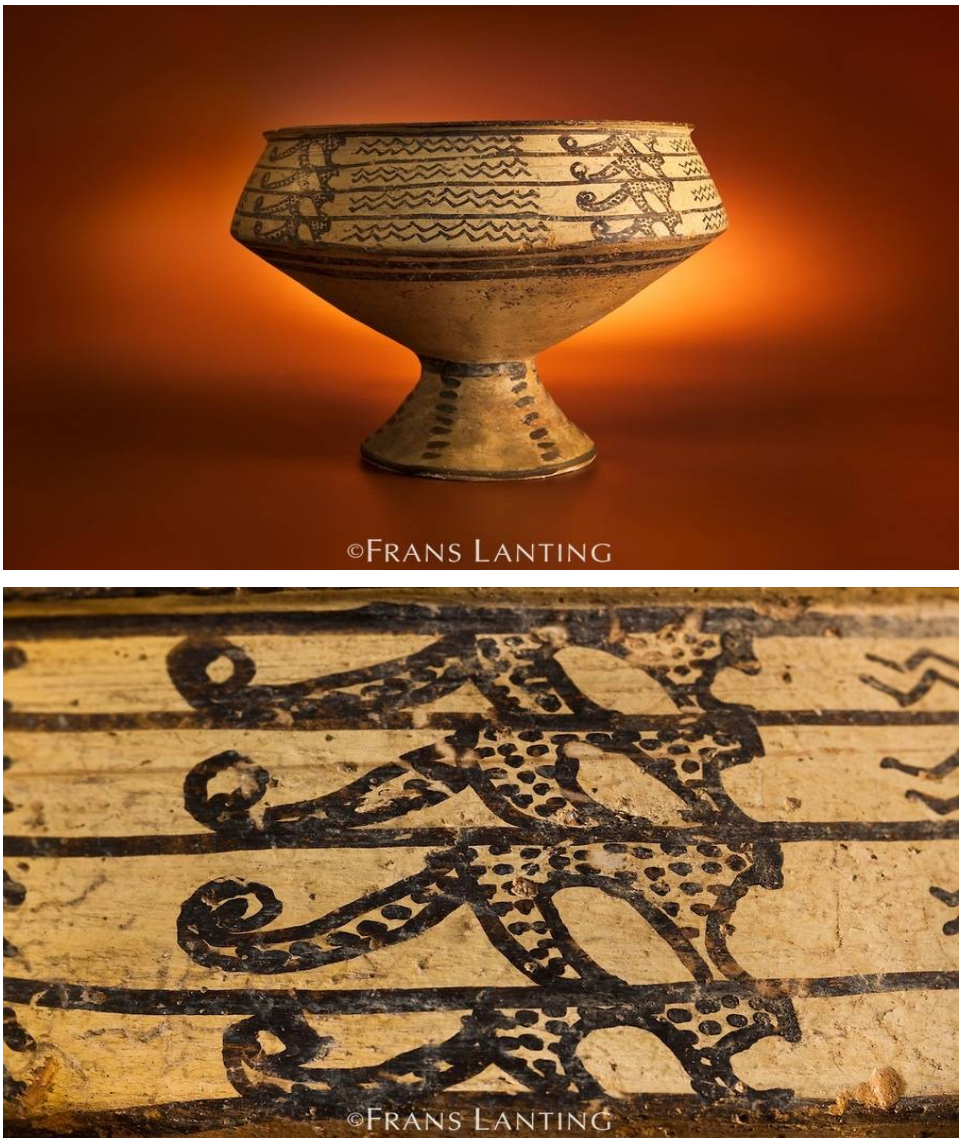


Figure 8. Artifact from Halil-Rud Civilization, third millennium BC that depicts two cheetahs surrounding a man in priest dress. National Museum, Tehran, Iran. This mysterious artifact is referred to as "stone handbag", and it is speculated is that priests used to carry these during ceremonies. (Photograph by Frans Lanting/ Cheetahs on the Run).



Figures 9. Ancient pottery from fourth millennium BC that depicts cheetahs. National Museum, Tehran, Iran (Photograph by Frans Lanting)

Figure 10. Cheetah road sign, Naybandan Wildlife Reserve, Iran. Photograph by Frans Lanting.

Figure 11. Five-year-old Koshki Rescued cub grew up in a reserve in northeast Iran. He is one of only two Asiatic cheetahs living in captivity. A thick tuft of fur on his shoulders, needed for bitter winters on the high steppes of central Iran, sets him apart from African cheetahs. (Hunter. 2012). Photograph by Frans Lanting.

Figure 12. An Asiatic Cheetah Minutes After Hunting A Chinkara Gazelle, Photographed By Ravar's Game Wardens In Winter 2011 In A Salt Pan (Kerman Province of Iran).

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RESEARCH ARTICLE

Livelihood Pattern of People Affected By Tsunami in the Intermediate Shelters in Andaman Islands, India

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ABSTRACT

After Tsunami the Andaman administration constructed 744 intermediate shelters and allotted 705 units to affected people. They are from different islands, language and with low literacy levels. This study was done in 2008-09 with mixed methods in order to provide wider information to Government and NGOs regarding their current livelihood pattern. For the analysis, the occupational status, monthly income, employment in a year and additional sources of income were considered. In spite of the efforts of Government and NGOs, a large proportion of them have the same pattern of livelihoods they had prior to tsunami. Their income generating opportunities and ability to sustain them could deteriorate further when they will be relocated in permanent houses located way from towns and markets and the ration given by the Govt. will be stopped. The findings of this study could enable the Government and NGOs to facilitate appropriate livelihood opportunities for them.

Key word: Intermediate shelters, pre and post Tsunami, Andaman & Nicobar Islands

INTRODUCTION

The tsunami in the Indian Ocean in 2004 was a disaster to which there was enormous worldwide response, millions of dollars raised are still being spent in post disaster rehabilitation programs. The tsunami caused widespread loss of life, infrastructural damage and loss of livelihoods across Andaman. During the Rehabilitation phase, one of the

important components of the program in these areas is economic rehabilitation of the survivors or displaced. This includes enabling them to return to their previous livelihoods or find new avenues. It also includes identifying hitherto non-breadwinners like women in some cases and starting them off, as small entrepreneurs (Sumeeta Banerji, *et al, (2007*). Individuals, households and communities are exposed to unpredictable events that can undermine livelihoods and cause them to fall into poverty or destitution. Some of these events have a sudden onset (e.g. earthquakes) while others develop over a longer period (e.g. conflict, soil erosion), but all can have negative effects on livelihoods (FAO & ILO (2009).

The impact of the tsunami was greatest on the poor, as they have the fewest resources and their ability to recover is the weakest. While some have been able to adapt and shift to other livelihoods post tsunami, for many, their situation just got worse. Not only were lives lost, but so too were household and productive assets (such as boats, ponds, marketing facilities and jetties). These losses reduced the ability of households to earn income and sustain livelihoods (Robert S. Pomeroy, *et al*, (2006). Nearly a year after millions of people were affected by the Indian Ocean tsunami, a significant number continue to face substantial decrease in livelihoods and household income, a follow-up survey has revealed (Times Foundation, 2006).

The US-based Fritz Institute in a report stated that all respondents reported devastating losses in family income as a consequence of the tsunami. There remains a significant decrease in livelihoods and household incomes in all affected areas. Further the Survey results showed 83 percent of affected families in Indonesia had a decrease of over 50 percent of their family income, as did 59 percent of respondents in Sri Lanka and 47 percent in India. In addition to losing their livelihoods, the vast majority of families are still living in temporary shelters or camps. In Indonesia, 100 percent of the affected families are still living in camps or temporary shelters, as are 92 percent in India and 78 percent in Sri Lanka (European Commission's Humanitarian Aid Department (ECHO), 2005). Having these as the background, the present study done in 2008- 09 has tried to explore the pattern of livelihood of those who are residing in intermediate shelters of Andaman Islands.

MATERIALS AND METHODS

The scope of this research is restricted to only those intermediate shelters which are located in and around Port Blair. These are in five places namely, Bambooflat, Namunaghar, Chouldari, Brichgunj and Wandoor. In all these shelters those relocated continue to live. The other areas were not included because of the administrative procedures (The Protection of Aboriginal Tribes Act, 1956, which inter alia recognizes the right of Nicobarese over their lands and prohibits access and residence of non- Nicobaris in Nicobar except with the permission of the Deputy Commissioner), extensive travel to the interior islands and the time constraints of the study.

This is a population based study where primary data is obtained using both qualitative and quantitative methods. The quantitative component of the research study included a cross sectional survey of the selected households in the study area using simple random sampling. The qualitative data was obtained through Focus group discussions (FGD) and interviews conducted at different shelters. For the relief and rehabilitation of the people affected by tsunami, the Andaman administration constructed 744 intermediate temporary shelters and allotted 705 units to the affected people in and around Port Blair at 5 locations. All the individual households were numbered. A complete list of these 705 units (households) forms the sampling frame of the study. The sample size for the quantitative data was calculated taking the population size as 705, Expected frequency as 50%, worst acceptable as 45% and CI 95 % using statistical software EPI INFO version 3.3.2. The Sample size obtained was 249. Out of the total 705 households, the sample of 249 was selected by simple random sampling using SPSS software. Of these, 237 were considered as valid and used for data entry and analysis. For the qualitative data, information was obtained from an additional number of 105 people.

RESULTS

Age and sex of the respondents

The study had 79.7 percent male and 20.3 percent female respondents which is evident from table 1. The age of the respondents varied from below 20 years to more than 60 years. Of these, 62.4 percent were between 31-50 years of age. The table also shows that about 90 percent of the respondents were between 21 to 60 years which is considered to be the productive age group in the society. From the data it can be inferred that in about a fifth of the families, women are the heads of the households.

Marital status

About 83 per cent of the heads of households are married, of which 1.6 per cent are those who have re -married. There is a slight decrease in the number of married people and a slight increase of widowed persons after tsunami.

Literacy level of heads of household

The data from table 3 reveals that about 16 per cent are illiterate, 72 per cent are educated up to secondary level only, about 9 per cent have attained higher secondary level of education and only about 3 per cent have done some diploma, technical or graduate studies. On the whole about 52 per cent of them have done middle or high school level of education. This shows that by and large the educational status of most of the heads of families is indeed quite low.

Shelter and religion

From table 4, it is evident that majority (77 per cent) of the respondents belong to Hindu, followed by 13 per cent and 10 per cent from Christian and Muslim religions respectively. In Bambooflat a substantial percentage of households are Hindus followed by Christians and Muslims. While Hindus and Christians are in all the shelters, Muslims are only in Bambooflat and Namunaghar shelters.

Shelter and Communities

The people in the shelters have originally come from different states of the main land such as Tamil Nadu, Bihar, Bengal, Andra Pradesh, Utter Pradesh etc. Table 5 shows that the Tamils are more in Bambooflat and Brichgunj shelters where as Bengalies are more in Namunaghar and Chouldari/Wandoor shelters. On the whole as shown in table 5, Tamil are 21.9 percent, Ranchi are 14.8 percent, Bengali are 33 percent, Telugu are 18.1 percent, Tribal 0.4 percent, U.P 1.3 percent and others including Nepali and Kaka Muslims are about 10.5 percent.

Status of Family in India

The family is a complex and dynamic institution in India for many decades, but the people usually look at the type of family (Nuclear and Joint) to judge its strength in the community. Most of the micro level studies have stated that the joint family in India is one of the common features among the higher castes. A study conducted in Karnataka, by Caldwell in 1984 showed that, joint families are more common among those households which owned agricultural land.

Types of Families and Communities

As seen in table 6, except the tribal family, all the communities have nuclear families and joint families. Among the individual communities the change in the type of families after tsunami is very marginal, but collectively the increase in nuclear families is substantial.

Type of family and shelters

Table 7 shows that before tsunami 73 per cent of the families lived in a nuclear family system and 27 per cent enjoyed their life in a joint family system. In the post tsunami period the table shows that about 84 per cent live as nuclear families and only 16.5 percent live in joint families.The table also shows that there has been a considerable increase in nuclear families in Brichgunj shelter where there has been no change in the type of families in Chouldari/Wandoor shelter. In the communities nuclear families are more than joint families. This has been so before and after the tsunami. After tsunami the joint families decreased by 11 per cent. This change is significant.

The table also shows that there has been a considerable increase in nuclear families in Brichgunj shelter where there has been no change in the type of families in Chouldari/Wandoor shelter. In the communities nuclear families are more than joint families. This has been so before and after the tsunami. After tsunami the joint families decreased by 11 per cent. This change is significant.

Period	Nuclear family	Joint family	Total	Chi-square 7.14 p value 0.0075 Highly Sig.
Pre tsunami	173	64	237	
Post tsunami	198	39	237	

The FGDs revealed that the allotted intermediate shelters and which are about 11 x 10.5 feet (approx. 45 sq. ft/per room) are not sufficient for more than three members (two adult and one child) in a family. It was stated that this contributed to people living as nuclear families in the shelters.

Ownership of house

According to the data, before tsunami almost 84 per cent of the families lived in their own houses with whatever they had while the others lived in rented houses, government quarters or relatives' homes. After tsunami, according to table 8, nearly 82 per cent of respondents were residing in the intermediate shelters which were made up of tin sheets; only 11 per cent of the respondents have shifted to permanent shelters followed by 5.5 per cent of respondent still living in their home where tsunami affected them. From this analysis it is evident that due to insufficient space in the shelter rooms some of the families are still living in the place were tsunami affected them. Though 24 per cent of respondents expressed that the shelter is not located in a proper place, there is no proper ventilation, no arrangements for transport, no waste disposal system built and that it is very far away from all kinds of facilities, whereas 65 percent of the respondents were quite comfortable in the same location. None of the families from the Chouldari/ Wandoor and Brichgunj shelters have moved into the permanent shelters.

"There has been lot of criticism on the kind of intermediate shelters which were handed over, made of CGI sheets, which are unbearably hot and too congested with only one room. Nicobarese are not using these shelters, however, non-tribal who lost their homes have no other alternative. Official view is, due to heavy monsoon in the islands that was the only structure which could be built in short time and which can withstand the strong monsoons" (Ganguly, Meenakshi, 2005).

Types of houses

Shelter/ house is one of the basic necessities of life for people and it is a critical determinant for survival in the initial stages of disaster. In this respect the administration took tremendous efforts to accommodate the tsunami victims in different places in and around Port Blair. As per table 9, before tsunami about 73 percent of the families lived in RCC/Semi RCC houses, while others lived in wooden, tin roofed houses, thatched houses and huts. For all the tsunami victims the administration has provided intermediate shelters which were built with tin sheets not only for the roofs but also for the walls. So there is a ten fold increase in those living in tin rooms after tsunami.

Training programs for income generation by Govt and NGOs

In order to help the tsunami victims, Govt. and NGOs have taken many efforts to impart training which would generate income for immediate needs and for their sustainability. In fact, the Govt started with tailoring training which only less than one per cent could attend and benefit. But more 40 per cent of the people who lived in shelters were able to access various trainings which were given by NGOs. Of these 34.6 per cent of the respondents benefited through gardening and coconut toy making training. The difference between the training imparted by the Government and the NGOs was captured in one of the FGDs as "the Govt. trainings and supports were organized in the earlier stage of our settlement in intermediate shelters. Many of us were able to undergo the trainings conducted by NGOs because they organized the training later on based on the needs appropriate for men and women, separately".

Occupational status of respondents - Pre and Post Tsunami

Usually in a society, the status and respect of a person depends on his income and employment. Besides, these income and employment are the important parameters not for the status alone but to measure the standard of living of persons. In order to understand the standard of living of the people in the shelters, it is essential to find out the present main sources of income and additional sources of income of the sample respondents in relation to what they were before tsunami.

From table 11, it can be observed that before tsunami 22 per cent of the respondents worked in government, 33 per cent as daily wage labourers, 15 per cent engaged in petty business followed by the remaining 30 per cent engaged in various activities such as farming, fishing, self employment, contractor, getting pensions, Anganwadi work, electrician, street vendors and so on. After tsunami, there has been very little variation in the percentages. But due to damage to agricultural land and marine resources those who were farming and fishing resorted to daily wages, self employment and other income generating activities.

Occupation and periodicity of Income

In the post tsunami situation, with respect to employment and sources of income, table 12 shows that about 20 per cent of the respondents received their income from government departments on monthly basis. In the daily wage labourer category, 35 per cent (84 nos.) of the respondents earned income on monthly basis in spite of the differences in their jobs and only 2 per cent on daily basis. Overall it can be inferred that nearly 70 per cent of the people in the shelters are earning on a monthly basis and that the remaining 29.6 per cent of them receive income daily, seasonal or piece work basis. In the intermediate shelters almost 99.6 per cent of the respondents are engaged in some livelihood activities outside the shelters which have contributed to sustain their families.

Monthly income

In accordance with the European Commission's Humanitarian Aid Department (ECHO) study in Sri Lanka, the respondents of this study stated that they intend to return to their original homes and villages in order to resume their previous economic activities. But the Govt. restricted their return to the places from where they were brought as they are tribal areas. This has led to a decline in their ability to generate income. Table 13 reveals that the percentage of those whose income is above Rs.10000 has decreased from 3.4 to 2.1; that whose income is between 6001 to 10000 has decreased from 18.1 to 14.8; those whose income is between 3001 to 6000 has decreased from 68.8 to 38 after tsunami. But the percentage of those whose income is upto 3000 has increased from 23 to 107 households.

As expected, the dual disasters of earthquake and tsunami disrupted the livelihoods of all the respondents who were in the intermediate shelters. Most of them experienced difficulties restarting their livelihoods. As many as (80 per cent) respondents had taken any kind of work available while 6 per cent had borrowed to reinvest in their businesses. In shelters more respondents have relied on daily wage employment (37 per cent) while 7 per cent had taken up new (temporary) jobs. The pattern of livelihood of the people in the intermediate shelters changed due to accessibility to the source of work and availability of work at the location of shelters. Basically all the shelters were located about 15-20 km away from the main city.

Number of days of employment

Table 14 shows that 73 per cent of respondents got employment more than 240 days in a year in the Intermediate shelters. 20 per cent of respondents were able to engage themselves in work for 100-240 days in a year. Six per cent of the respondents received pension from the government and they were not engaged in any days of employment. This shows that a substantial proportion of those in the intermediate shelters are having more than 100 days of employment in keeping with 100 days employment program advocated by the Government.

Additional income of respondents

Additional income is another important factor which will enhance the socio-economic status of a family. The additional sources of income have been petty business, private jobs, tailoring etc. Table 15 shows that before tsunami nearly 14 per cent of the respondents generated additional income through various sources such as petty business, private job, milk and egg selling, anganwadi work, fishing. But post tsunami, this has decreased to less than 8 per cent of the respondents having additional sources of income.

Amount of Additional income Earned

The additional income is not based on a particular activity but from different kinds of initiatives. People with various regular occupations are involved in a number of other income generation activities and have been successful in increasing their income. The FGDs revealed that those who had previous additional sources of income had motivation develop additional sources of income. It is also observed that those who had experiences and skills before tsunami have been able to generate additional income for their families. Table 16 shows that nearly 6 per cent of respondents were able to enhance their family income up to Rs.3,000 per month. Another 2 per cent of the respondents were in a position to increase their family income above Rs.3,000 with additional income. Though the additional income is not too much, it shows that a small proportion of those in the intermediate shelters are motivated and trying to increase their income through various sources.

Current loan outstanding

A total of 21 (8.8 per cent) out of 237 families had taken loan from various sources for certain purposes and 17 (7 per cent) of them have repaid the loan amount. Table 16 shows that only 4 (1.7 per cent) out of 237 families still have loan outstanding of more than Rs.10,000, at banking institutions and NGOs, which were mainly disbursed for business activities. All the 6 women heads of households who had taken loan have repaid their loan and so do not have the burden of outstanding loan.

Savings in the shelters

While the government and the NGOs working in the shelters have been trying to enable those in the intermediate shelters to be self supportive, it is encouraging to see that those in the shelters have not only had their needs met, but also have been able to save their money.It was interesting to note from the FGDs that in the intermediate shelters most of them are aware about the concept of saving and investment for unexpected events and future needs. From table 18, it is clear that almost 92 per cent of the respondents saved their hard earned money in different forms. Nearly 91 per cent of them found it is very safe and secure to deposit the money in banking sector. It is also seen that 33 per cent of respondents saved upto Rs.5,000 during their stay at shelters; about 26 per cent of them saved Rs.5000 to Rs.10,000 and about 30 per cent, more than Rs.10,000 after tsunami.

Jewels in families

Table 20 shows that 85 per cent of the families have gold jewels and nearly 3 per cent of them have silver jewels in their homes now, some of which have been purchased during their stay in the shelters.

DISCUSSION

Most of the people are living in Govt. provided intermediate shelters and three years after tsunami some of them got permanent shelters. The devastating tsunami made great impact not only to their health, family structure and children's education, but also to a great extent on livelihoods of those in the intermediate shelters in Andaman. This has been compounded by the non-availability of basic services such as education, market, health and sanitation and public transport.The intermediate shelters have been erected by the Government in five locations in and around Port Blair. Those in the intermediate shelters are from various communities, with different languages and religion and from different geographical locations of the islands. Apart from the fact that all of them were displaced by the disaster, there does not seem to have any unifying factor binding them. Within the shelters also they are living as sub groups, not mingling with others except when necessary. Thus in spite of the heterogeneity of the residents there, there are small homogenous groups.

The respondents were the heads of the families. The educational statuses of most of the heads of families were very low. A sizable proportion of respondents were married and a few are widowed. Majority of them were men. Most of them were very willing to respond to the questions asked during data collection. They looked confident and seemed to have overcome the effects of the losses they had experienced to a large extent. As the permanent accommodations are being built and a few had already shifted into them, they are expectantly waiting for their turn to move in.Trainings were organized in order to generate income to cope with their present needs and to develop sustainable livelihoods. The trainings given by Govt. have not been accessed by the respondents due to its nature and the time of when they were arranged. But the trainings which were organized by NGOs after the people had settled to some extent, were, accessed by nearly half of the respondents. This is mainly because of the various skills imparted and time of implementation. In fact the trainings given to the people in shelter have not made any additional income to

their livelihoods. Actually, those trained have not been able to implement the skills acquired and generate income as they have no avenues to market the products. This is due to the fact that the intermediate shelters are located in remote areas, without proper transportation and far away from towns and markets. The trainings should be planned according to the choice of the people keeping in mind the location where these emergency shelters were constructed. The trainings given by Govt. and NGOs were not tailor made to meet the needs of those affected by Tsunami. People from different communities and languages lived in the intermediate shelter. The people earned their living by doing a variety of jobs some of which such as farming and fishing are community specific. They also had petty businesses to contribute to their income. These also depended on the locations where they lived and the cultivable land they possessed or the fishing they could do in the costal areas.As Government provided accommodation in intermediate shelters after tsunami, more households have opted to live as nuclear families. Thus joint families prior to tsunami chose to become nuclear families after tsunami and made the most out of the situation. Nuclear family setup has considerably affected their family income because of reduction of earning members in families.

Currently a small proportion have Government jobs while others are daily wage labourers, engaged in petty business and in various activities such as farming, fishing, self employment, contractor, getting pensions, Anganwadi work, electrician, street vendors and so on. In spite of differing occupational background a large proportion of them have work for more than hundred days a year and receive their income in monthly intervals. This shows that there is some stability in their income. As most of them do not have technical expertise, they have to engage in jobs that do not pay much. Also as jobs are not available near the intermediate shelters, they have to travel long distances in search of the same. As the educational levels of the heads of households are quite low and they were set in their traditional ways of earning their livelihood, though the Government offered training and NGOs trained a number of them in livelihood skills, most of them have not been able to earn their livelihood using those skills. However their families have benefited by having kitchen gardens which are expected to contribute towards their nutritional status though it has not been contributing to income generation to a large extent. The shelters are in places away from other villages and there are very limited job opportunities apart from construction of the permanent accommodations but have been able to make substantial financial progress though they are still in the intermediate shelters. The fact that most of them now have a monthly income has helped them to organise their lives in a better way.

The additional income is not based on particular activity but from different kinds of initiatives. People with various regular occupations are involved in a number of income generation activities and have been successful in increasing their income. Those who had previous additional sources of income/knowledge have motivated them to earn more and those who had experiences and skill before tsunami have been able to generate additional income for their family.Those in the shelters are fully conscious of the fact that should save money so that it will be useful in times of need. They have not only been able to meet their financial needs through various income generating activities, but have also been thoughtful to save money in banks and post office. In addition to this a substantial proportion has gold jewelry some of which has been purchased during their stay in the intermediate shelters. This type of saving could be due to the practice of those from south India who invest in gold which could be handy in times of calamities.

CONCLUSION

This paper made an attempt to find out the livelihood pattern of the people who were residing in intermediate shelters in Andaman Islands. The low literacy level and much focused traditional ways of generating income in the past has not been beneficial to explore new income generation activities in spite of livelihood trainings arranged for them by the Government and the NGOs. Most of the families in shelters were able to find livelihood activities according to their abilities. Most of the heads of households have monthly income irrespective of government jobs, petty business or being labourers. A small proportion has additional income as well. The main concern among

thosewhose livelihood depends upon petty business and daily wage is the fear of again losing their livelihood options because of shifting to permanent shelters elsewhere. In the permanent shelters, no separate allotment has been made for commercial purpose. When they move to their permanent houses, they will face insufficient place for starting petty business and will have new environment to adjust. Over a period of time the people in intermediate shelters coped with the situation and were able to generate income with their present form of livelihoods. Though the people are eager to occupy the permanent accommodation, they do not have provision for commercial purposes or for starting petty businesses. This is a matter of great concern especially after the monthly provisions given by the Government will be totally stopped.

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Table 1.Age Sex distribution of respondents in each shelter

	10-20 yrs			21-30 yrs			31-40 yrs			41-50 yrs			51-60 yrs			61 and above		
Name of shelters	**M**	**F**	**Total**	**M**	**F**	**Total**	**M**	**F**	**Total**	**M**	**F**	**Total**	**M**	**F**	**Total**	**M**	**F**	**Total**
Bambooflat	0	0	0	8	2	10	32	3	35	17	2	19	9	3	12	4	1	5
Namunaghar	1	1	2	9	3	12	8	4	12	10	2	12	6	0	6	4	1	5
Chouidari/ Wandoor	0	0	0	6	1	7	11	3	14	11	3	14	3	0	3	6	4	10
Brichgunj	0	0	0	6	0	6	28	9	37	3	2	5	4	4	8	3	0	3
Total	**1 (50)**	**1 (50)**	**2 (100)**	**29 (83)**	**6 (17)**	**35 (100)**	**79 (81)**	**19 (19)**	**98 (100)**	**41 (82)**	**9 (18)**	**50 (100)**	**22 (76)**	**7 (24)**	**29 (100)**	**17 (74)**	**6 (26)**	**23 (100)**
Row percentage of total			**0.84**			**14.77**			**41.35**			**21.1**			**12.24**			**9.7**

Source: Primary data. Note: The figures (values) in parentheses indicate the percentage

Table 2 .Marital status of the respondents

	Marital status									
	Pre Tsunami					Post Tsunami				
Name of Shelter	**Un-married**	**Married**	**Separated**	**Widowed**		**Un-Married**	**Married**	**Separated**	**Widowed**	**Re-married**	**Total**
Bambooflat	2	72	0	7		2	67	1	7	4	**81 (34)**
Namunaghar	2	44	1	2		4	38	3	4	0	**49 (21)**
Chouldari / Wandoor	2	40	1	5		0	39	0	9	0	**48 (20)**
Brichgunj	1	53	1	4		1	52	0	6	0	**59 (25)**
Total	**7 (3)**	**209 (88)**	**3 (1)**	**18 (8)**		**7 (3)**	**196 (82.8)**	**4 (1.6)**	**26 (11)**	**4 (1.6)**	**237 (100)**

Source: Primary data. Note: The figures (values) in parentheses indicate the percentage

Table 3.Shelter and Educational status of head of family

Name of Shelter	**Educational status of head**							
	Illiterate	**Upto V**	**VI-X**	**XI & XII**	**Diploma**	**Technical**	**Graduate**	**Total**
Bambooflat	9	16	37	13	1	1	4	81 (34)
Namunaghar	14	13	18	2	0	0	2	49 (21)
Chouldari/ Wandoor	5	11	28	4	0	0	0	48 (20)
Brichgunj	9	8	39	3	0	0	0	59 (25)
Total	37 (16)	48 (20.2)	122 (51)	22 (9)	1 (0.4)	1 (0.4)	6 (3)	237 (100)

Source: Primary data. Note: The figures (values) in parentheses indicate the percentage

Table 4 .Religious backgrounds of respondents

Name of shelter	Religion of the respondent			
	Christian	Muslim	Hindu	Total
Bambooflat	16	12	53	**81 (34)**
Namunaghar	8	12	29	**49 (21)**
Chouldari/ Wandoor	1	0	47	**48 (20)**
Brichgunj	6	0	53	**59 (25)**
Total	**31 (13)**	**24 (10)**	**182 (77)**	**237 (100)**

Source: Primary data. Note: The figures (values) in parentheses indicate the percentage

Table 5.Community wise classification of respondents

Names of shelters	Community							
	Tamil	Ranchi	Bengali	Telugu	Tribal	UP	Others	Total
Bambooflat	23	15	7	19	1	3	13	**81 (34)**
Namunaghar	7	9	15	6	0	0	12	**49 (21)**
Chouldari/ Wandoor	2	4	38	4	0	0	0	**48 (20)**
Brichgunj	20	7	18	14	0	0	0	**59 (25)**
Total	**52 (21.9)**	**35 (14.8)**	**78 (33)**	**43 (18.1)**	**1 (0.4)**	**3 (1.3)**	**25 (10.5)**	**237 (100)**

Source: Primary data. Note: The figures (values) in parentheses indicate the percentage

Table 6.Distribution of respondents according to type of family and communities

Community	Type of family				
	Pre tsunami		Post tsunami		
	Nuclear	Joint	Nuclear	Joint	Total
Tamil	40 (16.9)	12 (5.1)	44 (18.6)	8(3.4)	52 (21.9)
Ranchi	31(13.1)	4 (1.7)	32 (13.5)	3(1.3)	35 (14.8)
Bengali	63 (26.6)	15 (6.3)	68 (28.7)	10 (4.2)	78 (32.9)
Telugu	25 (10.5)	18 (7.6)	35 (14.8)	8 (3.4)	43 (18.1)
Tribal	0	1 (0.4)	1 (0.4)	0	1 (0.4)
UP	1 (0.4)	2 (0.8)	2 (0.8)	1 (0.4)	3 (1.3)
Others	13 (5.5)	12 (5.1)	16 (6.8)	9 (3.8)	25 (10.5)
Total	**173 (73)**	**64 (27)**	**198 (83.5)**	**39 (16.5)**	**237 (100)**

Source: Primary data. Note: The figures (values) in parentheses indicate the percentage

Table 7.Distribution of respondents by types of family

Names of Shelters	Type of families									
	Pre tsunami					Post tsunami				
	Nuclear		Joint			Nuclear		Joint		Total	
	Nos	%	Nos	%		Nos	%	Nos	%	Nos	%
Bambooflat	56	23.6	25	10.55		63	26.58	18	7.59	81	34
Namunaghar	34	14.3	15	6.33		40	16.88	9	3.8	49	21
Chouidari/Wandoor	44	18.6	4	1.68		44	18.57	4	1.69	48	20
Brichgunj	39	16.5	20	8.44		51	21.52	8	3.38	59	25
Total	**173**	**73**	**64**	**27**		**198**	**83.55**	**39**	**16.5**	**237**	**100**

Source: Primary data.

Table 8. Ownership of house

Name of shelter	Ownership of house								
	Pre-tsunami					Post-tsunami				
	Own	Rented	Govt. quarters	Relative home		Inter-mediate shelter	Relative Home	Permt. Shelter	Own house	Total
Bambooflat	65	5	10	1		56	0	24	1	**81 (34)**
Namuna-ghar	43	3	3	0		43	2	3	1	**49 (21)**
Chouldari/ Wandoor	41	4	3	0		47	0	0	1	**48 (20)**
Brichgunj	50	8	1	0		48	1	0	10	**59 (25)**
Total	**199 (84)**	**20 (8.4)**	**17 (7.2)**	**1 (0.4)**		**194 (81.9)**	**3 (1.3)**	**27 (11.4)**	**13 (5.5)**	**237 (100)**

Source: Primary data. Note: The figures (values) in parentheses indicate the percentage.

Table 9.Shelter Area and type of house

Name of Shelter	Type of house										
	Pre-tsunami							Post-tsunami			
	RCC	Semi-RCC	Thatched	Tin	Wooden	Hut type		Tin	Thatched	Semi-RCC	Total
Bambooflat	24	47	3	1	6	0		56	0	25	**81 (34)**
Namunaghar	3	35	1	4	3	3		43	1	5	**49 (21)**
Chouldari/ Wandoor	0	26	8	10	4	0		47	1	0	**48 (20)**
Brichgunj	4	32	1	6	14	2		48	11	0	**59 (25)**
Total	**31 (13.08)**	**140 (59.07)**	**13 (5.49)**	**21 (8.86)**	**27 (11.4)**	**5 (2.1)**		**194 (81.8)**	**13 (5.5)**	**30 (12.7)**	**237 (100)**

Source: Primary data. Note: The figures (values) in parentheses indicate the percentage

Table 10. Livelihood Trainings for shelter people

Types of training	Organized by NGO			Organized by Government	
	No. of respondents	Percentage		No. of respondents	Percentage
Not attended	134	56.5		235	99.2
Handicrafts	3	1.3		-	-
Tailoring	13	5.5		2	0.8
Computer training	1	0.4		-	-
Beautician training	1	0.4		-	-
Gardening & coconut toy making	82	34.6		-	-
Masala items	2	0.8		-	-
Pickle making	1	0.4		-	-
Total	237	100		237	100

Table 11.Pre and post Tsunami occupational status of respondents

Occupation	Pre tsunami			Post tsunami	
	No. of respondents	Percentage		No. of respondents	percentage
No employment	-	-		1	0.4
Govt. Servant	53	22.4		48	20.3
Daily wage Labour	79	33.3		87	36.7
Petty business	35	14.8		33	13.9
Farmer	16	6.8		10	4.2

Fishing	23	9.7		14	5.9
Self employment	9	3.8		11	4.6
Contractor	4	1.7		4	1.7
Pensioner	7	3.0		12	5.1
Others	4	1.7		15	6.3
Anganwadi worker	7	3.0		-	-
Carpenter	-	-		2	0.8
Total	237	100.0		237	100.0

Table 12.Occupation and periodicity of income of respondents in post tsunami period

Present Occupation	Type of income						
	No	**Daily**	**Weekly**	**Monthly**	**Piece work**	**Seasonal**	**Total**
No employment	1 (0.4)	-	-	-	-	-	1 (0.4)
Govt. servant	-	-	-	48 (20.3)	-	-	48 (20.3)
Daily wage Labourers	-	3 (1.3)	-	84 (35.4)	-	-	87 (36.7)
Petty business	-	33 (13.9)	-	-	-	-	33 (13.9)
Farmer	-	2 (0.8)	-	2 (0.8)	-	6 (2.5)	10 (4.2)
Fishing	-	13 (5.5)	-	1 (0.4)	-	-	14 (5.9)
Self employed	-	6 (2.5)	-	5 (2.1)	-	-	11 (4.6)
Contractor	-	-	-	2 (0.8)	1 (0.4)	1 (0.4)	4 (1.7)
Pensioner	-	-	-	12 (5.1)	-	-	12 (5.1)
Others	-	2 (0.8)	2 (0.8)	10 (4.2)	-	1 (0.4)	15 (6.3)
Carpenter	-	-	-	2 (0.8)	-	-	2 (0.8)
Total	**1 (0.4)**	**59 (24.9)**	**2 (0.8)**	**166 (70.0)**	**1 (0.4)**	**8 (3.4)**	**237 (100)**

Source: Primary data. Note: The figures (values) in parentheses indicate the percentage

Table 13.Monthly incomes of the respondents

Monthly income of family/household	Pre-tsunami period			Post-tsunami period	
	No. of Respondents	Percentage		No. of Respondents	Percentage
Upto Rs.3000	23	9.7		107	45.1
Rs.3001-Rs.6000	163	68.8		90	38.0
Rs.6001-Rs.10000	43	18.1		35	14.8
Rs.10001 & Above	8	3.4		5	2.1
Total	237	100.0		237	100.0

Table 14. Number of days of Employment in a year

Present occupation	No. of days of employment				
	No	1-99 days	100-240 days	241 days & Above	Total
No	1 (0.4)	-	-	-	1 (0.4)
Govt. Servant	-	-	-	48 (20.3)	48 (20.3)
Daily wage Labourers	-	-	28 (11.8)	59 (24.9)	87 (36.7)
Petty business	-	-	2 (0.8)	31 (13.1)	33 (13.9)
Farmer	-	-	6 (2.5)	4 (1.7)	10 (4.2)
Fishing	-	-	7 (3.0)	7 (3.0)	14 (5.9)
self employed	-	-	1 (0.4)	10 (4.2)	11 (4.6)
Contractor	-	-	-	4 (1.7)	4 (1.7)
Pensioner	12 (5.1)	-	-	-	12 (5.1)
Others	-	3 (1.3)	3 (1.3)	9 (3.8)	15 (6.3)
Carpenter	-	-	1 (0.4)	1 (0.4)	2 (0.8)
Total	**13 (5.5)**	**3 (1.3)**	**48 (20.3)**	**173 (73.0)**	**237 (100)**

Source: Primary data. Note: The figures (values) in parentheses indicate the percentage

Table 15.Additional sources of income to the respondents

Source of additional income	PRE TSUNAMI		POST TSUNAMI	
	No. of respondents	Percentage	No. of respondents	Percentage
No	203	85.7	219	92.4
Petty business	9	3.8	8	3.4
Private	5	2.1	6	2.5
Milk & egg	16	6.8	0	-
Anganwadi	1	0.4	0	-
Fishing	1	0.4	0	-
Tailoring	0	-	1	0.4
Others	2	0.8	3	1.3
Total	**237**	**100**	**237**	**100**

Table 16.Amount of additional income

	Additional income (in Rs)	PRE-TSUNAMI		POST -TSUNAMI	
		No. of respondents	Percentage	No. of respondents	Percentage
1	No income	203	85.7	219	92.4
2	500-1000	19	8.0	4	1.7
3	1001-2000	5	2.1	4	1.7
4	2001-3000	9	3.8	5	2.1
5	3001 & Above	1	0.4	5	2.1
	Total	237	100	237	100

Table17. Current outstanding of respondents

Sex of respondent	No	10000-20000	20000 above	Total
Male	185 (78.1)	1 (0.4)	3 (1.3)	189 (79.7)
Female	48 (20.3)	0	0	48 (20.3)
Total	233 (98.3)	1 (0.4)	3 (1.3)	237 (100)

Source: Primary data. Note: The figures (values) in parentheses indicate the percentage

Table 18.Current forms of saving of respondents

Form of saving	No. of respondents	Percentage
No where	19	8.0
Post office	1	0.4
Bank	216	91.1
Purchase land etc	1	0.4
Total	237	100.0

Table 19. Amount of savings

Amount of saving (Rs.)	No. of respondents	Percentage
No saving	19	8.0
Rs.500- Rs.1000	6	2.5
Rs.1000- Rs.5000	78	32.9
Rs.5000- Rs.10000	62	26.2
Rs.10000- Rs.20000	51	21.5
Rs.20000 & above	21	8.9
Total	**237**	**100.0**

Table 20. Savings in form of jewels

Sex of respondent	Jewels				
	No	**Artificial**	**Silver**	**Gold**	**Total**
Male	13 (5.5)	10 (40.2)	2 (0.8)	164 (69.2)	**189 (79.7)**
Female	5 (2.1)	1 (0.4)	4 (0.4)	38 (16.0)	**48 (20.3)**
Total	**18 (7.6)**	**11 (4.6)**	**6 (2.5)**	**202 (85.2)**	**237 (100)**

Source: Primary data. Note: The figures (values) in parentheses indicate the percentage

Table 21.Shows Baseline characteristics of the respondents

Characteristics		No. in (%)	
		Pre tsunami (n=237)	**Post tsunami (n=237)**
Sex			
	Male	189 (79.7)	189 (79.7)
	Female	48 (20.3)	48 (20.3)
Age			
	10 - 20 yrs	2 (0.8)	2 (0.8)
	21 - 30 yrs	34 (14.3)	35 (14.1)
	31 - 40 yrs	98 (41.4)	98 (41.4)
	41 - 50 yrs	50 (21.1)	50 (21.1)
	51 - 60 yrs	33 (13.9)	29 (12.2)
	61 & above	20 (8.4)	23 (9.7)
Marital Status			
	Unmarried	7 (3.0)	7 (3.0)
	Married	209 (88.2)	196 (82.7)
	Separated	3 (1.3)	4 (1.7)
	Widowed	18 (7.6)	26 (11.0)
	Remarried	--	4 (1.7)
Religion			
	Christian	31 (13.1)	31 (13.1)
	Muslim	24 (10.1)	24 (10.1)
	Hindu	182 (76.8)	182 (76.8)
Educational qualification			
	Illiterate	37 (15.6)	37 (15.6)
	Upto V	48 (20.3)	48 (20.3)
	VI – X	122 (51.5)	122 (51.5)
	XI – XII	22 (9.3)	22 (9.3)
	Diploma	1 (0.4)	1 (0.4)
	Technical	1 (0.4)	1 (0.4)
	Graduate	6 (2.5)	6 (2.5)
Type of family			

	Nuclear	173 (73)	198 (83.5)
	Joint	64 (27)	39 (16.5)
Type of ration card			
	BPL	140 (59.1)	--
	APL	97 (40.9)	--

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RESEARCH ARTICLE

Occurrence of Gastric Disorders in Dogs with Vomiting

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ABSTRACT

Vomiting is the most common complaint in small animal practice and can present considerable challenges to the diagnostic and therapeutic skills of a clinician. One hundred dogs brought to Teaching Veterinary Clinical Complex were screened for the aetiologies of vomiting. Detailed anamnesis (age, sex, breed, diet, duration of illness, nature, colour and consistency of vomitus), clinical examination with physiological parameters, physical examination of the animal, laboratory procedures with routine faecal examination, wet blood and blood smear examination, haematology and serum biochemistry and diagnostic imaging techniques were carried out.

Key words: Vomiting, serum biochemistry, anamnesis.

INTRODUCTION

Vomiting is the most common complaints in small animal practice and can present considerable challenges to the diagnostic and therapeutic skills of a clinician. Clinical signs and physical examination findings are insufficient for a clinician to arrive at a definitive diagnosis. Clinical findings help to arrive at a tentative diagnosis and to adopt a strategy for symptomatic treatment.Diagnosis of correct etiological agents or factors leading to gastric disorders has been a subject of utmost interest. A multi-dimensional approach to evaluate cases of vomiting involves history taking, detailed clinical examination, blood smear examination, haemato-biochemical and electrolyte analysis,

diagnostic imaging and special diagnostic procedures.A thorough study of all the above aspects and diagnosis is very essential for the effective therapeutic management of canine gastric disorders.

Gastric diseases are due to inflammation, ulceration, neoplasia or obstruction. The gastric disorders were gastritis (superficial, atrophic, and hypertrophic), gastric ulcers, gastric dilatation and gastric dilatation-volvulus, obstruction (e.g., foreign body, pyloric mucosal hypertrophy, external compression), gastric hypomotility, gastric polyps, gastric neoplasia (De novo, 2003). Gastric disorders manifests as a variety of clinical signs like vomiting, haematemesis, diarrhoea, melena, retching, anorexia, hypersalivation, abdominal distension and abdominal pain (Simpson, 2010).
The main object of this study was to

Classify the disorders and diseases associated with vomiting in dogs

- Types of gastric disorders in vomiting dogs
- To determine the effect of age, sex, breeds, on the distribution of gastric problems in dogs with vomiting.
- To determine the diet, duration of illness, nature, colour and consistency of vomitus in gastric disorders.

MATERIALS AND METHODS

One hundred dogs brought to the Teaching Veterinary Clinical Complex, Pookode with a complaint of vomiting were screened for gastric involvement. Detailed anamnesis(age, sex, breed, diet,duration of illness, nature, colour and consistency of vomitus), clinical examination with physiological parameters, physical examination of the animal, laboratory procedures with routine faecal examination, wet blood and blood smear examination, haematology and serum biochemistry were carried out.Haematology was performed in Auto Blood Analyser of eosVet, Exigo,Boule Medical AB, Sweden. Radiography was performed in machine of capacity 500 mAs and 40 kvs with an digital x-ray processing unit of Care-Stream Company. Ultrasound was performed with 2.5, 3.0, 5.0 and 7.5 MHz transducer of e-saoate company (Model : MyLab70VetXv).

RESULTS

The results revealed that only 42 per cent of the vomiting cases had primary gastric involvement followed by renal disorders and infection (16 per cent each), intestinal disorders (9 per cent), haemoprotozoans (7 per cent), snake bite (3 per cent), reproductive disorders, liver disorders, and poisoning (2.3 per cent each) and pancreatic disorders (1 per cent). Disorders associated with vomiting in dogs are presented in the table 1 and figure 1. In gastric disorders gastritis was seen in 47.61 per cent (N=42, n= 20) of the gastric disorder dogs followed by gastric ulcers in 14.28 per cent (N=42, n=6), gastric foreign body in 9.52 per cent (N=42, n=4), and gastric dilatation and volvulus 9.52 per cent (N=42, n=4) of the gastric disorders dogs. Other gastric disorders diagnosed were gastric dilatation, gastric impaction, gastric perforation, gastric neoplasms, gastric abscess, pyloric stenosis, gastric polyp, and pyloro-gastric intussusception which constituted 2.38 percent (N=42, n=1) each. Types of gastric disorders in dogs with vomiting are presented in table 2 and figure 2.

It was observed that gastric disorders were more common in young-adult age group (1-3 years, 42.85 per cent), followed by adult age group (3-6 years, 35.71 per cent), young (< 1year, 14.28 per cent), and aged (> 6 years, 7.14 per cent). The results of age wise occurrence of the gastric disorders are presented in the table 3 and figure 3. Among the different breeds under the study, the highest occurrence was seen in Labrador (26.19 per cent) followed by Rottweiler (21.42 per cent), German shepherd (14.28 per cent), Doberman (7.14 per cent), Pug and Mastiff (4.76 per cent each) and Boxer, Bull dog, Dalmatian, Golden retriever, Great Dane, Non-descript, Pomeranian and Spitz (2.38 per cent each).

The results of breed wise occurrence of the gastric disorders are presented in the table 4 and figure 4. The occurrence of gastric disorders in male dogs was 66.6 per cent while in females it was 33.4 per cent and is presented in the table 5 and figure 5. In the present study, gastric disorders were more prevalent in dogs maintained on a homemade diet (42.85 per cent) followed by mixed (30.95 per cent), and commercial (26.19 per cent) diets. The results of occurrence of gastric disorders in dogs based on the diet are presented in the table 6 and figure 6. Based on the duration of vomiting the conditions were categorized into per-acute (1 day), acute (2-5 days), sub-acute (5-7 days) and chronic (more than 7 days). The occurrence of per-acute, acute, sub-acute and chronic form of gastric disorders were 16.66, 42.85, 11.90, and 28.57 per cent respectively and are presented in the table 7 and figure 7.

The nature of vomiting was productive in 50 per cent of the cases followed by non-productive in 42.85 per cent and projectile in 7.14 per cent. The colour of vomitus included whitish (30.95 per cent), yellowish (21.42 per cent), watery (26.19 per cent), blood tinged (11.90 per cent), and brown (9.52 per cent). The consistency of the vomitus was viscid in 64.28 per cent, watery in 23.80 per cent and frothy in 11.90 per cent and the results are presented in table and figures 8. 9. 10. respectively.

DISCUSSION

Gastric disorders are common in dogs and the underlying etiology of each case differs significantly. The present study was carried out in 100 dogs brought to the Teaching Veterinary Clinical Complex, Pookode. The occurrence of gastric disorders in dogs with vomiting was found to be 42 per cent. Malancus*et al.* (2010) observed similar findings with 33 percent of gastric involvement in gastro-intestinal disorders in dogs.Disorders associated with vomiting in the present study were gastric disorders (42 per cent) renal disorders and infection (16 per cent each), intestinal disorders (9 per cent), haemoprotozoans (7 per cent), snake bite (3 per cent), reproductive disorders, liver disorders, and poisoning (2.3 per cent each) and pancreatic disorders (1 per cent).Malancus*et al.* (2010) reported that out of 98 cases with gastrointestinal disorders 33 had gastric disease, 23 had intestinal disease and the remaining 42 dogs had gastrointestinal disorders and ultrasound-diagnosed disease were represented gastritis 24 cases, pyloric hypertrophy (three patients), enteritis (22 cases) and intussusception (two cases).

Rakha*et al.* (2015) reported that the vomiting is caused due to foreign body ingestion (34.5 per cent), food indiscretion (18.5 per cent), drugs (12.3 per cent), pneumonia (10.8 per cent), liver (9.2 per cent), poisoning (4.6 per cent), motion sickness (3 per cent), IBD (9 per cent) and renal (0.9 per cent). The author also suggested that males were more prone to gastro-intestinal problems than females with more occurrences in aged dogs. Infectious causes were the second most disorder causing vomiting in the present study and this was in accordance with Rakha*et al.* (2015) and this might be due increased incidence of parvo-viral infection during the study period. Increased incidences of vomiting due to renal disorders (16%) were also observed in the present study when compared to Rakha*et al.* (2015). Where they observed only 0.6 per cent of the total cases.Gastric disorders seen in vomiting dogs were gastritis (47.61 percent), gastric ulcers (14.28 percent), and gastric foreign body (9.52 percent). This was in contrary with the findings of Rakha*et al.* (2015) where he observed gastric foreign body (34.5 percent), followed by gastritis (30.8% percent). Increased occurrence of gastritis observed in the present study may be due to voracious and indiscriminate eating habit or variation in the composition of the diet.Gastric disorders were commonly observed in young-adult group followed by adult group and this was in contrary with the findings of Mahesh *et al.* (2015) where they observed more gastro-intestinal lesions in aged dogs. High occurrence of gastric disorders in young-adult dogs may be due to more number of young-adult enrolled in the present study and voracious or indiscriminate feeding habits in young-adult dogs.

The incidence of gastric disorders was found to be high in Labrador, followed by Rottweiler, German shepherd and Doberman. This might be due to over presentation of the Labrador in the study area and this was in good agreement

with Mahesh *et al.* (2015).The present study revealed that males over represent the affected population, contributing 66.66 per cent of the cases with gastric involvement. This corroborates with the reports of Patnaik *et al.* (1977), Twedt (1992), Cornell and Selcer, (2002) and Pietra*et al*. (2003), Jankowski *et al.* (2015) and Mahesh *et al.* (2015). This may be due to over-presentation of male dogs in this area.

Gastric disorders were commonly seen in dogs fed with home-made diet and may be due to variation in the composition of daily diet. Most of the dogs presented to the clinics with vomiting as a complaint were of acute form (45.33 per cent) this may be due to more occurrence of gastritis. Productive vomiting was seen in 50.00 per cent of the dogs due to more prevalence of gastritis. Non-productive vomiting was seen in 42.85 per cent of the cases which was consistent finding in GDV (Brooks, 2016). The colour of vomitus was whitish (40.80 percent), might be due to chyme and was seen primarily in gastritis, followed by watery (26.19 percent) seen in gastritis and GDV, yellowish (21.42 percent) due to bile reflux, blood tinged and brown (11.90 and 9.52 per cent respectively) may be due to bleeding from the gastric mucosa, gastric foreign bodies and gastric neoplasms. This was also observed by Halfacree (2010), Parrah*et al.* (2013) and Nath*et al.* (2015). The consistency of the vomitus was viscid (64.28 per cent) due to gastritis, watery (23.90 per cent) due to anorexia and frothy (11.90 percent) due to unproductive vomiting.

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Table 1. Disorders associated with vomiting in dogs

Sl No.	DISEASES		No. of Cases	% of Cases
a)	**GASTRIC Disorders**		42	42
b)	**Non Gastric disorders**			
1.	Intestinal	Parasites	3	3
		Obstruction	2	2
		Paralytic ileus	3	3
		Anal adenitis	1	1
2.	Haemoprotozoans	Babesiosis	3	3
		Ehrlichiosis	2	2
		Microfilariosis	2	2
3.	Infectious	Bacterial	4	4
		Viral	12	12
4.	Liver disorders		2	2
5.	Kidney disorders		16	16
6.	Reproductive disorders		2	2
7.	Pancreatic disorders		1	1
8.	Snake bites		3	3
9.	Poisoning		2	2
	Total		100	100

Table 2. Types of gastric disorders in vomiting dogs

Sl No.	GASTRIC DISORDERS	No. of Cases	% of Cases
1.	Gastritis	20	47.61
2.	Gastric Ulcer	6	14.28
3.	Gastric foreign bodies	4	9.52
4.	Gastric dilatation and volvulus	4	9.52
5.	Gastric dilatation	1	2.38
6.	Gastric impaction	1	2.38
7.	Gastric perforation	1	2.38
8.	Gastric neoplasm	1	2.38
9.	Gastric abscess	1	2.38
10.	Pyloric stenosis	1	2.38
11.	Gastric polyp	1	2.38
12.	Pyloro-gastric intussusception	1	2.38
	Total	**42**	**100**

Table 3. Age-wise occurrence of Gastric disorders in dogs with vomiting

AGE	Group I Gastritis	Group II Gastric ulcer	Group III Gastric foreign body	Group IV GDV	Group V Other gastric disorders								Total	%
					GD	GI	GP	GN	GA	PS	GP	PGI		
Young	1	0	4	0	0	0	0	0	0	0	0	0	5	11.90
Young-adult	10	1	0	3	0	1	0	0	1	1	0	0	17	40.47
Adult	8	4	0	1	1	0	1	0	0	0	0	0	15	35.71
Aged	1	1	0	0	0	0	0	1	0	0	1	1	5	11.90
Total	20	6	4	4	1	1	1	1	1	1	1	1	42	100

Table 4. Breed-wise occurrence of Gastric disorders in dogs with vomiting

Sl No.	Breeds	Gastric disorder in dogs	
		No. of Animals	Percentage
1.	Labrador	11	26.2
2.	Rottweiler	9	21.4
3.	German shepherd	6	14.2
4.	Doberman	3	7.14
5.	Pug	2	4.76
6.	Bull Mastiff	2	4.76
7.	Boxer	1	2.38
8.	Bull dog	1	2.38
9.	Dachshund	1	2.38
10.	Dalmatian	1	2.38
11.	Golden retriever	1	2.38
12.	Great Dane	1	2.38
13.	Non-descript	1	2.38
14.	Pomeranian	1	2.38
15.	Spitz	1	2.38
	Total	**42**	**100**

Table 5. Sex-wise occurrence of gastric disorders in dogs

Sex	Gastritis	Gastric ulcer	Gastric foreign body	GDV	Other gastric disorders								Total	%
					GD	GI	GP	GN	GA	PS	GP	PGI		
Male	13	4	3	3	0	0	1	1	0	1	1	1	28	66.7
Female	7	2	1	1	1	1	0	0	1	0	0	0	14	33.3

Table 6. Occurrence of gastric disorders in dogs based on the diet

DIET	Gastritis	Gastric ulcer	Gastric foreign body	GDV	Other gastric disorders								Total	%
					GD	GI	GP	GN	GA	PS	GP	PGI		
Home	8	4	2	1	1	0	1	0	1	0	0	0	18	42.85
Commercial	5	1	0	2	0	0	0	1	0	1	1	0	11	26.19
Mixed	7	1	2	1	0	1	0	0	0	0	0	1	13	30.95
Total	20	6	4	4	1	1	1	1	1	1	1	1	42	100

Table 7. Classification of gastric disorders in dogs based on the duration of illness

Duration	Gastritis	Gastric ulcer	Gastric foreign body	GDV	Other gastric disorders								Total	%
					GD	GI	GP	GN	GA	PS	GP	PGI		
Per acute	1	0	1	4	0	0	0	0	0	0	0	1	7	16.67
Acute	12	3	1	0	1	0	0	0	1	0	0	0	18	42.85
Sub-acute	2	1	1	0	0	0	1	0	0	0	0	0	5	11.90
Chronic	5	2	1	0	0	1	0	1	0	1	1	0	12	28.57
Total	20	6	4	4	1	1	1	1	1	1	1	1	42	100

GD: Gastric dilatation; GI: Gastric intussusception; GPe: Gastric perforation; GN: Gastric neoplasm; GA: Gastric abscess; PS; pyloric stenosis; GP : Gastric polyp; PGI : Pyloro-gastric intussusceptions

Table 8. Nature of vomiting in dogs with gastric disorders

Nature	Gastritis	Gastric ulcer	Gastric foreign body	GDV	Other gastric disorders								Total	%
					GD	GI	GP	GN	GA	PS	GP	PGI		
Productive	16	2	2	0	1	0	0	0	0	0	0	0	21	50
Non productive	4	4	2	4	0	1	1	1	1	0	0	0	18	42.85
Projectile	0	0	0	0	0	0	0	0	0	1	1	1	3	7.14
Total	20	6	4	4	1	1	1	1	1	1	1	1	42	100

Table 9. Colour of vomitus in dogs with gastric disorders

Colour	Gastritis	Gastric ulcer	Gastric foreign body	GDV	Other gastric disorders								Total	%
					GD	GI	GP	GN	GA	PS	GP	PGI		
Whitish	7	0	1	1	0	0	1	0	1	1	1	0	13	30.95
Watery	5	0	0	3	1	1	0	0	0	0	0	1	11	26.19
Yellow	6	2	1	0	0	0	0	0	0	0	0	0	9	21.42
Blood tinged	0	2	2	0	0	0	0	1	0	0	0	0	5	11.90
Brown	2	2	0	0	0	0	0	0	0	0	0	0	4	9.52
Total	20	6	4	4	1	1	1	1	1	1	1	1	42	100

Table 10. Consistency of vomitus in dogs with gastric disorders

Consistency	Gastritis	Gastric ulcer	Gastric foreign body	GDV	Other gastric disorders								Total	%
					GD	GI	GP	GN	GA	PS	GP	PGI		
Viscid	15	4	3	0	1	0	0	1	1	1	1	0	27	64.28
Watery	5	2	0	0	0	1	1	0	0	0	0	1	10	23.80
Frothy	0	0	1	4	0	0	0	0	0	0	0	0	5	11.90
Total	20	6	4	4	1	1	1	1	1	1	1	1	42	100

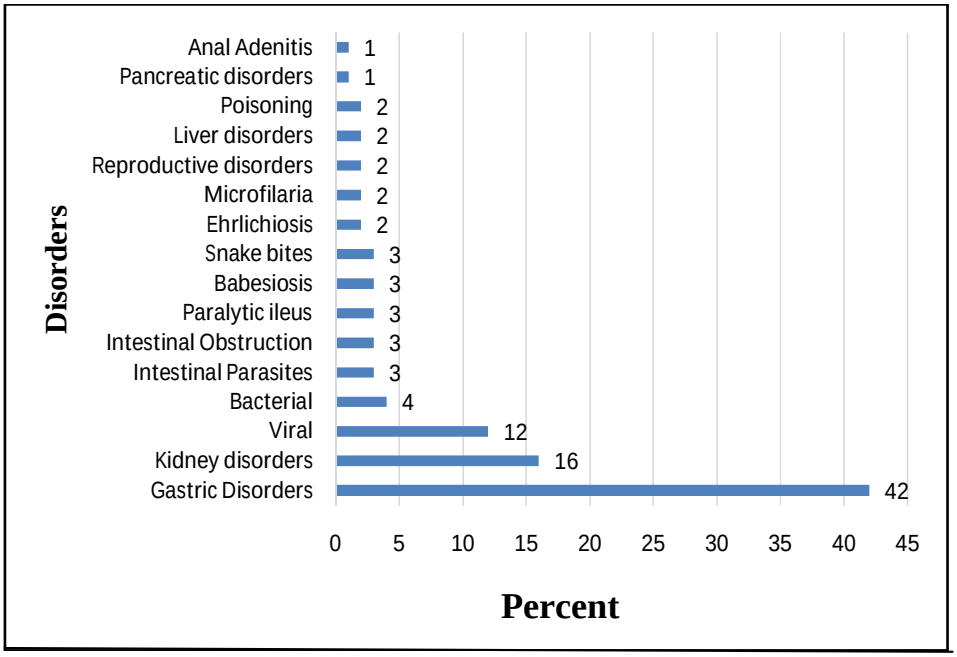

Figure 1. Disorders associated with vomiting in dogs

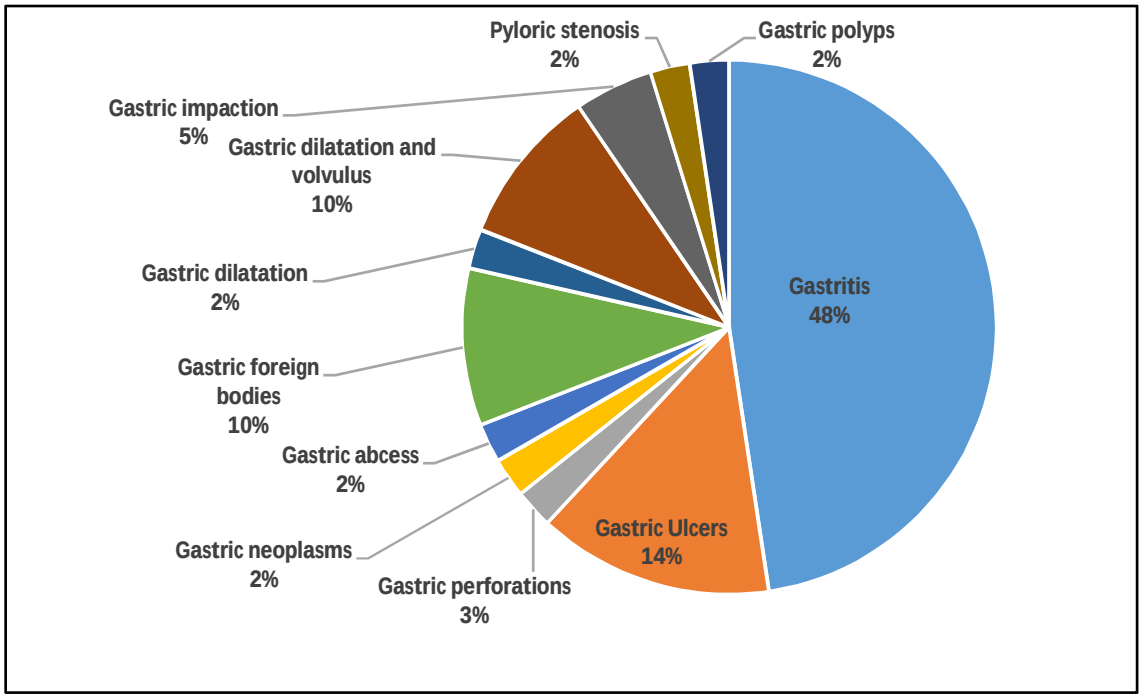

Figure 2. Types of gastric disorders in vomiting dogs

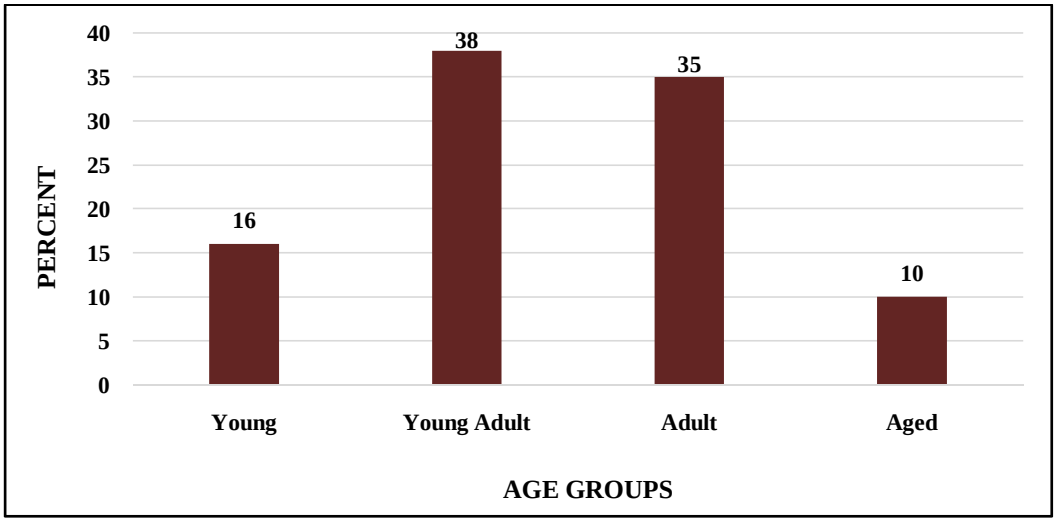

Figure 3. Age-wise occurrence of gastric disorders in dogs

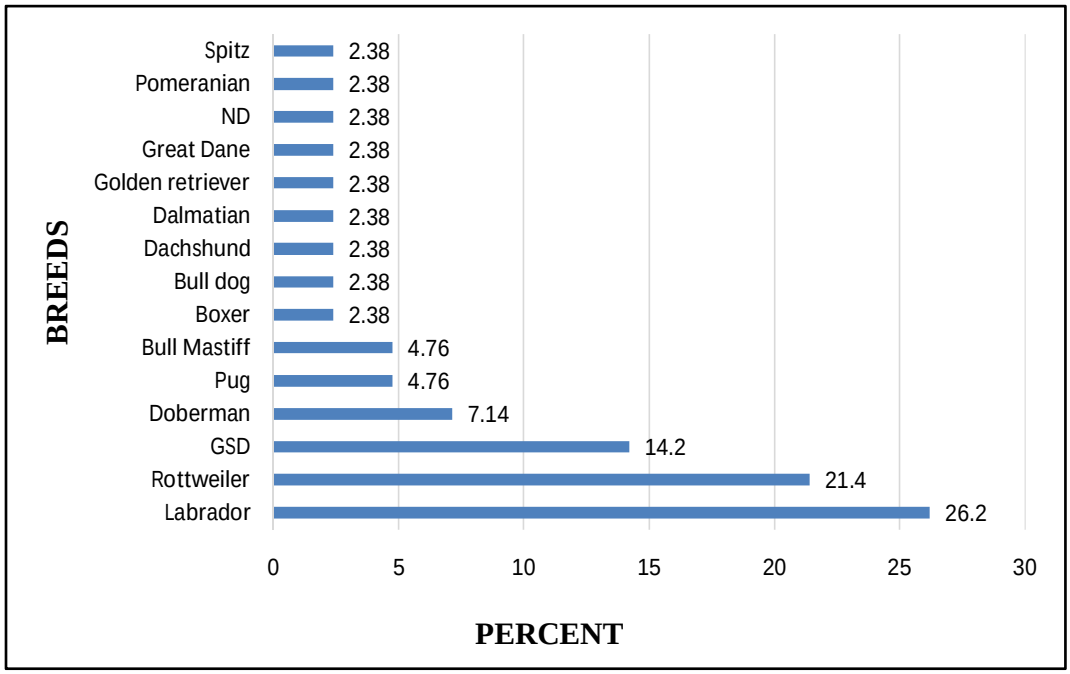

Figure 4. Breed-wise occurrence of gastric disorders in dogs

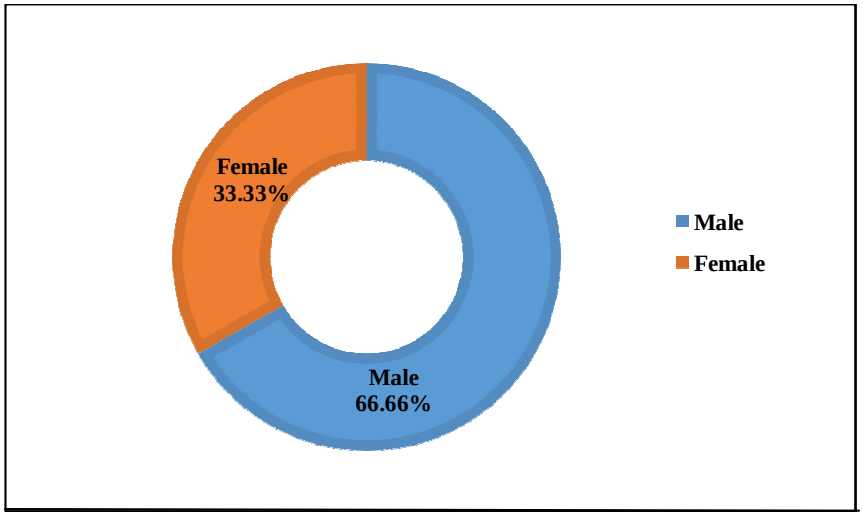

Figure 5. Sex-wise occurrence of gastric disorders in dogs

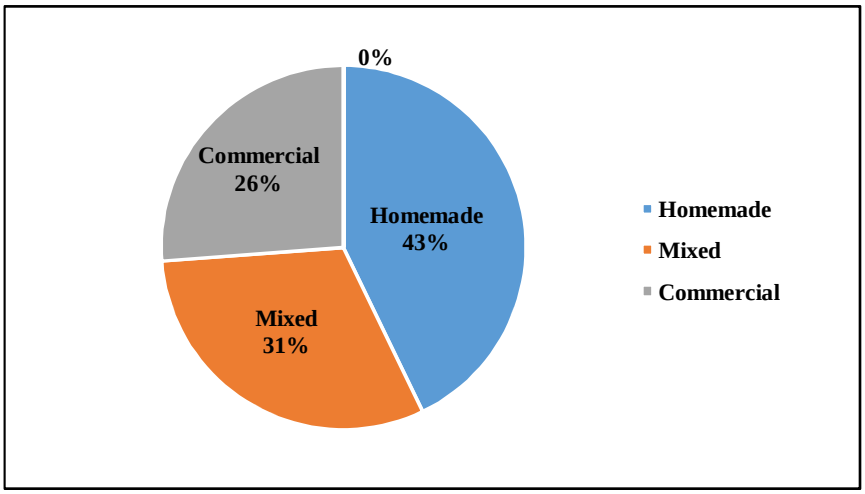

Figure 6. Occurrence of gastric disorders based on the diet in dogs

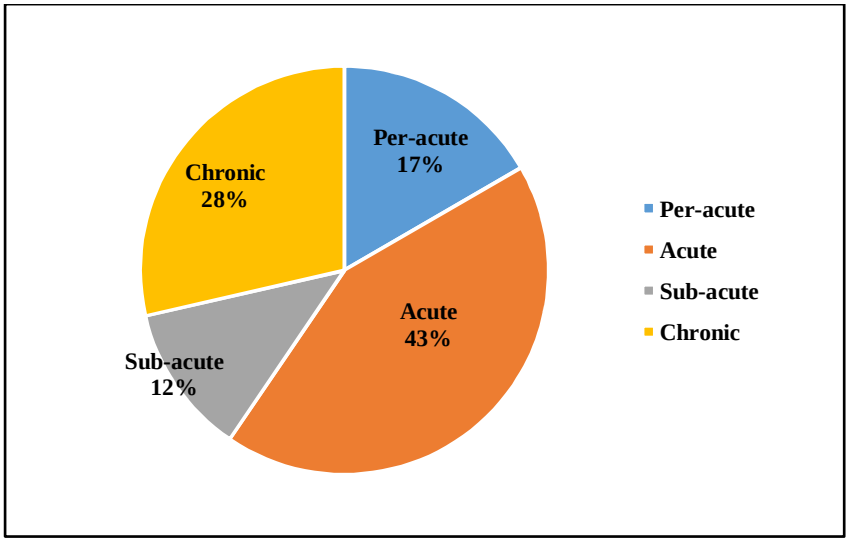

Figure 7. Classification of gastric disorders based on the duration of illness

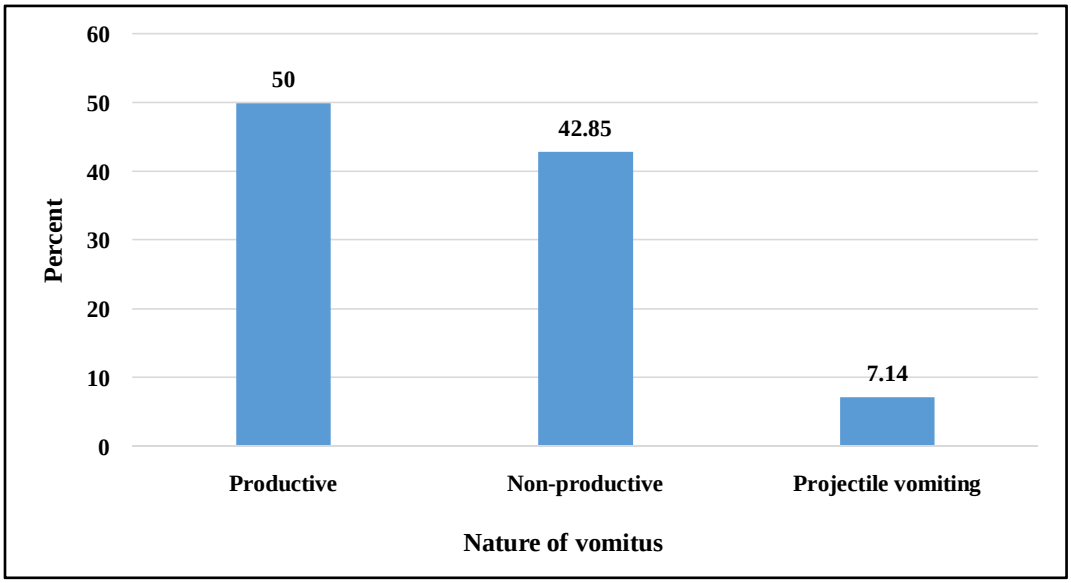

Figure 8. Nature of vomiting in dogs with gastric disorders

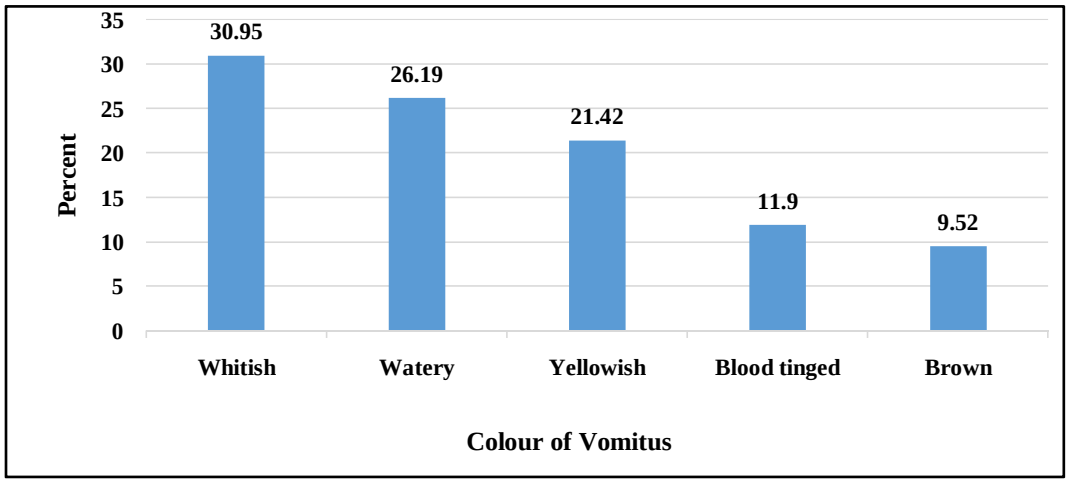

Figure 9. Colour of vomitus in dogs with gastric disorders

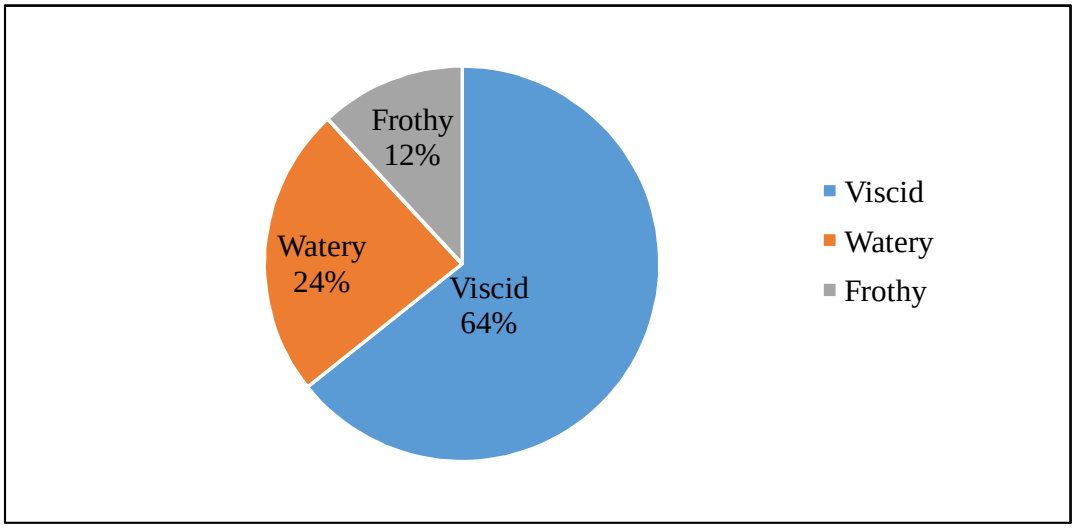

Figure 10. Consistency of vomitus with gastric disorders

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RESEARCH ARTICLE

Effective Treatment of Dyeing Effluent by using Chemical Coagulants

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ABSTRACT

Textile dyeing units require large quantity of water to color their products and release detached dye molecules, salts and acids as colored wastewater to the environment and cause pollution. In the present study, textile effluent was collected from fabric dyeing unit and the physical-chemical characteristics such as pH, color, odor, total solids, total dissolved solids, total suspended solids, total hardness, alkalinity, chemical oxygen demand and chloride were found to be 10.3, wine red,objectionable, 5200mg/l, 3800mg/l, 1400mg/l, 430mg/l, 730mg/l, 1120mg/l respectively. The parameters were compared with BIS standard effluent discharge limit. The heavy metals such as chromium, lead and nickel were found to be below detectable level in the effluent. The effluent was subjected to treatment using different dosages (0.2g, 0.4g, 0.6g, 0.8g and 1.0g) of chemical coagulants -calcium oxide and ferric chloride for the removal of color and TDS only. Accurately, 0.6g of CaO treatment showed about 100% of color and 89% of TDS removal. The ferric chloride treatment showed the color pollution even 0.3g treatment and so on due to oxidation of iron salts. Among the treatments, 0.6g of CaO is an effective coagulant dose for the treatment of dye effluent.

Key words: - Physico-chemical parameters, Coagulation, TDS, color, dye effluent

INTRODUCTION

Water is a major natural resource which plays a vital role in the growth and development of all living organisms. The population explosion, rapid growth of various industries, climate change and urbanization increase the demand for potable water (Xiaolei Qu *et al*., 2013; Wu *et al*., 2013). Textile industry is one of the major polluter of the environment which utilize huge amount of water and generate colored wastewater with toxic dye materials (Ali and El-Mohamedy, 2012). The unwanted dye substances from the dyeing process are released into the environment especially aquatic system with high concentration of color, pH, biological oxygen demand, turbidity, heavy metals, chemical oxygen demand, and noxious compounds. The dyes present in the effluent are difficult to degrade due to high molecular weight and its structure (Akshaya *et al*., 2012). About 30,000 tons of various synthetic dye materials were manufactured and used for industrial purpose. From this, approximately 1-10% of dye substances were discharged into the environment after process (Beltran- Heredia *et al*., 2011).

Synthetic dyes are more water soluble and detectable in water even in very low concentration. These dyes affect the transparency of water and prevent the sunlight penetration to the aquatic system which affects the photosynthesis process of aquatic vegetation (Sarwan *et al*., 2012). Dyes are very difficult to degrade because of its complex aromatic structure, strong coloring nature and resistance to fade (Akhil *et al*., 2012).Coagulation- flocculation is a traditional method widely used for the effective treatment of dyeing effluent (Solanki *et al*., 2013). The coagulating chemicals alter the physical condition of the dispersed particles and facilitate their deduction through sedimentation (Huang *et al*., 2009). In the present study, the dyeing effluent was treated using chemical coagulants for the reduction of color and total dissolved solids.

MATERIALS AND METHODS

Collection of dyeing Effluent

The textile dyeing effluent was obtained from a typical dyeing unit located at Tamilnadu.Dye effluent was collected in Pre-cleaned polyethylene bottle and stored at 4ºC for further analysis.

Physico-chemical Characterization of Effluent

The physical and chemical characteristics such as pH, color, total solids, total dissolved solids, total suspended solids, alkalinity, total hardness, chemical oxygen demand, chloride were analyzed based on the standard analytical method. The heavy metals like chromium, lead and nickel were examined by Atomic absorption spectroscopy (APHA, 2012).

Properties of Chemical Coagulants

The chemical coagulants used for the study was obtained from Merck and the chemicals are analytical grade and used without changing the nature and purity of the chemicals (Table-1).

Effect of Chemical coagulants on the removal of color and TDS from the effluent

The impurities present in the dye effluent were higher than the BIS permissible effluent discharge limit. The sample was subjected to treatment with chemical coagulating agents includes calcium oxide and ferric chloride. The effluent was taken in series of Erlenmeyer flask and added different dosages of 0.2g, 0.4g, 0.6g, 0.8g and 1.0g of chemical coagulants such as calcium oxide and ferric chloride followed by mixed well and kept it for one hour of retention time. After that, the sample was filtered through Whatman No.42 filter paper and the filtrate was subjected to analyze the removal of color and TDS.

RESULTS AND DISCUSSION

Physico-chemical characteristics of the dyeing effluent

The physical and chemical parameters were analyzed and depicted in the table-2. The color of the sample was found to be wine red color due to the presence of red reactive dyes (Red F3B, R3B dyes) for dyeing process. The pH of the effluent was alkaline (11.2) which indicates the presence of salts and heavy usage of dyes substances. The pH instability affects the buffering capacity of the aquatic system and cause harmful effect to the living things. Neetika Mathur and Ashwani Kumar (2013) also reported the alkaline nature of the textile effluent.

Arul *et al*., (2011) also reported that the pungent and unpleasant odour in their sample. Total solids, total dissolved solids and total suspended solids were found to be 5200mg/l, 3800mg/l and 1400mg/l respectively. The elevated concentration of dissolved solids increases the turbidity and prevents the light penetration leads to oxygen depletion which causes severe effect to the aquatic flora and fauna. Similar result was reported by Arun Prasad and Bhaskara Rao (2010).

The hardness of the dyeing effluent was 430mg/l signify the occurrence of calcium ions and magnesium ions as $CaCO_3$ and its adverse effect was not reported (Neeta *et al*., 2011). Alkalinity and COD of the sample was 730mg/l and 2120mg/l. Higher COD content estimate the presence of biologically resistant organic pollutants. Goyal Varsha *et al*., (2013) reported the COD value from the range of 1170mg/l to 3998mg/l in their result. The heavy metal such as chromium, zinc and lead were found to be below detectable level in the sample.

Effect of CaO on the removal of color and TDS from the effluent

The colour causing substances from the effluent should have been degraded when the dye effluent was treated with different dosages of CaO. Complete colour removal was noticed when the dye effluent was treated with 0.6g, 0.8g and 1g of CaO treatment. This removal must have been achieved by the oxidation process of hydroxyl radicals donated by CaO. Similarly Inthorn *et al.* (2001) have reported that the hydroxyl radicals generated are capable of degrading the contaminants especially chromophore or chromogen through the process of oxidation during the spent wash treatment.About 80% of TDS was removed from the dye effluent when it was treated with 0.6g of CaO. It must be the optimum dosage as it has resulted in the highest removal of TDS, after that when increase the dosage of (0.8g, 1g) CaO, has increased the TDS content of dye effluent. It may be the excess amount of CaO will have dissolved in water and might have increased the TDS content of dye effluent after the addition of excess CaO.

Similarly Vasanthy *et al.* (2006) have reported that 0.75 g of CaO is the optimum dosage for the highest removal of TDS and COD from the 5% sugar wash.

Effect of $FeCl_3$ on the removal of color and TDS from the effluent

About 90% of color reduction and 78% of TDS reduction were obtained in the treatment of dye effluent with 0.2g of $FeCl_3$. When increase the dosage of coagulants slightly turbidity and yellow colour were developed followed by TDS also increased. It may be by the excess addition of ferric chloride, the unbound or non-precipitated iron molecules lead to the turbidity of the solution (Zhen Liang *et al*., 2009). Ferric chloride is water soluble, pH dependant, hydrolyzing metallic salts which coagulate the pollutants through charge neutralization (Peavy *et al*., 1985; Abo-Farha, 2010). The parameters were analyzed after treatment and notable reduction was observed. The pH of the sample was reduced to acidic due to the reaction of chloride ions present in Ferric chloride with hydrogen ions and produce hydrochloric acid which involved in the decrease of pH in alkaline dye effluent (Lenntech, 2009).

CONCLUSION

In the present study, the level of impurities was higher in dye effluent than the BIS-permissible effluent discharge limit. The chemical coagulants showed distinctive reduction of color and TDS from the effluent. Among the treatments, 0.6g of CaO treatment showed good removal of colour and TDS. And it doesn't cause any secondary pollution like colour unlike ferric chloride. Hence, CaO can be used as effective coagulants for the treatment of dye effluent. Also the sludge can be further used to make cement and brick manufacturing industries.

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Table 1. Properties of Calcium oxide and Ferric chloride

S.No.	Properties	Calcium Oxide	Ferric chloride
1.	Molecular Formula	CaO	$FeCl_3$
2.	Molecular Weight	56.0774g/mol	162.24g/mol
3.	Solubility	Water soluble	Water soluble
4.	Melting point	2570ºC	304 ºC
5.	Boiling point	2850 ºC	316 ºC

Table.2- Physical and chemical characteristics of dyeing effluent

S.No.	Parameters	Results	BIS standard
1	Color	Wine red color	Colorless
2	Odor	Objectionable	Odorless
3	pH	11.2	6-8
4	TS	5200mg/l	-
5	TDS	3800mg/l	500mg/l
6	TSS	1400mg/l	-
7	Alkalinity	730mg/l	-
8	Hardness	430mg/l	600mg/l
9	COD	2120mg/l	250mg/l
10	Chloride	1233mg/l	250mg/l
11	Chromium	Below Detectable Level	2.0mg/l
12	Zinc	Below Detectable Level	5.0mg/l
13	Lead	Below Detectable Level	0.1mg/l

Table- 3. Effect of chemical coagulants on the removal of impurities from the effluent

S.No.	Dosage (g)	Calcium oxide			Ferric chloride		
		pH	Color (%)	TDS (%)	pH	Color (%)	TDS (%)
1.	0.2	Alkaline	75	58	7.8	90	78
2.	0.4	Alkaline	89	63	7.2	79	61
3.	0.6	Alkaline	100	80	6.6	72	48
4.	0.8	Alkaline	100	73	5.1	58	43
5.	1.0	Alkaline	100	72	4.0	39	31

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RESEARCH ARTICLE

Performance Evaluation of Chaetoglobosin Biomolecule against Late Blight Disease on Potato

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ABSTRACT

Chaetoglobosin is a secondary metabolite produced by the fungus *Chaetomium globosum.* In this present experiment the efficacy of chaetoglobosin was tested against the fungus *Phytophthora infestans* causing late blight disease on potato in four different concentrations viz., 500, 1000, 1500 and 2000 ppm. *Invitro* evaluation of Chaetoglobosin against *P.infestans* recorded 58.32 per cent inhibition of mycelial growth at 2000 ppm concentration whereas at 1500 ppm 51.31 per cent mycelial inhibition over control was observed. In the case of biomass production chaetoglobosin at 2000 ppm and 1500 ppm concentrations recorded 54.86 and 33.45 per cent respectively. Among the different treatments in the bioefficacy trial, azoxystrobin in combination with chaetoglobosin recorded 82.01 per cent disease reduction (11.20 PDI) over control. The combination of azoxystrobin with metalaxyl was more effective in controlling the late blight disease of potato which recorded 86.87 per cent disease reduction (8.17 PDI) over control. Combined application of chaetoglobosin with metalaxyl showed 79.61 per cent disease reduction (12.69 PDI). The individual application of azoxystrobin, chaetoglobosin and metalaxyl recorded 77.97, 62.90 and 72.87 per cent disease reduction over control respectively.

Key words: - Chaetoglobosin, Llight, Phytophthora, bioefficacy.

INTRODUCTION

Chaetomium species are normally found in soil and organic compost and it is one of the largest genera of saprobic ascomycetes with more than 300 species distributed worldwide (Arx *et al.,* 1986; Soytong and Quimio, 1989). *Chaetomium* species are potential degraders of cellulosic residues and other organic materials (Domsch *et al.,* 1972). In

addition, the antagonistic abilities of *Chaetomium* species against several phytopathogens have been well established. *Chaetomium* spp have also been reported to be potentially antagonistic to seed borne pathogens (Dhingra *et al.,* 2003; Aggarwal *et al.,* 2004; Park *et al.,* 2005). The emergence of antibiotic resistant microorganisms has resulted in an urgent need to find out newer antimicrobial drugs with novel modes of action against phytopathogens (Guo *et al.,* 2008). It is apparent that an enormous and relatively untapped source of biological diversity is represented by microbial endophytes which are the promising sources of novel and natural products for use in agriculture and industry. The range of chemicals produced by endophytes is diverse. Like their host plants, synthesize a wealth of secondary metabolites which are not directly involved in the metabolism of the micro-organisms; but, play a role in the fitness and survival of themselves and their hosts (Schulz *et al.,* 2008). Endophytes are known to produce biologically highly active alkaloids, peptides, steroids, terpenoids, isocoumarins, quinones, phenylpropanoids, lignans, phenols, phenolic acids, aliphatic compounds, lactones, and others (Gunatilaka, 2006; Strobel *et al.,* 2004; Tan and Zou, 2001 and Zhang *et al*., 2006). Ting *et al.,* (2010) reported that fungal endophytes have the ability to produce several types of volatile metabolites, which were found to inhibit the growth of *F. oysporum* f.sp. *cubens*e Race 4.Chaetoglobosin is a secondary metabolite isolated from the fungus *Chaetomium globosum.* Sibounnavong *et al.,* (2011) reported that Chaetoglobosin-C had showed greater antifungal activity against *F. oxysporum* f. sp. *lycopersici*, with an effective dose (ED50) of 5.98 μg / ml. Di Pietro *et al.,* (1992) reported that *C. globosum* had produced chetomin, which effectively inhibited *Pythium ultimum*, the damping-off pathogen in sugar beet. *C. globosum* strain KMITL 0802 has been shown to produce chaetoglobosin - C (Kanokmedhakul *et al.,* 2002). Park *et al.,* (2005) isolated chaetoviridin A from *C. globosum* strain F0142 and successfully contained rice blast, wheat leaf rust and tomato late blight pathogens. Soytong (1992) and Soytong *et al.,* (2001) showed that a specific isolate of *Chaetomium cupreum* had produced secondary metabolites, which were found to significantly suppress tomato wilt caused by *F. oxysporum* f.sp. *lycopersici* in Thailand. The same isolate also produced rotiorinols A to C and rotiorin, which exhibited antifungal activity against *Candida albicans* (Kanokmedhakul *et al.,* 2006). *Chaetomium cochlioides* - strains VTh01 and CTh05 produced sufficient quantities of chaetoglobosin and exhibited antimicrobial activities against a *Phytophothora* sp, *Colletotrichum gloeosporioides*, and *F. oxysporum* f. sp. *lycopersici* (Pornsuria *et al.,* 2008).With this background the present investigation was carried out to estimate the efficacy of chaetoglobosin biomolecule against *Phytophthora infestans* causing late blight disease in potato.

MATERIALS AND METHODS

Determination of antifungal activity of chaetoglobosin

The antifungal activity of chaetoglobosin produced by the endophytic *Chaetomium* isolate Ch-1 was evaluated under *in vitro* by poisoned food technique (Schmitz, 1930). An eight mm diameter mycelial disc of seven d old pathogen culture was aseptically placed at the centre of Petri plates containing the PDA medium amended with the extracted chaetoglobosin at 500, 1000, 1500 and 2000 ppm. Growth medium inoculated without chaetoglobosin served as control. The plates were incubated at 28 ± 2°C until the control plate was fully covered by the test fungus. Each treatment was replicated four times. The per cent growth inhibition of the test pathogen was calculated by using the following formula:

$$I = \frac{C-T}{C} \times 100$$

Where, I = Per cent inhibition

C = Growth in control

T = Growth in treatment

Efficacy of Chaetoglobosin on Biomass Production of *P. infestans*

One hundred ml of potato dextrose broth was transferred to 250 ml Erlenmeyer flasks, autoclaved at 1.4 kg / cm^2 for 20 min and cooled at room temperature. The test fungicides were suspended aseptically in sterile distilled water and added to the broth to get the required final concentration of 500, 1000, 1500 and 2000 ppm. The flasks were inoculated separately with a 15 d old nine mm disc of the respective isolate of *P.infestans*. The flasks were incubated at room temperature (28 $\pm$ 2 ^{o}C) for 20 d. Three replications were maintained for each isolate. The mycelial mat was filtered through Whatman No. 1 filter paper, dried in the hot air oven at 60^{o}C for 24 h till a constant weight was obtained. The mycelial dry weight was calculated as per procedures suggested by Awadhiya, 1991.

Bioefficacy of Chaetoglobosin in Comparison to Azoxystrobin against Late Blight disease on Potato

The chaetoglobosin extracted from the endophytic *Chaetomium* and a new formulation of azoxystrobin obtained from Willowood Agrochemicals Pvt.Ltd, NewDelhi were used in the present study. These two biomolecules were compared with commercially available fungicide metalaxyl against late blight of potato. All the fungicides at 0.2 per cent concentration (2 g / l) were sprayed as per the treatments given below:

Treatments:

T1	Azoxystrobin
T2	Chaetoglobosin
T3	Metalaxyl
T4	Azoxystrobin + Metalaxyl
T5	Azoxystrobin + Chaetoglobosin
T6	Metalaxyl + Chaetoglobosin
T7	Control

RESULTS AND DISCUSSION

Chaetoglobosin at 2000 ppm concentration recorded 58.32 per cent inhibition of mycelial growth of *P.infestans,* whereas at 1500 ppm it was recorded 51.31 per cent mycelial inhibition over control. In the case of biomass production, chaetoglobosin at 2000ppm concentration showed the highest inhibition of biomass production of *P. infestans* (54.86 per cent). The next highest inhibition recorded at 1500 ppm exhibited 33.45 per cent reduction of biomass production. Results of this experiment clearly revealed that the antifungal potential of chaetoglobosin increased with a concomitant increase in the concentration used. Soytong, (2007) reported that the bioactive compound extracted from *Chaetomium cochliodes* and *C. cupreum* inhibited plant pathogenic fungi, *Phytophthora palmivora* (root rot of Pomelo) and *Fusarium oxysporum* f. sp. *lycopersici* (tomato wilt). Charoenpoen *et al.,* (2010) reported that crude extract of *Chaetomium lucknowense* CLT significantly inhibited the mycelial growth and conidial production of *F.oxysporum* f. sp. *lycopersici.* The bioefficacy study of the present experiment reveals that, azoxystrobin in combination with chaetoglobosin recorded 82.01 per cent disease reduction (11.20 PDI) over control. Combined application of chaetoglobosin with metalaxyl showed 79.61 per cent disease reduction (12.69 PDI). But, the combination of azoxystrobin with metalaxyl showed the maximum reduction of late blight incidence on potato which recorded 86.87 per cent (8.17 PDI) over control. The individual application of azoxystrobin, chaetoglobosin and metalaxyl recorded 77.97, 62.90 and 72.87 per cent disease reduction over control respectively. Through this study it is evident that the biomolecule chaetoglobosin is having the ability to control the fungal pathogens. But the ability is comparatively lesser than the commercially available fungicides. It may be improved by identifying proper extraction procedure and ideal concentrations towards the ecofriendly management of plant diseases.

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Table 1.*In vitro* evaluation of chaetoglobosin against *P. infestans*

Chaetoglobosin concentration (ppm)	*P. infestans*	
	Mycelial growth (mm)	Per cent inhibition over control
500	67.17[d] (55.04)	25.36
1000	61.35[c] (51.56)	31.83
1500	43.82[b] (41.45)	51.31
2000	37.51[a] (37.76)	58.32
Control	90.00[e] (71.56)	-

Mean of four replications
Values in parentheses are arcsine-transformed
In a column, means followed by same letter are not significantly different at the 5 per cent level by DMRT

Table 2.Efficacy of chaetoglobosin on biomass production by *P. infestans*

Biomolecules (ppm)	*P. infestans*	
	Mycelial dry weight (mg)	Percent inhibition over control
500	406.33[g]	15.94
1000	375.66[f]	22.28
1500	321.66[e]	33.45
2000	218.00[d]	54.86

Mean of three replications
In a column, means followed by same letter are not significantly different at 5 per cent level by DMRT

Table.3 Bioefficacy of chaetoglobosin against *P. infestans* on potato

Treatment	PDI				Per cent decrease over control
	Before Spray	After I spray	After II spray	After III Spray	
Azoxystrobin 0.2%	32.26 [d] (36.03)	23.65 [c] (29.09)	18.99 [d] (25.83)	13.71 [d] (21.73)	77.97
Chaetoglobosin 0.2 %	30.54 [b] (35.39)	28.82 [f] (32.46)	27.34 [f] (31.52)	23.09 [f] (28.72)	62.90
Metalaxyl 0.2%	35.63 [g] (37.25)	27.64 [e] (31.71)	22.07 [e] (28.02)	16.88 [e] (24.25)	72.87
Azoxystrobin 0.2% + Metalaxyl 0.2 %	31.79 [c] (35.86)	22.50 [a] (28.31)	14.92 [a] (22.72)	8.17 [a] (16.60)	86.87
Azoxystrobin 0.2% + Chaetoglobosin 0.2 %	33.15 [e] (36.36)	23.27 [b] (28.84)	16.35 [b] (23.85)	11.20 [b] (19.55)	82.01
Metalaxyl 0.2% + Chaetoglobosin 0.2 %	29.37 [a] (34.95)	26.22 [d] (30.80)	18.33 [c] (25.35)	12.69 [c] (20.86)	79.61
Control	34.13 [f] (36.72)	41.56 [g] (40.14)	49.89 [g] (44.93)	62.24 [g] (52.08)	0.00

Mean of three replications

Values in parentheses are arcsine-transformed

In a column, means followed by same letter are not significantly different at the 5 per cent level by DMRT

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RESEARCH ARTICLE

Image Mining in Tumor Detection in Brain using Sushisen in Arima Model

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ABSTRACT

Detection of brain tumour is very common casualty in current scenario of health maintenancehumanity.Image segmentation is used to extract the abnormal tumour portion in brain. Brain tumor is an abnormal mass of tissue in which cells grow and multiply uncontrollably, apparently unregulated by mechanisms that control cells. Several techniques have been developed for detection of tumor in brain. Our main concentration is on the techniques which use image mining in sushisen (SSS) algorithms arima model segmentation to detect brain tumor. The experiment is performed on 240 tumor contained brain MR images from the Internet Brain Segmentation Repository.Discrete Wavelet Transform was applied on the training sets using BRATS 2016. Tumor classification and segmentation from brain computed tomography image data is an important but time consuming task performed by medical experts.

Key words: - Brain Tumor, SSS, Image Segmentation, sushisen, arima,

INTRODUCTION

Segmentation of medical images is challenging due to poor image contrast and artifacts that result in missing or diffuse tissue boundaries. We present a discrete wavelet based sushisen algorithm is proposed to detect the MR brain Images. First, MR images are enhanced using discrete wavelet descriptor, and then sushisenalgorithm is applied to detect the tumor pixels. A sushisen algorithm is then used in order to determine the best combination of information extracted by the selected criterion. The present approach uses k-Means unsupervised clustering methods into Sushisen Algorithms for guiding this last Evolutionary Algorithm in his search for finding the optimal or sub-

optimal data partition task that as we know, and requires a non-trivial search because of its intrinsic NP-complete nature. To solve this task, the appropriate sushisen coding is also discussed since this is a key aspect in the implementation. Our purpose is to demonstrate the efficiency of Sushisen Algorithms to automatic and unsupervised image segmentation.Some examples in human MRI brain tumor segmentation are presented and overall results discussed.

Medical imaging is performed in various modalities, such as magnetic resonance imaging (MRI), computed tomography (CT), ultrasound etc. Segmentation is typically performed manually by expert physicians as a part of treatment planning and diagnosis. Due to the increasing amount of available data and the complexity of features of interest, it is becoming essential to develop automated segmentation methods to assist and speed-up image-understanding tasks.

Related Work

Image segmentation is a low-level image processing task that aims at partitioning an image into homogeneous regions. How region homogeneity is defined depends on the application. A great number of segmentation methods are available in the literature to segment images according to various criteria such as grey level, color, or texture (Gonzales and woods 2016). Several automated methods have been developed to process the acquired images and identify features of interest, including intensity-based methods, region-growing methods and deformable contour models. Intensity-based methods identify local features such as edges and texture in order to extract regions of interest. Region-growing methods start from a seed-point on the image and perform the segmentation task by clustering neighborhood pixels using a similarity criterion. Recently, researchers have investigated the application of sushisen algorithms into the image segmentation problem.

To improve the image quality we can use any one of the filtering technique (Mostafa et al 2016).Magnetic Resonance (MR) image enhancement are mainly used for reconstruction of missing or corrupted parts of MR images, image de-noising and image resolution enhancement. While using Magnetic Resonance (MR) images resolution enhancement face many problems like Resolution enhancement of MR images (512 x 512 pixels times more),conservation of sharp edges in the image and conservation and highlighting of details. There are two designed and tested methods used for image resolution enhancement: Discrete Fourier Transform (DFT) and Discrete Wavelet Transform (DWT). Recently wavelets have been successfully used in a large number of biomedical applications (Mostafa et al 2016 and Bealy 2016). The multi-resolution framework makes wavelets into very powerful compression and filter tool and the time and frequency localization of wavelets makes it into a powerful tool for feature detection. This chapter, 2D discrete wavelet transform is used for removing noise from MRI brain image. The performance of an Image Denoising System using Discrete Wavelet Transform (DWT) is experimentally analyzed for four levels of DWT decomposition.Some works have applied sushisen algorithms (SSS) to image processing and to segmentation particularly. As segmentation can be seen as a process which finds out the optimal regions partition of an image according to a criterion, SSS are well adapted to achieve this goal. Indeed, SSS is particularly efficient when the search space is really important and when the criterion to optimize is numerically complicated which is always the case in image processing. The main advantages of using SSS for segmentation lie in their ability to determine the optimal number of regions of a segmentation result or to choose some features such as the size of the analysis window or some heuristic thresholds. The SSS proposed by Holland (2016) is a general-purpose global optimization technique based on randomized search (Franti 2016). They incorporate some aspects of iterative algorithm.

Proposed Approach

A sushisen algorithm is based on the idea that natural evolution is a search process that optimizes the structures it generates. An interesting characteristic of SSS is their high efficiency for difficult search problems without being stuck in local extreme. In a SSS, a population of individuals, described by some chromosomes, is iteratively updated by applying operators of selection, mutation and crossover to solve the problem. Each individual is evaluated by a fitness function that controls the population evolution in order to optimize it. SSS can be used to find out the optimal label of each pixel, to determine the optimal parameters of a segmentation method, or to merge regions of a fine segmentation result. Concerning the fitness function, it can be an unsupervised quantitative measure of a segmentation result or a supervised one using some a priori knowledge. In this chapter, we deal with a general scheme for MRI brain tumor image segmentation that involves a SSS. SSS is used here as an optimization method for the optimal combination of segmentation results whose quality is quantified through an evaluation criterion. We use a general scheme to define segmentation methods by optimization.

Algorithms

1. MRI (x, order=c(0,0,0), seasonal=c(0,0,0).
2. xreg=NULL, include.mean=TRUE, include.drift=FALSE.
3. include.constant, lambda=model$lambda, transform.pars=TRUE.
4. fixed=NULL, init=NULL, method=c("CSS-ML","ML","CSS"), n.cond,
5. optim.control=list(), kappa=1e6, model=NULL) auto.arima(x, d=NA, D=NA, max.p=5, max.q=5,
6. max.P=2, max.Q=2, max.order=5, max.d=2, max.D=1,
7. start.p=2, start.q=2, start.P=1, start.Q=1.
8. stationary=FALSE, seasonal=TRUE.
9. arima.errors(z)>BRAIN TUMOR
10. ic=c("aicc", "aic", "bic"), stepwise=TRUE, trace=FALSE.
11. approximation=(length(x)>100 | frequency(x)>12), xreg=NULL.
12. test=c("kpss","adf","pp"), seasonal.test=c("ocsb","ch").
13. allowdrift=TRUE, allowmean=TRUE, lambda=NULL, biasadj=FALSE.
14. parallel=FALSE, num.cores=2).
15. fit <- Arima(one,order=c(3,1,0))
16. plot(forecast(fit,h=20))
17. seasonal=list(order=c(0,1,1),period=12),lambda=0)
18. plot(forecast(air.model,h=48))
19. Apply fitted model to later data
20. Forecast accuracy measures on the log scale.
21. in-sample one-step forecasts.
22. accuracy(model)
23. out-of-sample one-step forecasts.
24. accuracy(model2).
25. out-of-sample multi-step forecasts
26. accuracy(forecast(air.model,h=48,lambda=NULL),
27. log(window(s,start=1957)))
28. out.fit <- auto.arima(FTSF)
29. out.errors <- arima.errors(GAM)
30. par(mfrow=c(2,1))
31. plot(FTSF).
32. plot(GAM).

Algorithm Analysis

xreg	must have the same number of rows as x.
include.mean	The default is TRUE for undifferenced
series,	FALSE for differenced ones
include.drift	a linear regression with ARIMA errors is fitted. The default is FALSE.
include.constant	If TRUE, then include.mean is set to be TRUE for undifferenced series and
include.drift	is set to be TRUE for differenced series. included regardless of the value
x	a univariate time series
d	Order of first-differencing. If missing, will choose a value based on KPSS test.
D	Order of seasonal-differencing. If missing, will choose a value based on test.
max.p	Maximum value of p
max.q	Maximum value of q
max.P	Maximum value of P
max.	Q Maximum value of Q
max.order	Maximum value of p+q+P+Q if model selection is not stepwise.
max.d	Maximum number of non-seasonal differences
max.D	Maximum number of seasonal differences
start.p	Starting value of p in stepwise procedure.
start.q	Starting value of q in stepwise procedure.
start.P	Starting value of P in stepwise procedure.
start.Q	Starting value of Q in stepwise procedure.
Stationary	If TRUE, restricts search to stationary models.
Seasonal	If FALSE, restricts search to non-seasonal models.
ic	Information criterion to be used in model selection.
Stepwise	If TRUE, will do stepwise selection (faster). Otherwise, it searches over all models.
Non-stepwise	selection can be very slow, especially for seasonal models.

Discrete Wavelet Transform

An advancement of wavelet theory has taken the interest of researchers in its application to image enhancement which is done by noise removing and edge enhancement. Wavelet basis function enables DWT based filtering procedures to adapt to spatial variations. Wavelets are functions generated from one single function Ψ by dilations and translations. The basic idea of the wavelet transform is to represent any arbitrary function as a superposition of wavelets. Any such superposition decomposes the given function into different scale levels where each level is further decomposed with a resolution adapted to that level.

$$(x + a)^n = \sum_{k=0}^{n} \binom{n}{k} x^k a^{n-k} \qquad (1)$$

The DWT is identical to a hierarchical sub band system where the sub bands are logarithmically spacedin frequency and represent octave-band decomposition. By applying DWT, the image is actually divided i.e., decomposed into four sub bands. These four sub bands arise from separable applications of vertical and horizontal filters. Figure 1(a) shows the sub bands labeled LH1, HL1 and HH1 represent the finest scale wavelet coefficients, i.e., detail images while the sub band LL1 corresponds to coarse level coefficients, i.e., approximation image. Figure 1(b) shows the next coarse level of wavelet coefficients, the sub band LL1 alone is further decomposed and criticallysampled.

Image Decomposition

Wavelet Based Denoising method relies on the fact that noise commonly manifests itself as fine-grained structure in the image and DWT provides a scale based decomposition. Thus, most of the noise tends to be represented by wavelet co-efficient at the finer scales. Discarding these coefficients would result in a natural filtering of the noise on the basis of scale. Because the coefficients at such scales also tend to be the primary carriers of edge information, this method threshold the DWT coefficients to zero if their values are below a threshold. These coefficients are mostly those corresponding to noise. The edge relating coefficients on the other hand, are usually above the threshold. The Inverse DWT of the thresholded coefficients is the denoised image.

Wavelet thresholding is a signal estimation technique that exploits the capabilities of wavelet transform for signal denoising. It removes noise by killing coefficients that are insignificant relative to some threshold, and turns out to be simple and effective, depends heavily on the choice of a thresholding parameter and the choice of this threshold determines, to a great extent the efficacy of denoising. Threshold Selection plays main role in denoising. A small threshold may yield a result close to the input, but the result may still be noisy. A large threshold on the other hand, produces a signal with a large number of zero coefficients. This leads to a smooth signal. Paying too much attention to smoothness, however, destroys details and in image processing may cause blur and artifacts. Some of thresholding methods are: (i) Hard thresholding, (ii) Soft thresholding, (iii) Semi-soft Thresholding and (iv) Quantile thresholding. In our implementation, soft thresholding method is used to analyze the performance of denoising system for DWT decomposition, since soft thresholding results in better denoising performance than other denoising methods.Thresholding leads to less severe distortion of the object of the interest than other thresholding methods. Several approaches have been suggested for setting the threshold for each band of the waveletdecomposition. A common approach is to compute the sample variance (σ^2) of the coefficients in each band and set the threshold to some multiple of standard deviation (σ) for that band. Thus, to implement a soft threshold of the DWT coefficients for a particular wavelet band, the coefficients of that band should be thresholded.

Sushisen Algorithm for Image Segmentation

Sushisen algorithms determine the optimal value of a criterion by simulating the evolution of a population until survival of best fitted individuals. The survivors are individuals obtained by crossing-over, mutation and selection of individuals from the previous generation. We think that SSS is a good candidate to find out the optimal combination of segmentation results for two main reasons. First one is due to the fact that an evaluation criterion is not very easy to differentiate. SSS is an optimization method that does not necessitate to differentiate the fitness function but only to evaluate it. Secondly, if the population is important enough considering the size of the search space we have good guarantees that we will reach the optimal value of fitness.

SSS is a special form of local search that models our own understanding of evolution. In essence a number of simultaneous agents (the population) each having an encoded state (the chromosome) perform a random walk (mutations) around the search space, while forming new solutions from combinations of existing solutions (crossover) and, thus adjusting and refocusing the efforts of the search on exceptionally good areas once located. A few important choices are made during any application of sushisen algorithms, involving how to encode the population (binary, integer, decimal, etc), how to mutate the population (mutate all genes, some genes, etc), how to select the parents for crossovers (roulette wheel, tournament selection), how to perform those crossovers (uniform, single-point), and finally what fitness function to use for evaluation. Though these choices seem complex, in situations where the energy functional has hundreds or even thousands of dependent variables and parameters these few choices can yield nearly optimal values for all variables and parameters concerned.

Execution of the sushisen algorithm is carried out in four steps

1. Definition of the genotype, initial population (segmentation results) and computation of the fitness function (evaluation criterion) of eachindividual.Genotype: the K-means segmentation result of an image S_1 is considered as an individual described by the class of eachpixel.
2. Initial population: a set of individuals characterized by their genotypes. It is composed of the segmentation results tocombine.
3. Fitness function: this function enables us to quantify the fitness of an individual to the environment by considering itsgenotype.
4. The fitness computation process consists of two phases. In the first phase, the clusters are formed according to the centers encoded in the chromosome under consideration. This is done by assigning each point $x_i, i=1,2,....n$ to one of the clusters C_j with centre z_j such that

$$e^{-i\omega t} = x_{y^2} + x^2 + {}^{n}_{1}Y + \text{xi} + \text{Cj} \qquad (2)$$

All ties are resolved arbitrarily. After the clustering is done, the cluster centres encoded in the chromosome are replaced by the mean points of the respective clusters. In other words, for cluster C_i, the new centre z_i * is computedas

$$Z_i^* = (x\ X\ a)^n = \sum_{k=0}^{n} \binom{n}{k} x^k a^{n-k}{}_{xC} X_i,\ i=1,2,...,K \qquad (3)$$

These z_i * s now replace the previous z_i s in the chromosome. Subsequently, the clustering metric M is computed as follows:

Similarity Measure

$$Mim(D,Q) = \sum (s_i * i_i)\partial \text{.....(1)}$$

$$Mim(D,Q) = \frac{\sum_i (s_i * i_i)\,\partial}{\sqrt{\sum_i s_i^{\,2} * \sum_i s_i^{\,2}\delta}} \text{.....(2)}$$

$$Mim(D,Q) = \frac{2\sum_i (s_i * i_i)}{\sum_i s_i^{\,2} + \sum_i s_i^{\,2}}\partial \text{.....(3)}$$

$$Mim(D,Q) = \frac{\sum_i (s_i * i_i)}{\sum_i s_i^{\,2} + \sum_i i_i^{\,2} - \sum_i (i_i * i_i)}\partial \text{.....(4)} \qquad (4)$$

s=Images;*i*=Arima;∂=Brain Tumor;*D*=Dataset in MRI Q;H_k=Time;

N_{HB}=Accuracy Result;i = True Positive;x,y=FalsePositive;Zi=SushisenAlgorithm;Xj=Arima Model

$$F_k(u,v) = MRI\{f_k(i,j)\},$$

If w(i,j) = 1 then

$\Delta Q_e(\frac{F_k(x,y)}{\Delta})$ x , y $\in H_k\ 1 \le k \le N_{HB}$

F_k (x,y) =

$F_{k(x,y)}$ x , y $\notin H_k\ 1 \le k \le N_{HB}$

If w (i,j) = 0 then

$\Delta Q_o(\frac{F_k(x,y)}{\Delta})$ x , y $\in H_k\ 1 \le k \le N_{HB}$

F_k (x,y) =

$F_{k(x,y)}$ x , y $\notin H_k\ 1 \le k \le N_{HB}$

$$M \circledcirc X\ j \circledcirc Z i \qquad (5)$$
$$x i C i$$

The fitness function is defined as *f*'=1/M, so that maximization of the fitness function leads to minimization of M.

Selection of Individuals

The selection process selects chromosomes from the mating pool directed by the survival of the fittest concept of natural sushisen systems. In the proportional selection strategy adopted in this article, a chromosome is assigned a number of copies, which is proportional to its fitness in the population, which then goes into the mating pool for further sushisen operations. Roulette wheel selection is one common technique that implements the proportional selection strategy

Mathematical Analysis

Definition 1. Asequence MRI diagram is a tuple $H=(C,E,M,L,W)$ where: C isa finite set of components; E is a finite set of events; M is a finite set of messages. For any message $g \in M$, let $g!$ and $g?$ represent the sending and receiving for g respectively. for any $e \in E$, it corresponds to a send event or receive eventfor a message g, denoted by $\tau(e) = g!$ or $\tau(e) = g?$; $L: \mathrm{E} \to C$ is labeling function which maps each event $\mathrm{e} \in E$ to a component $\mathrm{L}(e) \in C$; W is a finite set whose elements are of the form (e,e') where $\mathrm{e}, e' \in E$ and $\mathrm{e} \neq e'$,which represents a visual order displayed in H .The temporal order of the message flow is described by the event sequence of asequence diagram, which is called the trace of a sequence diagram[8].For a sequence diagram $H=(C,E,M,L,W)$, a event sequence $e_0 \hat{} e_1 \hat{} \ldots \hat{} e_n$ is the trace of H, iff (1)all events in E occur in this sequence and each eventoccurs only once, and (2) $\mathrm{e}_0, e_1, \ldots, e_n$ satisfy the visual order defined by W .

Definition 2.Aninteraction overview diagram is a tuple $I=(D,H,A,F)$ where: D is a activity diagram; $H=\{h_1,h_2,...,h_m\}$ is a set of sequence diagrams, which is associated with the node of activity diagram D; $A=\{a_1,a_2,...,a_m\}$ is the set of nodes of activity diagram D; $F:H\to A$is a label function which associates with eachsequence diagram $h_i\in H(1\le i\le m)$ with a node $F(h_i)\in A$of activity diagram D.

Definition 3.A semanticsequence diagram is a tuple $SH=(C,E,M,f_T,S,L,W)$ where: C and E are a finite set of components and events respectively; M is a finite set of messages; f_T is a label map associating each message $g\in M$ to type set T, i.e. $f_T(g)=t$ and $t\in T$, where $T=\{Im,Om,Hm,Ir,Or,Hr,Ie,Oe,He\}$; $S:M\times T\to\{I_g,O_g,\kappa\}$ is a semantics map associating each $g\in M$to a semantic information according to themessagetype $t\in T$, i.e. $S(\langle g,f_T(g)\rangle)=\lambda,\lambda\in\{I_g,O_g,\kappa\}$; $L:E\to C$is labeling function which maps each event $e\in E$to a component $L(e)\in C$; W is a finite set whose elements are of the form (e,e') where $e,e'\in E$ and $e\neq e'$, which represents a visual order displayed in SH.In the definitionabove, the method semantics andmethod type mapping are added to sequence diagram. The semantics information of message $g\in M$(i.e. $S(\langle g,t\rangle)$) is receivingsemantics I_g (resp. sendingsemantics O_g and emptysemantics κ)if $f_T(g)=Im$ (resp. $f_T(g)=Om$ and $f_T(g)\neq Om\cup f_T(g)\neq Im$). For any message $g\in M$, $g!$and $g?$represent the sending and receiving for g respectively.And for any $e\in E$, $\tau(e)=g!$is corresponding to a sending eventand $\tau(e)=g?$isa receiving eventfor a message. In addition, the symbol Im (resp. Om and Hm)of type set T means that the type of action is input(resp. output and internal) method. Similarly, the symbols Ir (resp. Or and Hr) and Ie (resp. Oe and He) represent the type of action are input(resp. output and internal) return action and input (resp. output and internal) exception action respectively. According to the relations between the event and message ofsequence diagram which is given in definition 1, we can know that the trace of the semanticsequence diagram will not only contain the order information but also the semantic information after the semantics constrain is added to SH.

Definition4. The event sequencewithsemanticsconstrain, with the form of $(e_0,S(\langle\tau(e_0),t\rangle))^\wedge(e_1,S(\langle\tau(e_1),t\rangle))^\wedge...^\wedge(e_n,S(\langle\tau(e_n),t\rangle))$, is called the trace of $SH=(C,E,M,f_T,S,L,W)$, if the following conditions are satisfied: (1) $\{e_0,e_1,...,e_n\}=E$, and for any $i,j(i\neq j,0\le i,j\le n),e_i\neq e_j$; (2) for any $i(0\le i\le n)$, $S(\langle\tau(e_i),t\rangle)$ satisfies the semantic map described by S;and (3) each e_{i+1}takes place after e_i for any $i(0\le i\le n-1)$.

Condition(1) of definition 4 states that the event sequencewithsemantic constrain contains all the events of E and that each event occurs only oncein this sequence. And the condition(2) states that the semantics map of $\tau(e_i)$ is not empty if its type is input method or output method, i.e. $S(\langle\tau(e_i),t\rangle)=I_g$ (resp. O_g), if $f_T(\tau(e_i))=Im$ (resp. Om), and $S(\langle\tau(e_i),t\rangle)=\kappa$ for other type $f_T(\tau(e_i))\notin\{Im,Om\}$.The idea of condition (3) is that the sequence $e_0,e_1,...,e_n$ mustsatisfy the visual order defined by W.

Definition 5.A interaction overview diagram with semantics constrain is a tuple $SI=(D,SH,A,F)$ where D is a activity diagram; $SH=\{sh_1,sh_2,...,sh_m\}$ is a set of semantic sequence diagrams, which is associated with the node of activitydiagram D; $A=\{a_1,a_2,...,a_m\}$ is the set of nodes of activitydiagram D; $F:H\to A$is a label function which associates each semantic sequence diagram $sh_i\in H(1\le i\le m)$ with a node $F(sh_i)\in A$of activity diagram D. According to the relations betweenthe interaction overview diagram and the sequence diagram, it can deduce that

the event sequence of an interaction overview diagram is the composition ofevent sequences of semantic sequence diagram. And the order of this composition should obey to the nodes ordering inthe activity diagram. The definition of the trace of the interaction overview diagram with semantic constrain is given below.

Definition 6. Let $\eta_i = \left(e_0, S\left(\left\langle \tau(e_0), t\right\rangle\right)\right)^{\wedge}\left(e_1, S\left(\left\langle \tau(e_1), t\right\rangle\right)\right)^{\wedge}...^{\wedge}\left(e_n, S\left(\left\langle \tau(e_n), t\right\rangle\right)\right)$ bea semantic event sequence of $sh_i\left(1 \le i \le n\right)$. Aset of the event sequences $\eta_1{}^{\wedge}\eta_2{}^{\wedge}...{}^{\wedge}\eta_n$ is called the trace of an interaction overview diagram with semantic constrain $SI = \left(D, SH, A, F\right)$, iff the following conditions hold:(1) for each $\eta_i\left(1 \le i \le n\right)$, η_i is atrace of $sh \in SH$; (2)for two nodes $a_i, a_j \in A\left(i \ne j, 1 \le i, j \le n\right)$ and its corresponding semantic sequence diagram sh_i, sh_j, if a_j takes place after a_i then the semantic event sequence η_j of sh_j also takes place after η_i.

In the definition above, condition (1) means that each semantic event sequence η_i shouldsatisfies definition 4, and condition (2)states that the order of $\eta_1, \eta_2, ..., \eta_n$ must obey to the nodeordering of activity diagram D.

Definition 7. An interface automaton $A = \left\langle V_A, v_A^{init}, \Sigma_A^I, \Sigma_A^O, \Sigma_A^H, \delta_A \right\rangle$ consists of the following elements: V_A is a set of states; $v_A^{init} \in V_A$ is the initial state. A is empty iff $v_A^{init} = \phi$; Σ_A^I, Σ_A^O and Σ_A^H are mutually disjoint sets of input, output,and internal actions. We denote by $\Sigma_A = \Sigma_A^I \cup \Sigma_A^O \cup \Sigma_A^H$ the set of all actions; $\delta_A \subseteq V_A \times \Sigma_A \times V_A$ is a set of transitions.If $a \in \Sigma_A^I$(resp. $a \in \Sigma_A^O$, $a \in \Sigma_A^H$), then $\left(v, a, v'\right)$ is called input(resp. output, internal) transitions. In order to describe the semantics of method actions, [10] further classify actions into method actions(denoted by Σ_A^m), return actions (denoted by Σ_A^r) and exception actions (denoted by Σ_A^e). Then the set Σ_A^m, which represents the method actions of A,is defined by $\Sigma_A^m = \Sigma_A^{\mathrm{Im}} \cup \Sigma_A^{O\mathrm{m}} \cup \Sigma_A^{H\mathrm{m}}$, where $\Sigma_A^{Im} \subseteq \Sigma_A^I$, $\Sigma_A^{Om} \subseteq \Sigma_A^O$ and $\Sigma_A^{Hm} \subseteq \Sigma_A^H$ are resp. actions of public provided methods, call of environment public methods and calls of private methods. Furthermore, a state sequence $v_0 \xrightarrow{a_0} v_1 \xrightarrow{a_1} ... \xrightarrow{a_{n-1}} v_n \xrightarrow{a_n} v_{n+1}$ is a behavior of A iff $v_0 = v_A^{init}$ and for each $i\left(0 \le i \le n\right)$, there is $\left(v_i, a_i, v_{i+1}\right) \in \delta_A$.We can use interface automaton networks(*IANs*) to model the designs of component-based software system. An*IANs* consists of a set of interface automata which represent the abstractions of software components. Notice that the composition of two interface automata may induce deadlock situations caused by the potential protocol incompatibilities, i.e. one automaton may produce an output eventthat is an input event ofanother automaton, but this output event is not accepted by the latter one. Tocompute the compatible composition of interface automata , [6] has presentedan algorithmtoremove the set of incompatible states recursively. As a resultwe can denote by $comp\left(N\right)$ the compatible*IANs*. The definition of *IANs*and its behavior are givenas follows.

Definition 8. Interface automaton networks(*IANs*) is a tuple $N = (K, W)$,where $K = \left\{A_1, A_2, ..., A_n\right\}$ is a set of composable interfaceautomata; $W = \left\{Shared\left(A_i, A_j\right) \mid 1 \le i, j \le n, i \ne j\right\}$ is a set of all shared actions.

Definition 9. Let $comp\left(N\right) = (K, W)$ be a compatible *IANs*. A state sequence is a behavior of $comp\left(N\right)$ iff $\overline{v}_0 \in v_{comp(N)}^{init}$, and for each $i\left(0 \le i \le n\right)$, there is $\left(\overline{v}_i, a_i, \overline{v}_{i+1}\right) \in \delta_{comp(N)}$ $\overline{v}_0 \xrightarrow{a_0} \overline{v}_1 \xrightarrow{a_1} ... \xrightarrow{a_{n-1}} \overline{v}_n \xrightarrow{a_n} \overline{v}_{n+1}$.

In order to correct describe the semantic information of component-based systems, [10] gave the definition of the semantics of the operations.

Definition 10.Given an interface automaton A , an *input semantics* $I_a = (P_a, S_a, Q_a, E_a)$ of action $a \in \Sigma_A^{\mathrm{Im}}$ is defined by: a precondition $P_a \subseteq \mathbf{Z}^{\psi_A^{\mathrm{i}}(a)}$, a specification $S_a \subseteq \mathbf{Z}^{\psi_A^{\mathrm{i}}(a) \cup \wedge_A(a) \cup \psi_A^{\mathrm{o}}(a)}$, a termination post-condition $Q_a \subseteq \mathbf{Z}^{\psi_A^{\mathrm{i}}(a) \cup \wedge_A(a) \cup \psi_A^{\mathrm{o}}(a)}$, and an exception post-condition $E_a \subseteq \mathbf{Z}^{\psi_A^{\mathrm{i}}(a) \cup \wedge_A(a) \cup \psi_A^{\mathrm{o}}(a)}$. An *output semantic* $O_b = (P_b, Q_b)$ of an action $b \in \Sigma_A^{Om}$ is that a precondition $P_b \subseteq \mathbf{Z}^{\psi_A^{\mathrm{i}}(b)}$ and a post-condition $Q_b \subseteq \mathbf{Z}^{\psi_A^{\mathrm{i}}(b) \cup \psi_A^{\mathrm{o}}(b)}$. The symbol $\psi_A^{\mathrm{i}}(a) = \{i_1, ..., i_k\}$ is the set of input parameters of action a . The set of return parameters $\psi_A^{\mathrm{o}}(a)$ of a is the singleton $\{o\}$. The set of attributes used by a is denoted by $\wedge_A(a)$ if a $\in \Sigma_A^{\mathrm{Im}} \cup \Sigma_A^{Hm}$. In terms of [10], the signature of method action $a \in \Sigma_A^m$ is a $(i_1 : \mathbf{Z}[i_1], ..., i_k : \mathbf{Z}[i_k]) \to o : \mathbf{Z}[o] \# e$, where the type of $v \in \mathrm{V}$ is defined by $\mathbf{Z}[v]$, i.e. $v : \mathbf{Z}[v]$.

Definition 11.**A *SIA* is a tuple** $B = (A, f_s)$ **,** where: A is an interface automaton of B , i.e. $B.A = (V_{B.A}, v_{B.A}^{\mathrm{Init}}, \Sigma_{B.A}^I, \Sigma_{B.A}^O, \Sigma_{B.A}^H, \delta_{B.A})$; $f_s : \Sigma_{B.A} \to \{I_m, O_m, \kappa\}$ is a map associating each operation tosemantic. If operation $a \in \Sigma_{B.A}^{\mathrm{Im}}$(resp. $a \in \Sigma_{BA}^{\mathrm{Om}}$, $a \notin (\Sigma_{B.A}^{\mathrm{Im}} \cup \Sigma_{BA}^{\mathrm{Om}})$) ,then f_s associating operation to an input semantics I_m (resp. an output semantics O_m , empty semantics κ).The definitions of $\Sigma_{B.A}^{\mathrm{Im}}$, $\Sigma_{B.A}^{\mathrm{Om}}$and $\Sigma_{B.A}^{H\mathrm{m}}$ have been given in 2.2.1. The set V_{B} of B and the set $v_B^{\mathrm{Init}} \in V_{\mathrm{B}}$ are equal respectively to $V_{\mathrm{B.A}}$ and $v_{B.A}^{Init} \in V_{\mathrm{B.A}}$. Obviously, the set Σ_B of actions of B is defined by $\Sigma_B = \Sigma_{B.A}^I \cup \Sigma_{B.A}^O \cup \Sigma_{B.A}^H$,and the set Σ_B^m of method actions of B is definedby $\Sigma_B^m = \Sigma_{B.A}^{\mathrm{Im}} \cup \Sigma_{B.A}^{Om} \cup \Sigma_{B.A}^{Hm}$.And the set of transitions is $\delta_{\mathrm{B}} \subseteq V_B \times \Sigma_B \times f_s(\Sigma_B) \times V_B$. An action $a \in \Sigma_B$ is enabled at state $v \in V_B$ if there is a transition $(v, a, f_s(a), v') \in \delta_B$.According to the component-based designs approach, new software designs are created by combining existing modules with new components which provides both new functionality and interface information. The composition rule of *SIA*sis the sameas that of componentscomposition in [6]. According to the definition of *IANs*(see definition 8) and the formalization of *SIA*(see definition 11), we can easily obtain the formalism of semantics extended interface automata networks (*SIANs*).

Definition 12.A*SIAN*sis a tuple $SN = (B, S)$, where $B = \{B_1, B_2, ..., B_n\}$ is a set of composable semantic extended interface automata (*SIAs*), and $W = \{Shared(B_i.A, B_j.A) \mid 1 \le i, j \le n, i \ne j\}$ is a set of allshared actions. The states, input, output and internal actions in the*SIANs* are as follows:the set of states of SN is $V_{SN} = (V_{B_1} \times V_{B_2} \times ... \times V_{B_n})$, astate $\overline{v}$ in V_{SN} is $\overline{v} = (v_1, v_2, ..., v_n)$ $(v_i \in V_{B_i}, 1 \le i \le n)$; the initial state of SN is $v_{SN}^{\mathrm{Init}} \in V_{SN}$, and $v_{SN}^{\mathrm{Init}} = (v_{B_1}^{\mathrm{Init}}, v_{B_2}^{\mathrm{Init}}, ..., v_{B_n}^{\mathrm{Init}})$; the set of actions of SN is $\Sigma_{SN} = \Sigma_{SN}^I \cup \Sigma_{SN}^O \cup \Sigma_{SN}^H$, where the set of input actions is $\Sigma_{SN}^I = (\cup_{1 \le i \le n} \Sigma_{B_i}^I) / W$, the set of output actions is $\Sigma_{SN}^O = (\cup_{1 \le i \le n} \Sigma_{B_i}^O) / W$, and the set of internalactions is $\Sigma_{SN}^H = (\cup_{1 \le i \le n} \Sigma_{B_i}^H) \cup W$.

RESULTS

Proposed system shows the results of two types of experiments. First, System uses soft thresholding based DWT for denoising MR images. Fig.3(a) shows the original MR image without denoising and Fig.3 (b) shows the enhanced image for single level of decomposition. Moreover, magnetic resonance images are lesser noise densities corrupted images, single level of DWT decomposition is sufficient for this type of images. During the decomposition σ = 5,SNR = 43.5 and σ = 50, SNR = 20.3, while σ value increases value of SNR will be reduced gradually.

Secondly, we present some sushisen segmentation results of human MR brain tumor T1 and T2 weighted and PD modality of images. For all the following experimental results, we set the value of the selection probability to 8%, the crossing-over probability to 60% and the mutation probability to 5%. This method has been tested with more than 100 real MRI images. There are two aspects to check whether the proposed segmentation algorithm has to be used in real time clinical applications: accuracy and reliability. The segmentation results of the wavelet based sushisen algorithm are shown in Figure 7 the computation time, accuracy and their corresponding iterations are shown in Table 1. Figure 7 shows segmented images using the K-means algorithm with mean and variance as attributes with different numbers of clusters (NC) 2, 3, 6, 9, which constitutes the initial population for the SSS.

In this case, the genotype of an individual is a vector of size 262144 (the size of each image is 512 x 512 pixels). A gene corresponds to the label of each pixel in the considered segmentation result. Figure 1.3.e shows the final segmented result. The importance in this experiment point is that we did not specify the number of clusters we wanted. It has been automatically determined (NC=4).

System Description

The Toshiba Portege R600 U2530 laptop is powered by Intel Core 2 Duo SU9400, 1400 Mega Hertz (Mhz) processor. This Portege series laptop from Toshiba comes with 3072 Megabytes (MB) of RAM, which is expandable up to Megabytes (MB). Toshiba Portege R600 U2530 laptop or notebook PC has a 128Solid State Drive Gigabytes (GB) hard disk capacity and HDMI Port. The display of Toshiba Portege R600 U2530 is with 1280 x 800 pixels resolution. This Toshiba laptop has a battery life of hours and weighs around 1 kgs with the R Language version Software programming.

CONCLUSION

In this paper we focused on the interest of soft thresholding DWT for enhancement and sushisen algorithms for image segmentation. We showed that this kind of approach can be applied either for grey-level magnetic resonance images. The developed method uses the ability of SSS to solve optimization problems with a large search space (label of each pixel of an image). The developed method can also integrate some a prior knowledge (such as a local ground truth) if it is available. The developed method achieved SNR value from 100 to 140 and segmentation accuracy from 82 percent to 97 percent of detected tumor pixels based on ground truth.

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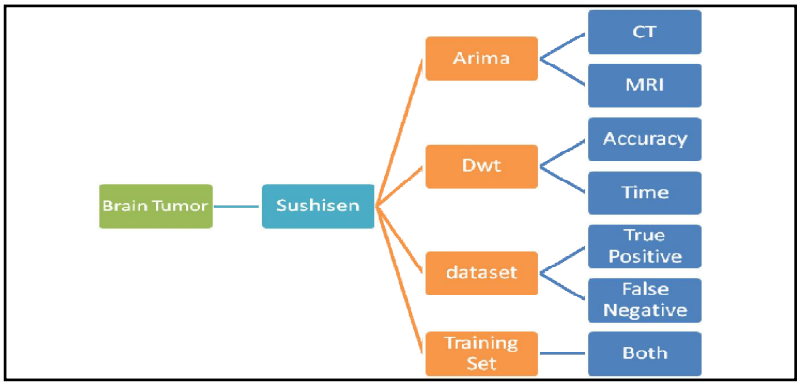


Fig.1. Flow for Sushisen Algorithm

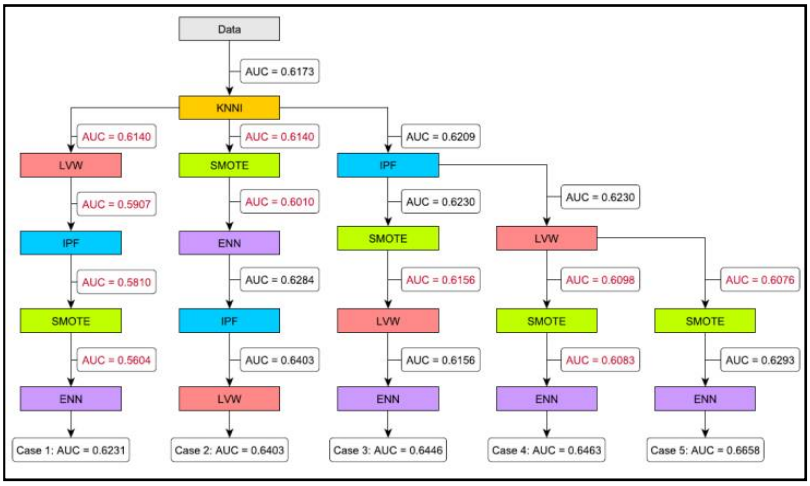
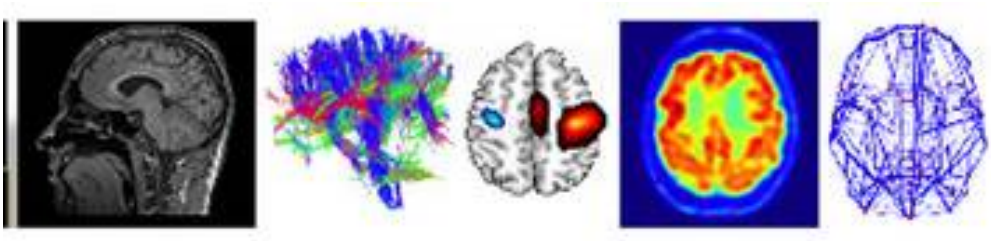


Fig.2. Image Analysis model methods



(a) **(b)**

Fig.3(a). Original T2 weighted MRI brain tumor image (before denoising), (b) Enhanced image using DWT algorithm (soft thresholding)

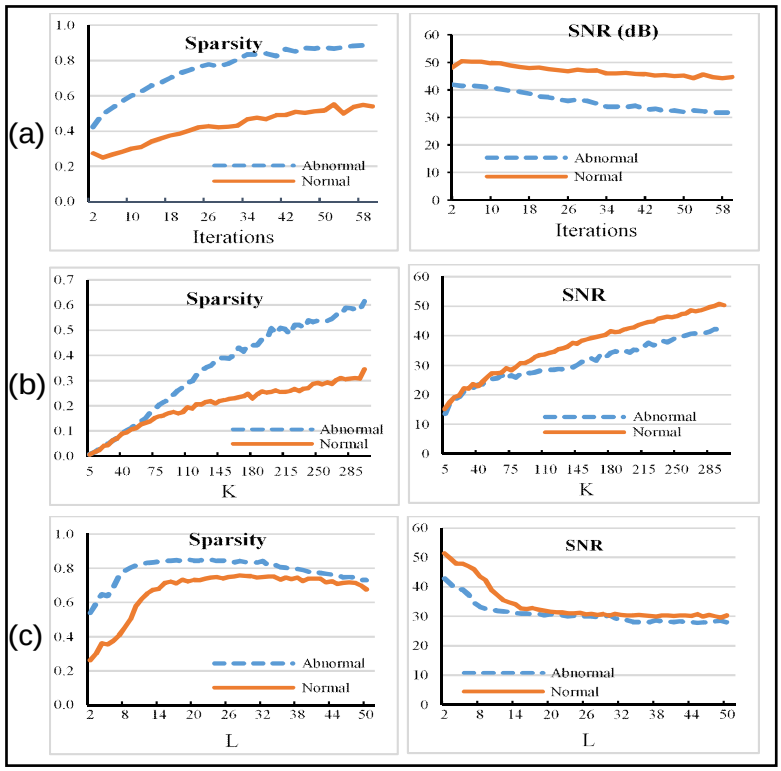


Fig.4. Normal and abnormal dictionaries performance on different set of training iterations.

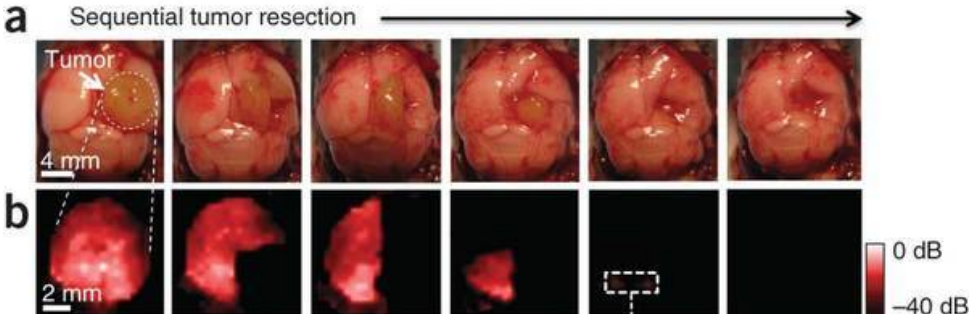


Fig.5. MRI reconstruction error based classifier analysis.

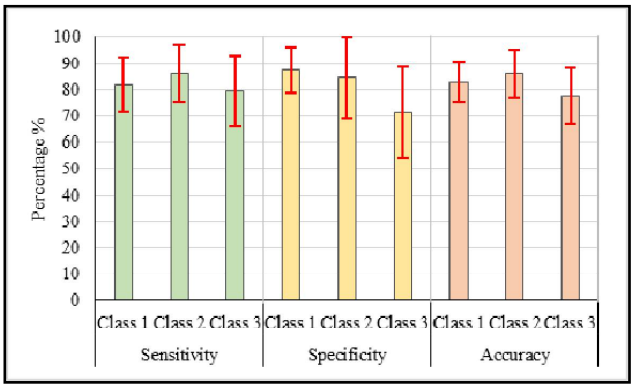


Fig .6.Classifier average performance.

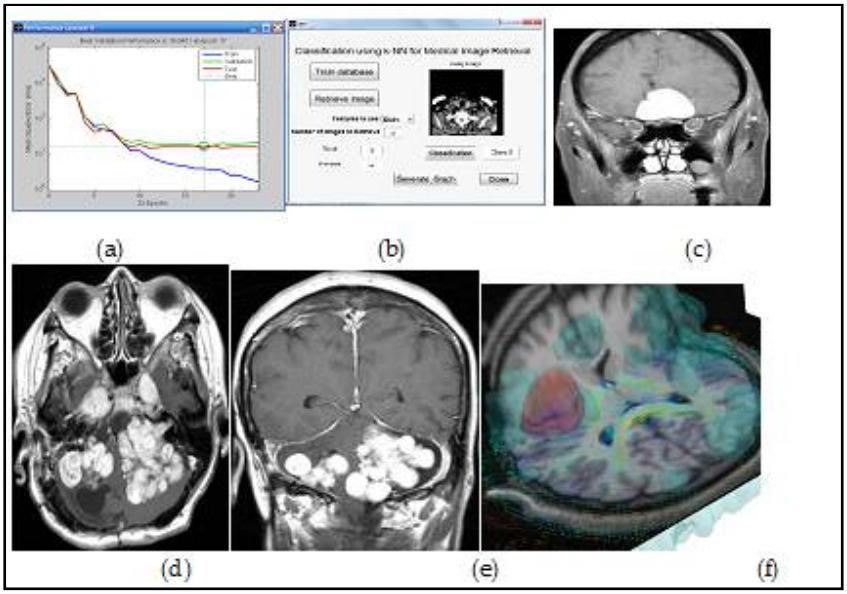


Fig .7(a).Enhanced T2 weighted MRI brain tumor image, (b-e) is segmented image using wavelet based SSS algorithm with NC=2,3,6,9 (Number of cluster) and (f) is a resultant segmented image with four cluster (white matter, gray matter, CSF and tumor(red)).

Table 1. The over all results

Input Query (Total=240) Query Class	Normal Class	Tumor Class	None
65 Normal Images	65	0	0
36 Tumoral Images	3	33	0

Table 2 .Classification Performance on QTDB Database

	Sensitivity	Specificity	Accuracy
Class 1	91.78	95.13	92.40
Class 2	92.09	94.67	92.30
Class 3	84.68	83.39	84.41

Table 3. Current Research Model

Dataset	Algorithm	Accuracy Rate
BRATS 2014	KNN	0.88
	WEAPON	0.81
	CASINO	0.82
BRATS 2015	C4.5	0.88
	WEAPON	0.84
	CASINO	0.79
BRATS 2016	New SSS	0.99
	WEAPON	0.96
	CASINO	0.84

Table 4.Comparitive Study with the Previous Algorithms

S.No.	Unit Phase	Kang-Kook Kong	Ki-Sang Hong	Yuan Dong	Chong Huang
1	Preparation	9603	9603	9603	7789
2	Investigation	3299	3299	3299	3309
3	Test	118	90	95	93
4	Indexing	Boolean	Cache	Frequency	Similar
5	AdaBoost	87.99	87.7	88.1	88.7

Few algorithms are showed good performance. But AdaBoost are showed Very excellent presentation.C.45, and K-Means showed relatively poor performance.

Table 5 .The Comparison Results of the Performance between Proposed Arima Method 2016 Dataset

Algorithm	***F-Measure***	Accuracy	***Time***	***Precision***
SVM	75,880865	14,510213	25,106339	69,725939
KNN	66,421235	11,462507	40,976129	55,163069
ADABOOST	73,546903	14,041202	25,280317	72,915196
C4.5	48,793725	7,233527	88,397341	34,736934
HITS	76,400945	15,559229	6,733586	95,998365
FUSSY	76,590380	14,971854	10,881221	84,803941
SUSHISEN	87,2025181	17.3203707	4,4992576	88,8057364
Rank	↓ 1st	↓ 1st	↓ 1st	↓ 2nd

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RESEARCH ARTICLE

Perception of Developed Android Fodder App among Livestock Farmers of Karnataka

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ABSTRACT

The livestock sector in India plays a multi-faceted role in the socio-economic development of rural households. Extension services provide critical access to the knowledge and technology that farmers require to improve the productivity. It is hence crucial to provide livestock farming community with the information in a quality and timely way. As smart phone penetration among farmers is increasing day by day, there is huge potential to use their smartphones as primary tool of intervention to deliver the knowledge/information. The present study focuses on development of Android Fodder App with a goal to break the literacy barrier and provide the detail information to livestock farmers on fodders suitable to Karnataka. To assess the farmers' perception level regarding Fodder app was done among 120 farmers

from all divisions of karnataka. The results indicated that majority of respondents had favourable attitude towards it and expressed that the relevance of contents as more relevant (56.66%), preciseness of content as very precise (72.50%), very simple (61.66%) in understanding of information, very good video (70.00%), audio (48.34%) quality, more credible information (71.66%), very effective in arousal of curiosity and interest (56.66%), very easy to use (55.83%), high perceived utility (54.16%), highly satisfied with the logical presentation of information (61.66%) and very effective in improving self confidence (50.83%) with overall favourable perception (76.66%). The study concludes that fodder app could effectively used as ICT enabled tool for information dissemination for farmers.

Key words: - livestock sector, socio-economic, Android Fodder App, Extension services,

INTRODUCTION

Livestock is symbolic to wealth and power across civilizations for centuries. Nearly two thirds of farm families in India are associated with one or the other form of livestock to sustain their livelihood. It is evident that animal husbandry in India has transformed from being an integral part of agriculture and has assumed a much broader role in the overall economy. The Gross Value Added (GVA) from livestock sector at constant prices (2011-12) was about 3593 billion during 2013-14 which is about 26.1% of the Gross Value Added from total agricultural and allied sector and 3.9% of the total GDP (BAHFS, 2015). However, the recent trend in livestock sector growth suggests that in order to meet the emerging demand for livestock based products, both in domestic and global markets, there is a need to reorient the production system by enhancing the efficiency and creating quality consciousness.

But the fact is even today, livestock are sustained on only crop residues obtained from Agriculture activities and as well as high cost inputs like concentrates. Cost of production can be brought down as well as production levels can be increased by reducing the concentrate cost and feeding the animals with good quality green fodders which can be grown exclusively to feed them. In this line lot of activities are adopted by many Universities and research institutions to penetrate this knowledge of growing of fodder for feeding animals. Focussing on wider reachability and quick penetration of knowledge into the social system Government of India has initiated Digital India programme which taps various digital tools as a means to disseminate information to the end users. As India is the second largest smartphone user country, it has a wide scope and potential in using it has an information disseminator tool. Although, smartphone penetration is increasing in rural areas but internet penetration is still long way to go and hence to cope-up with this barrier along with other bottlenecks like illiteracy and large population, android mobile app in vernacular language, which works offline will play a great role in overcoming information asymmetry existing among the group of farmers. With this concept in mind, inorder to create awareness and for adoption of better practices to improve their livelihoods, this new generation smart technology of Android Fodder App was developed.

The goal of this developed Android Fodder App is to provide the detail information to livestock farmers on fodders (such as cultivation practices, seed/ root slips rate, soil and weather suitable for specific fodders, fertilizer requirements, harvesting time and yield etc) in regional language with audio-visual support. Fodder app will provide information to farmers having android phone which operates without Internet Service onces downloaded in anywhere at any time, overcoming the constraints of place, time and character. This Fodder App spread mainly through farmer to farmer multiplier effect without depending on internet. Mobile based App on fodder production will enhance the availability of information and will further help in improving awareness, knowledge, better adoption of fodder cultivation practices, balanced ration feeding to maintain livestock health and efficiency. These in turn will catalyze the rural sector development and economic growth.

MATERIALS AND METHODS

The present study was conducted in Karnataka state, where all four divisions were covered. The study was carried out in three steps (a) Repository preparation for Fodder App (b) Development of "Android Fodder App" and (c) Assessment of perception of Android Fodder App among the livestock farmers. With experts consultation, Content was prepared and required pictures were collected for 16 fodders suitable to Karnataka. An Android Fodder App in vernacular language (Kannada) with audio-visual support, which could work offline once installed was developed using android platform and studio. This App was made available for downloading in Google's Playstore and could be transferred to others using "Shareit" and "Bluetooth" applications.

Perception of developed Android Fodder App was accessed among 120 livestock farmers of Karnataka. Thirty livestock farmers from each division having smart phones rearing livestock were selected for the study. To explore the perception level among them, an interview schedule was constructed in consultation with the experts and perception level of the Android Fodder App were studied and analysed.

RESULTS AND DISCUSSION

Farmers Perception about the "Android Mobile Fodder App"

Effectiveness of the Fodder App was assessed by analysing the comprehensive perception level of the farmers regarding fodder app. Hence, it is assumed that greater the perception of fodder app as perceived by its users in terms of satisfaction derived from the various components, greater would be the viewers' exposure and post exposure activities. On the basis of perception score, the farmers were respectively categorised into three degrees of groups. The detail of percentage distribution of the respondents under each category is furnished in tabulated.

Relevance

The Fodder App content was evaluated for its relevance to identified whether it reached the information needs of respondents. On perusal of Table 1, majority (56.66%, 66.6%, 53.33%, 55.00%) of respondents from divisions of Bengaluru, Belagavi, kalburgi and as well as total respondents respectively perceived that the contents provided in mobile app were more relevant followed by relevant (43.34%, 33.34%, 46.67%, 45.00%) respectively. But in case of Mysuru division, majority (56.66%) perceived the contents were relevant followed by more relevant (43.34%). None of the respondents from the study area opined that the contents as less relevant. In the process of content development, enough efforts were being made for more credibility of fodder app by gathering locally relevant and authentic information in consultation with Veterinary / Agriculture experts. Phand *et al.* (2013) has developed 'Animal Health Information System (AHIS)' and its perception was tested among the 120 dairy owners and reported that, 61.67 % of respondents opined the content of the AHIS was appropriate to the topic presented, followed by 35.00% who said it was relevant. But only 3.33% respondent felt it was not relevant to the topic, which may be due their higher knowledge level and their need for more details information about certain aspects of the topic.

Preciseness and Logical presentation of information

Preciseness and logical presentation of information are the important characteristics of extension information content to create and stabilize the interest among the farmers. Table 1 revealed that majority (72.50%) had perceived the content in mobile app had been presented in precise way, while 24.16 per cent of the respondents as very precise and only a meagre 3.34 per cent of them opined the contents as not precise i.e the content is lengthy and elaborate. while, majority (61.66%) were highly satisfied followed by 34.17 per cent and 4.17 per cent were satisfied and partially

satisfied with the logical presentation of information in mobile app. Fodder app, having each fodder variety with separate sub-icons (General information, Soil type, Weather condition, Cultivation requirements, Cultivation methods, Harvesting along with yield) was logically presented in precise way for simple understanding among the farmers. Phand *et al.* (2013) reported that 88.33% of respondents felt the content provided AHIS had been presented in a precise way. Regarding logical presentation, similar type of observations was reported by Meena *et al*., where majority (76.67%) were satisfied with logical presentation of information in their educational DVD developed on dairy farming practices.

Simplicity

In order to understand the subject, the content should be present in simple and farmer's language. The respondents were asked their opinion about the simplicity in understanding of information. The result in Table 2. Showed that the information provided in the mobile app was perceived to be very simple by majority (61.66%) of the overall respondents, followed by simple (35.00%) and difficult (3.34%) to understand with no variation of opinions among the divisions. Thammiraju and Sudhakar (2006) had developed 'Poultry Expert System' (PES) and its perceived complexity was tested among the sixty Veterinarians and Veterinary students, where he reported PES was easy in its operation, navigation and understanding of the content through simple language, compared to traditional way of using knowledge system.

Visual quality

Visuals are the ones which are most necessary to hold attention and interest of the farmers. With enough efforts, better quality visuals of various stages of fodder production were provided to impart complete picture for comprehensive understanding. The results showed in Table 2 indicated that among all, majority of respondents perceived that the video quality of mobile app was very good (70.00%) followed by good (24.17%) and poor (5.83%) quality.

Audio quality

The content of the Fodder App was supported by the voice backup in local language (Kannada), so that even illiterate users can also understand. The respondents were asked about the audibility of voice in terms of its clarity, pitch, speed and pronunciation. From the pooled figures in Table 2, it was found that 48.34 % of the respondent opined the audio quality of fodder app as very good, followed by good (46.66%) and only a meagre 5 per cent of them perceived it as poor. Phand *et al.* (2013) found that majority (60.00%) of the respondent expressed as very good voice quality of AHIS followed by 36.67% who informed as good and remaining 3.33% respondents reported it needs improvement.

Credibility of the information

It denotes the degree to which the respondents perceived the information included in the Android Fodder App is trustworthy or reliable. Table 3. results revealed that the information provided in the mobile app was perceived to be more credible by majority (71.66%) of the overall respondents, followed by credible (25.00%) and less credible (3.34%). Similar observation were made by Meena *et al*. (2014) and Sangappa (2015) where a good number (88.89% and 48.97%) of respondents were satisfied with credibility of the information provided in "Education DVD" and **"**Web-enabled Interactive Information Delivery System (WIIDS)" for Dairy Stakeholders respectively**.**

Ability to arouse curiosity and interest

One of the principles involved in interactive learning is arousal of curiosity of learner, which stimulates learning process. Fodder App was assessed for the same attribute, Respondents from all division expressed as either very effective or effective in arousal of curiosity where, on perusal of Table 3. more than half (56.66%) of the total respondents reported that the mobile app was very effective and rest 43.34 per cent reported as effective. None of the respondents from the study area found it as less effective in arousal of curiosity and interest. This is attributed to the fact that, the Fodder App had all essential elements of multimedia tools such as text, graphics, images, animations, video, audio backup, which are integrated enough to make it to have better ability in arouse curiosity and interest among the farmers.

User Friendliness

It refers to the degree of easiness to operate the Android Fodder App. On perusal of Table 3, majority (63.34%, 50.00%, 56.66% & 53.33%) of the respondents from Bengaluru, Mysuru, Belagavi, Kalburgi divisions found it as very easy to use followed by easy (36.66%, 43.34%, 40.00%, 43.33% & 40.83%) respectively. With respect to other response, none from Bengaluru division and only few from Mysuru (6.66%), equal number (3.34%) from Belagavi, Kalburgi divisions expressed as difficult to use it. Data of total respondents expressed that the mobile app was very easy (55.83%) to use followed by easy (40.83%) and rest (3.34%) as difficult to use it. Farmers, as the end users perspective, Fodder App had easily responsible icons, which made it effective farmer centric design. Thammiraju and Sudhakar (2006) reported that 80 % of veterinarians and 90% of veterinary students agreed that the developed 'Poultry Expert System' (PES) a user friendly design.

Perceived utility

Fodder app was developed for livestock owners to create awareness and to enhance their knowledge regarding various fodders orienting them to adopt the technology of growing fodders separately to feed their livestock, which results in balanced ration inturn leads to improved profitability. Glance on Table 4. Depletes that more than half (54.16%) of total respondents, perceived the mobile app with high utility and rest as moderate utility (45.84%) and none of the respondents of any categories found it with less utility. Similarly the system i.e. Animal Health Information System (AHIS) was developed and tested by Phand *et al.* (2013) reported that, AHIS was perceived by majority (85%) as more useful in taking decisions, especially when experts are not available as it educate them mainly on tentative diagnosis of animal diseases at their level, which ultimately help farmers to take decision such as application of first- aid measures inturn resulting in saving on economic losses by saving of time, money and efforts

Information dissemination

It denotes to degree of satisfaction by the respondents that this mode of information dissemination i.e. through smart phone as a android app is satisfactory or not. The data shown in Table 4 clearly indicated that majority (64.16%) of the total respondents expressed the dissemination of information through mobile app as highly satisfied followed by remaining (35.84%) as moderately satisfied and none of the respondents from study area found it as unsatisfied with this mode of information dissemination. Intervention of this new technology for dissemination of information made wider reachability to farmers. Fodder app act as digital book in farmers' hand, they can assess it anywhere at any time. Similarly, 'Android based apps', providing all the agricultural and horticultural crops' information in Kannada language was developed by Babu *et al.* (2015). "Farmers' App" in Gujarati languagewas designed by Vimal *et al.* (2014) providing horticulture information and 'M-learning app' for organic agriculture by Sotiris *et al*. (2014).

Improves self confidence

This refers to the opinion of the respondents that the Android Fodder App act as a tool to enhance confidence level in adopting the practices of growing fodder which is disseminated through the App. Table 4 indicted that more than half (50.83%) of respondents from the study area found it as very effective in improving self confidence followed by effective (43.33%) and less effective (5.84%). This fodder app creates awareness, educate the farmers and motivate them to grow fodder suitable to their area. Similar observation were made by Meena *et al*. (2014) and Sangappa (2015) where a good number (77.78% and 48.14%) of respondents were opined that their respective information system that are "Education DVD" and **"**Web-enabled Interactive Information Delivery System (WIIDS)"found effective in improving the self confidence.

Overall perception

Observations made on the overall perception of Fodder Mobile App as shown in Figure 1a, revealed that, a good number of the respondents from all divisions namely Bengaluru (76.66%), Mysuru (80.00%), Belagavi (80.00%) and Kalburgi (70.00%) had favourable perception followed by neutral perception (23.34%, 20.00%, 20.00% and 30.00%). Among the total respondents, three forth of respondents (76.64%) had perceived mobile app by favourable perception followed by neutral (23.33%) and none of the respondents from the study area had unfavourable perception as shown in Figure 1b. Farmers' friendly features, easy accessibility, more utility made favouring attitude towards it. The results were in consonance with that of Sangappa (2015) and Sasikala (2012). Similar observation were also made by Rathod *et al.* (2016), who reported that perception of scientists and extension experts was more favourable towards relevance, profitability and sustainability of mobile use in dairying however while the perception of farmers was less favourable towards mobile use.

CONCLUSION

The study focuses on finding the effectiveness of Fodder App by analysing farmers' perception level. Android smartphone fodder app, which works offline can be effectively used as intervention tool for overcoming information asymmetry existing among the group of farmers by leveraging the smartphone penetration in rural areas. Many farmers could be illiterates but they are best visual learners and hence such ICT solutions with audio visuals are ideal to overcome information dissemination challenges like illiteracy and wide diversity further coupled with poor internet penetration faced by extension services. Recent trends of increasing demand on livestock products made scientific information and knowledge as important factors for increased production capability. The effective dissemination of information on animal husbandry is most necessary to fetch the increase demands by delivering quality services. The result proves that this mode of information dissemination through app is satisfactory where majority of the respondents opined that the relevance of contents as more relevant (56.66%), preciseness of content as very precise (72.50%), very simple (61.66%) in understanding of information, very good video (70.00%), audio (48.34%) quality, more credible information (71.66%), very effective in arousal of curiosity and interest (56.66%), very easy to use (55.83%), high perceived utility (54.16%), highly satisfied with the logical presentation of information (61.66%) and very effective in improving self confidence (50.83%) with overall favourable perception (76.66%).

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Table 1: Relevancy, Preciseness and logical presentation of information

Sl. No.	Variables	Bengaluru Division		Mysuru Division		Belagavi Division		Kalburgi Division		Overall perception	
		f	%	f	%	F	%	f	%	f	%
1.	**Simplicity in understanding of information**										
	Very simple	21	70.00	19	63.33	17	56.66	17	56.66	74	61.66
	Simple	8	26.67	10	33.34	13	43.34	11	36.67	42	35.00
	Difficult to understand	1	3.33	1	3.33	0	0.00	2	6.67	4	3.34
2.	**Visual Quality**										
	Very good	21	70.00	21	70.00	23	76.67	19	63.34	84	70.00
	Good	8	26.67	7	23.34	6	20.00	8	26.66	29	24.17
	Poor	1	3.33	2	6.66	1	3.33	3	10.00	7	5.83
	Audio quality										
	Very good	15	50	18	60	13	43.33	12	40	58	48.34
	Good	15	50	10	33.34	14	46.67	17	56.67	56	46.66
	Poor	0	0	2	6.66	3	10.00	1	3.33	6	5.00

Table 2. Simplicity, visual and audio quality of fodder app

Sl. No.	Variables	Bengaluru Division		Mysuru Division		Belagavi Division		Kalburgi Division		Overall perception	
		f	%	f	%	f	%	f	%	f	%
1.	**Relevancy of the content**										
	More relevant	17	56.66	13	43.34	20	66.66	16	53.33	66	55.00
	Relevant	13	43.34	17	56.66	10	33.34	14	46.67	54	45.00
	Less Relevant	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
2.	**Preciseness of content**										
	Very precise	21	70.00	20	66.66	24	80.00	22	73.33	87	72.50
	Precise	8	26.66	9	30.00	5	16.66	7	23.33	29	24.16
	Not precise	1	3.34	1	3.34	1	3.34	1	3.34	4	3.34
3.	**Logical presentation of information**										
	Highly satisfied	15	50.00	16	53.33	21	70	22	73.33	74	61.66
	Satisfied	13	43.33	12	40	8	26.66	8	26.67	41	34.17
	Partially satisfied	2	6.67	2	6.67	1	3.34	0	0.00	5	4.17

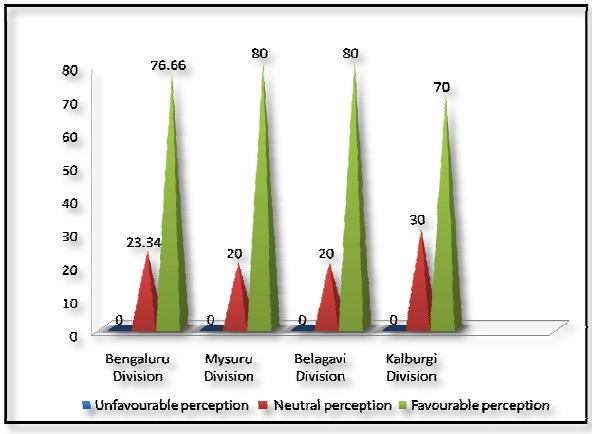

Fig. 1a. Division wise distribution of farmers based on overall Perception.

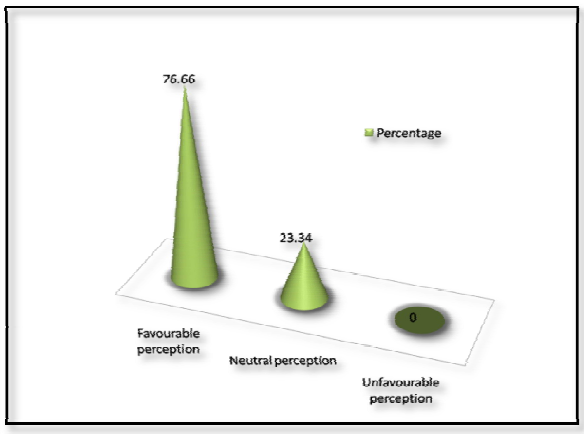

Fig. 1b. Distribution of total respondents based on overall Perception.

Table 3. Credibility, Arousal of curiosity and interest, User Friendliness of Fodder App

Sl.No	Variables	Bengaluru Division		Mysuru Division		Belagavi Division		Kalburgi Division		Overall perception	
		f	%	f	%	f	%	f	%	f	%
1.	**Credibility of the information**										
	More credible	23	76.66	21	70	20	66.66	22	73.33	86	71.66
	Credible	7	23.34	8	26.67	7	23.34	8	26.67	30	25.00
	Less credible	0	0.00	1	3.33	3	10.00	0	0.00	4	3.34
2.	**Arousal of curiosity and interest**										
	More credible	23	76.66	21	70	20	66.66	22	73.33	86	71.66
	Credible	7	23.34	8	26.67	7	23.34	8	26.67	30	25.00
	Less credible	0	0.00	1	3.33	3	10.00	0	0.00	4	3.34
3.	**User Friendliness**										
	Very easy	19	63.34	15	50.00	17	56.66	16	53.33	67	55.83
	Easy	11	36.66	13	43.34	12	40.00	13	43.33	49	40.83
	Difficult	0	0.00	2	6.66	1	3.34	1	3.34	4	3.34

Table 4. Perceived utility, Information dissemination, Improves self confidence of Fodder App rest, User Friendliness of Fodder App

1	**Perceived utility**										
	High utility	17	56.66	13	43.33	21	70.00	14	46.66	65	54.16
	Moderateutility	13	43.34	17	56.67	9	30.00	16	53.34	55	45.84
	No utility	0	0	0	0	0	0	0	0	0	0
2	**Information dissemination**										
	Highly satisfied	20	66.66	16	53.33	18	60.00	23	76.66	77	64.16
	Moderately satisfied	10	33.34	14	46.67	14	40.00	7	23.34	43	35.84
	Unsatisfied	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
3	**Improves self confidence**										
	Very effective	14	46.66	17	56.66	14	46.66	16	53.33	61	50.83
	Effective	15	50.00	9	30	14	46.66	14	46.67	52	43.33
	Less effective	1	3.34	4	13.34	2	6.68	0	0.00	7	5.84

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RESEARCH ARTICLE

Efficacy of Fungal and Bacterial Antagonists against Tomato Fruit Rot

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ABSTRACT

In the present experiment, the efficacy of fungus and bacterial biocontrol agents were tested against tomato fruit rot pathogens both under *in vitro* and *in vivo*. Totally six isolates of *Trichoderma viride* and one isolate of *T. harzianum, T. hamatum, T. konighi* were collected from different area of Madurai district and tested under *in vitro* by dual culture technique. Among them *Trichoderma viride* isolate 3 (*T.v* 3) was found to have maximum per cent mycelial growth inhibition against fruit rot pathogens viz., *Alternaria solani* (70.12), *Colletotrichum capsici* (72.11), *Fusarium solani* (71.46) and *Phytophthora capsici* (71.96) over control followed by *T. harzianum* recorded maximum per cent growth inhibition against *A.solani* (65.99) C*. capsici* (61.89), *F. solani* (60.37), and *P. capsici* (62.55) over control. Among the nine isolates of *Pseudomonas fluorescens* (*P.f 1- 9*) collected from various places of Madurai district *P.f* 3 was found to have maximum per cent growth inhibition against tomato fruit rot pathogens *viz., Alternaria solani* (82.91), *Colletotrichum capsici* (85.00), *Fusarium solani* (84.38) and *Phytophthotra capsici* (83.81) over control. Followed by *P.f* 7 showed 61.15, 60.57, 61.09, and 59.47 percent growth inhibition over control against the pathogens respectively.

Key words: - *Trichoderma*, *Pseudomonas*, Tomato, Fruit rot.

INTRODUCTION

In India, tomato ranks second among vegetables, in area and production and ranks first for processing of vegetable is concerned .The fruits are available in the market through out the year. The incredible increase in the attentiveness of tomato in the recent years, the crop is estimated to occupy an area of 4.54 lakh ha with a total production of 6.88 m. tonnes (Indian Horticulture Database, 2014). In Tamil Nadu the area under tomato was estimated to be about 27.20

thousand hactare with a production of 3,02,270 tones (National Horticulture Production database -2013). However, utilization of tomatoes for processing is low in India compared to other countries. Only 1.30 per cent of total fruits and vegetables produced in India are being processed against 40 per cent in some developing countries and 70 per cent in the developed countries (Negi *et al*., 2015).

Even though India is having fabulous potential in tomato production, a high proportion of harvested tomato fruits are being shattered before they reach the consumer echelon. In India, the loss of vegetables including those during storage and transit was estimated to be around Rs. 230 crores annually.Postharvest losses are due to many factors of which post harvest disease loss is considered as a principal origin. A substantial quantity of vegetable loss occurs every year due to microbial infection resultant in decay during in field, harvest, transit, storage and marketing (Busari *et al.,* 2015).Studies on post harvest loses of tomato were limited and fragmentary.The reports from areas showed that pre and post harvest spoilage of tomato is mainly due to *Alternaria* sp , *Colletotrichum* sp, *Fusarium* sp, *Phytophthora* sp ,*Penicillium* sp, *Rhizopus* sp , *Erwina* sp *Clavibactor michiganense* subsp *michignense*. In this background the present study was under taken to work out the efficacy of fungal and bacterial antagonists against tomato fruit rot pathogens.

MATERIALS AND METHODS

Isolation of antagonistic organisms

The antagonistic organisms were isolated from the rhizosphere and phylloplane region of tomato plants. The rhizosphere soil of tomato plants were collected from different places of Madurai district *viz.,* Agricultural College and Research Institute-Madurai, Palamedu, Alanganallur, Errampatti, hruparangundram, Melur, Usilampatti, Andipatti, and Kottampatti. From these samples serial dilutions were prepared to isolate fungal antagonist 10^3 dilution was used and for the bacterial antagonist 10^6 was used. *Trichoderma viride* selective medium was used to isolate fungal antagonist (0.20g Magnesium sulphate, 0.90g Dipotassium chloride, 3.00 g glucose, o.30 g Ridomil, 0.20g g quintozene (PCNB) 20.00g agar agar) Kings B medium was used to isolate bacterial antagonist (0.15 g rose Bengal, 0.25 g chloramphenicol, 20.00 g agar agar, 1000 ml distilled water, pH 7.0)

In vitro evaluation of antagonists on radial growth of mycelium

Six isolates of *Trichoderma viride* and other species like *T.harzianum T.konighi*, *T.hamatum* and nine isolates of *Pseudomonas fluorescens(P.f 1-9)* were tested against *Alternaria solani*, *Fusarium solani* , *Colletotrichum capsici* and *Phytophthora capsici* by dual culture technique (Dennis and Webster ,1971) on Potato Dextrose Agar(PDA) medium. The mycelial disc of pathogen with 3 mm diameter borer was placed at one end of petri dish, while at the opposite side, mycelial disc of target antagonistic fungus was placed. In case of bacteria one cm long streak of antagonistic bacteria was made two days before the inoculation of the pathogens. Petri dishes were incubated at room temperature (28 ± 2^0 C) and the linear growth of antagonist and pathogen was measured on seventh day after inoculation. The observation on inhibition of mycelial growth recorded in all the *in vitro* experiments. The percent inhibition of mycelial growth was calculated by using the formula described by Vincent (1927).

$$I = \frac{(C-T)}{C} \times 100$$

Where
I=percent inhibition of mycelial growth or spore germination
C=Mycelial growth / spore germination in control
T= Mycelial growth/ spore germination in treatment.

RESULTS AND DISCUSSION

Efficacy of fungal and bacterial antagonists against tomato fruit rot pathogens

To test the efficacy of fungal antagonist an experiment was worked out with six isolates of *Trichoderma viride* and *T. harzianum, T. hamatum, T. konighi* were collected from different area of Madurai district and tested under *in vitro* by dual culture technique (Dennis and Webster, 1971) in which *Trichoderma viride* isolate 3 (*T.v* 3) was found to have maximum per cent mycelial growth inhibition against fruit rot pathogens viz., *Alternaria solani* (70.12), *Colletotrichum capsici* (72.11), *Fusarium solani* (71.46) and *Phytophthora capsici* (71.96) over control followed by *T. harzianum* which recorded maximum per cent growth inhibition against *A.solani* (65.99) C*. capsici* (61.89), *F. solani* (60.37), and *P. capsici* (62.55) over control. In the side of bacterial antagonists, totally nine isolates of *Pseudomonas fluorescens* (*P.f 1- 9*) were collected tested against tomato fruit rot pathogens under *in vitro* by dual culture technique.In which *P.f* 3 was found to have maximum per cent growth inhibition against tomato fruit rot pathogens *viz., Alternaria solani* (82.91), *Colletotrichum capsici* (85.00), *Fusarium solani* (84.38) and *Phytophthotra capsici* (83.81) over control. Followed by *P.f* 7 which showed maximum per cent growth inhibition 61.15, 60.57, 61.09, and 59.47 over control against the pathogens respectively.

Although fungicides are primary means of controlling fruit rot diseases, they have recently come under special scrutiny as they pose potential oncogenic risks when applied to processed foods. So biocontrol is the effective alternative method in controlling fruit rot diseases and there was no health hazard in using them. In this phase different biocontrol agents were tested under *in vitro* and the preeminent one was used for further study. Dorby and Sen (1991) reported that biological control offers an alternative to the fungicide use for the control of post harvest diseases. The efficacy of nine fungal antagonists, which were isolated from the rhizosphere soil, was carried out by dual culture technique (Dennis and Webster, 1971). Among the six isolates of *Trichoderma viride* and *T.harzianum ,T.konighi*, *T.hamatum* tested by the above technique *T.v* 3 was found to have maximum growth inhibition percent against tomato fruit rot pathogens *viz*., *Alternaria solani* (70.12), *Colletotrichum capsici* (72.11), *Fusarium solani* (71.46) and *Phytophthora capsici* (71.96) over control followed by *T. harzianum* recorded maximum per cent growth inhibition 65.99, 61.89 , 60.37, and 62.55 against above pathogens respectively over control. This findings is in accordance with the result given by Jatav *et al* (2005) reported that the volatile compound present in the culture filtrate of *Trichoderma viride* inhibited the mycelial growth and sclerotia formation of *Sclerotinia sclerotiorum* in Pea. Nazam Waris Zaidi *et al.* (2002) reported that *Fusarium oxysporum f. sp. vasinfectum* in okra was found to be effectively controlled by *T. harzianum* under dual plate technique. Sharma (1998) observed that the biocontrol agents, *T. harzianum, T. viride* and *Gleocladium virens* were effective in reducing the mycelial growth of *F. oxysporum* f. sp. *zingiberi* and *P. aphanidermatum*, the causal agents of yellows and rhizome rot of ginger respectively. Usman *et al*. (1996) reported that among the eight species of *Trichoderma* tested, *T. harzianum* (180-2) was the best antagonist in inhibiting the mycelial growth of *P. aphanidermatum* caused rhizome rot in turmeric.

Among the nine isolates *P. f* (3) was found to be efficient and also recorded maximum per cent inhibition against fruit rot pathogens *viz., Alternaria solani* (82.91), *Colletotrichum capsici* (85.00), *Fusarium solani* (84.38), and *Phytophthora capsici* (83.81) over control. Followed by *P.f* 7 showed maximum per cent growth inhibition of 61.15, 60.57, 61.09 and 59.47against above pathogens respectively over control. Several workers have done their work in accordance with this result. Sohin et al ., (2000) reported that tomato fruits treated with three biological agents *viz* ., *Burkholderia sp* (BA-7), *Pseudomonas* sp (BA-8) and *Bacillus* sp.(BA-40) showed significant reduction in the infection by the pathogens . Jatar and Mathur (2005) reported that *B.subtilis* (5) was found to be effective against *F.solani* and *R.solani* in various crops. Txomlexoglou *et al.* (2002) reported that the isolates of *Bacillus* found to inhibit *Botrytis cinerea,* on detached tomato leaves and on whole plants both under *in vitro* and *in vivo* by the production of antifungal compound and biosurfactants. Geels and Schippers.,(1983) reported that *P.fluorescens* recorded high degree of growth inhibition against *Alternaria solani* and *Rhizoctonia solani* of tomato. Koffi *et al.,* (2015) reported that the *P.fluorescens* showing maximum antagonism against tomato fruit rot pathogens under *in vitro* condition.

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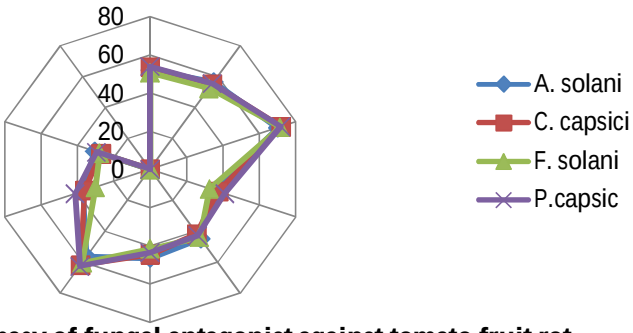

Fig.1.Efficacy of fungal antagonist against tomato fruit rot pathogens

Table.1 Efficacy of fungal antagonists against tomato fruit rot pathogens *in vitro*

S.no	Treatments	*A. solani*		*C. capsici*		*F. solani*		*P. capsici*	
	Fungal antagonist	Mycelial growth (cm)	Growth inhibition (%)	Mycelial growth (cm)	Growth inhibition (%)	Mycelial growth (cm)	Growth inhibition (%)	Mycelial growth (cm)	Growth inhibition (%)
1	*T.viride* 1	40.89	51.97	38.62	53.54	39.63	50.59	38.92	53.74
2	*T.viride* 2	36.99	56.55	37.26	55.18	38.11	52.49	37.62	55.29
3	*T.viride* 3	26.04	70.12	23.46	72.11	24.26	71.46	23.59	71.96
4	*T.viride* 4	54.75	35.69	51.98	37.47	53.96	32.72	49.50	41.17
5	*T.viride* 5	46.93	44.88	48.51	41.65	45.05	43.83	48.21	42.70
6	*T.viride* 6	45.54	46.51	46.13	44.51	46.93	41.48	47.52	43.52
7	*T.harzianum*	36.96	56.59	31.68	61.89	31.78	60.37	31.51	62.55
8	*T.konighi*	53.76	36.86	53.26	35.93	56.03	30.13	49.50	41.17
9	*T.hamatum*	59.00	30.70	60.69	26.99	57.82	27.90	59.40	29.40
10	control	85.14		83.13		80.20		84.14	
CD (P= 0.05)		**0.88**		**0.87**		**0.86**		**0.86**	

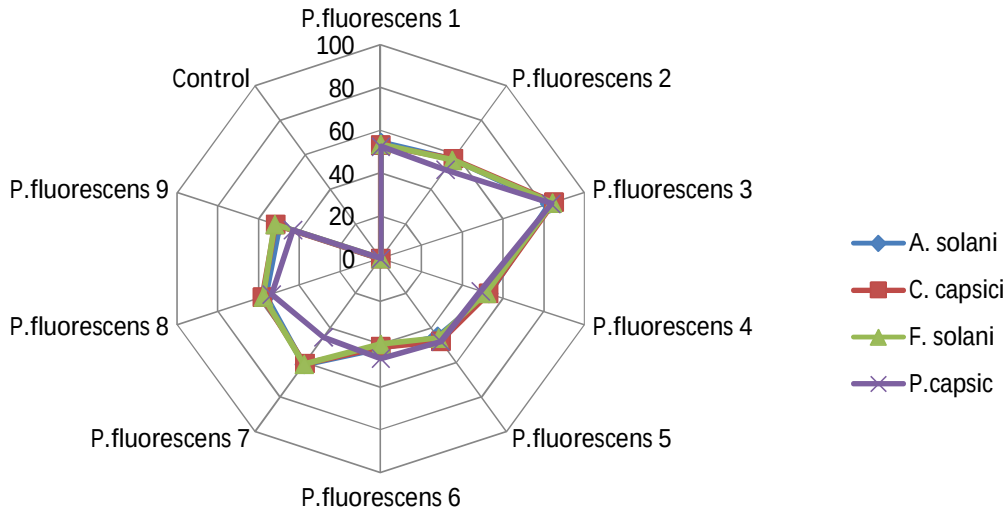

Fig.2.Efficacy of bacterial antagonist against tomato fruit rot pathogens

Table.2 Efficacy of bacterial antagonist against tomato fruit rot pathogens *in vitro*

S. no		*A. solani*		*C. capcici*		*F. solani*		*P. capcici*	
	Bacterial antagonist	**Mycelial growth (cm)**	**Growth inhibition (%)**	**Mycelial growth (cm)**	**Growth inhibition (%)**	**Mycelial growth (cm)**	**Growth inhibition (%)**	**Mycelial growth (cm)**	**Growth inhibition (%)**
1	*P.fluorescens* 1	39.80	54.33	39.50	53.04	39.60	53.41	41.04	52.46
2	*P.fluorescens 2*	37.13	57.40	35.64	57.63	36.63	56.90	42.03	51.30
3	*P.fluorescens 3*	14.89	82.91	12.61	85.00	13.27	84.38	13.97	83.81
4	*P.fluorescens 4*	40.99	52.97	39.60	52.92	40.89	51.89	44.01	49.01
5	*P.fluorescens 5*	48.02	44.90	43.96	47.74	45.94	45.94	45.00	47.87
6	*P.fluorescens 6*	50.69	41.84	49.50	41.15	50.99	40.01	45.99	46.72
7	*P.fluorescens 7*	33.86	61.15	33.17	60.57	33.07	61.09	46.98	45.57
8	*P.fluorescens 8*	37.92	56.49	35.15	58.21	35.93	57.73	39.97	53.70
9	*P.fluorescens 9*	43.76	49.79	40.59	51.75	40.69	52.12	48.96	43.28
10	Control	87.15	-	84.12	-	85.00	-	86.32	-
CD (P=0.05)		**0.80**		**0.79**		**0.79**		**0.78**	

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RESEARCH ARTICLE

Constraints of Dairy Farmers under Individual Milking System and Community Milking System in Kolar district of Karnataka

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ABSTRACT

The present study is mainly aimed at identify the constraints faced by the dairy farmers in production of milk through Individual Milking System (IMS) and Community Milking System (CMS). Three Individual Milking Cooperative Societies (IMCS) and three Community Milking Cooperative Societies (CMCS) each from Bangarpet and Kolar taluks of Kolar district were randomly selected. Ten respondents from each IMCS and CMCS were randomly selected. The findings of the study indicated cent per cent of the respondents expressed consumption of much time for milking as the major problem followed by other constraints like hand milking cause physical strain (95.00%), children and aged people cannot engage in milking activities (80.00%) and milker need to be there at all time (58.34%), In this context the constraints could be converted to advantage in convincing these farmers to adopt machine milking, either individually or community basis. Whereas in case of CMS all the farmers expressed, less number of milking machines as the major (100%) problem followed by less staff in society to help during milking activities (83.34%) and difficult to take the injured and sick animals to society for every milking (70.66%). The staff and milking machines can be increased by superior officers and constraint of bringing sick and injure animals to milking place may be addressed by maintaining one mobile/portable milking machine at community level, which can be taken to the farmers' house and returned.

Key words: - Clean milk production, Community Milking System, Constraints, Individual Milking System, Kolar, Milking machine.

INTRODUCTION

The dairy industry has evolved through consistent efforts and application of science and technology during pre and post independence period of India. Consequently, India has become leading milk producer in the world with 137.7 million tonnes in 2013-14. At the same time India is the second highest populated country with 1.267 billon persons in 2014. The per capita availability of milk is around 299 grams per day in 2012-13 (Basic Animal Husbandry and Fisheries Statistics, 2014) and almost its entire produce is consumed in the domestic market and the country is neither an importer nor an exporter, except in a marginal sense. (Research and Markets, 2015).Milk at the time of secretion from the mammary glands is usually free from microorganisms. However, microorganisms associated with the teat move up the teat canal and into the interior of the udder. This causes, even the aseptically drawn milk to contain microorganisms, mostly bacteria. Bacteria in aseptically drawn milk are usually limited in number and include mostly Micrococci, Lactococci, Staphylococci, Streptococci and Bacillus (Love 4 Cow Trust, 2011).

The hygienic practices at the time of milking are therefore one of the first and most important steps in clean milk production. Clean milk production results in milk that is safe for human consumption, free from disease producing microorganisms, has a high keeping quality and high commercial value and high quality base suitable for processing, resulting in high quality finished products. Milk needs to be protected from all possible sources of microbial contamination. Potential sources of contamination of milk are dung, water, utensils, soil, feed, air, milking equipment, animal and the milker. Contamination of milk can also occur during storage and transport (Kanyeka, 2014).Nanu *et al.* (2007) suggested that the hygienic practices followed during the production of milk at the point of production needs an improvement with regard to reduction in microbial count and in overcoming the impact of the harmful pathogens.Kolar-Chikkaballapura District Co-operative Milk Producers Union Ltd., (KOMUL) has installed “Bulk Milk Coolers and Community Milking Machines (Community milking system)” on pilot basis at society level in the year 2001 to get quality milk required for ultra high temperature processing packed at Kolar dairy under the brand name of Nandini (Good-Life).A study aimed at identify the constraints faced by the dairy farmers in production of milk through Individual Milking System (IMS) and Community Milking System (CMS) will throw light on further steps to be adopted towards milking practices.

MATERIALS AND METHODS

An exploratory research design was adopted for the study. Three Community Milking Societies (Machine Milking Societies) and three Individual Milking Societies (Hand Milking Societies) each from Bangarpet and Kolar taluk of Kolar district were randomly selected. Ten respondents from each Individual Milking Society and Community Milking Society were randomly selected. Thus 60 respondents from Individual Milking Society and 60 respondents from Community Milking Society were selected. In consultation with the subject matter specialists an interview schedule was developed and data were collected from the farmers of IMS and CMS and was then statistically analyzed for frequency and percentage.

RESULTS AND DISCUSSION

The data presented in Table 1 revealed that cent per cent of the respondents of IMS expressed consumption of much time for milking as the major problem followed by other constraints like hand milking cause physical strain (95.00%), children and aged people cannot engage in milking activities (80.00%), milker need to be there at all time (58.34%) and non cooperation of animal during milking in absence of regular milker (28.34%).

The results clearly indicated that the constraints were related to milking, milking time, milker and his presence. It invariable taken more time to milk cross breed cows as they are high yielding.This would increase if number of animals were more. Skilled worker and strain becomes indispensible. As a solution mechanization of milking is one way out.In this context the constraints could be converted to advantage in convincing these farmers to adopt machine milking, either individually or community basis.

Regarding the constraints under CMS (Table 2), all the farmers expressed, less number of milking machines as the major (100%) problem followed by less staff in society to help during milking activities (83.34%), difficulty in taking the injured and sick animals to society for every milking (70.66%), less space near society for gathering of animals (66.66%), longer distance of society from home (40.00%), spread of airborne diseases when animals gather for milking (13.34%) and spread of infections from one animal to other through teat cups of milking machine (11.66%). None of the respondents expressed the problems like, physical injury due to fighting of animal at the time of gathering, irregular electricity (power) supply, non-cooperation from animal during milking and incomplete milking.

From the results it is clearly understood that the constraints shifted from strain and time as in IMS to handling of machines and associated problems of bringing animal to milking place. Less staff in assisting milking would be the major constraints, especially during the initial period as the staff and farmers are trained and get experienced in handling the milking machine, the problem would reduce. Further adequate staff posted. The constraints of bringing sick and injure animals to milking place is bound to exist. This may be addressed by maintaining one mobile/portable milking machine at community level, which can be taken to the farmers' house and returned.Likewise, associated constraints like lack of space, long distance from home and spread of air born diseases can be managed by carefully planning the site, movement of animals, staggering the influx of animals, etc. the other constraint of spread of infection from one animal to other is expressed by few farmers and that too can be addressed by following strict aseptic practices. The findings were in agreement with the findings of Annegret (2004).

CONCLUSION

The constraints of farmers of IMS can be resolved by convincing these farmers to adopt machine milking, either individually or community basis and constraints of farmers of CMS can be solved by increasing staff size and number of milking machines adequately in the societies, mobile/portable milking machine can be taken to the farmers' house and returned in for milking sick/ injured animals, all the above efforts are to be taken up by officers of KMF.

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Table 1.Constraints faced by the farmers of Individual Milking System

Sl. No.	Constraints	Respondents No. = 60	
		f	%
1	Consumes more time for milking	60	100.0
2	Physical strain	57	95.00
3	Children and aged people cannot engage milking activities	48	80.00
4	Milker need to be there at all time	35	58.34
5	Non cooperation of animal during milking in absence of regular milker	17	28.34

Table 2: Constraints faced by the farmers of Community Milking System

Sl. No.	Constraints	Respondents No. = 60	
		f	%
1	Longer distance of society from home	24	40.00
2	Less number of milking machines	60	100.0
3	Less staff in society to help during milking activities	50	83.34
4	Difficult to take the injured and sick animals to society at every milking	43	71.66
5	Less space near society for gathering of animals	40	66.66
6	Physical injury due to fighting of animal at the time of gathering	00	00.00
7	Irregular electricity (power) supply	00	00.00
8	Spread of infections from one animal to other through teat cups of milking machine	07	11.66
9	Non-cooperation from animal during milking	00	00.00
10	Spread of airborne diseases when animals gather for milking.	08	13.34
11	Incomplete milking	00	00.00

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RESEARCH ARTICLE

Normalizing the Histopathology Architecture of the Rat Ovaries in PCOS Induced Rats

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ABSTRACT

Polycystic ovary syndrome (PCOS) affecting reproductive aged women and also known as Stein and leventhal syndrome. Reproductive, endocrine and metabolic systems are mostly affected in this disorder. The study aims to find out the ameliorating effect of the plant *Pergularia daemia* when given in combination with metformin which is an insulin sensitizer. *Pergularia daemia* is an herb which belongs to Asclepiadaceae family. Traditionally it used to treat various diseases like menstrual disorders, cough and asthma. Albino wistar strain rats were selected for the study and the PCOS was induced using letrozole. 1% CMC was given for the control Group rats and other four treatment group rats received letrozole once daily for 21 days. Group II was considered as PCOS model where no treatment was given and serves as a PCOS model. Group III was administrated with metformin for 15 days. Methanolic leaf extract of *Pergularia daemia* was administrated for 7 days in Group IV. Combined administration of methanolic leaf extract and metformin was given for 15 days in Group V rats. After the treatment period, the ovaries were removed and processed for histological observations. The result showed that the treatment group consisting of the combination of metformin and plant ameliorate PCOS condition than plant and /or metformin alone.

Key words: - Polycystic Ovary Syndrome, albino wistar rat, *Pergularia deamia*, metformin, letrozole,

INTRODUCTION

Polycystic ovary syndrome is a multifactorial, complex, endocrine and metabolic disorder (Homburg, 2009) affecting premenopausal women. Hyperinsulinimia and hyperandrogenemia is a main cause of PCOS. Oligo/amenorrhea, hirsutism, obesity, and enlarged ovaries with small multiple cysts that leads to anovulation (Amer, 2009; Sriwardene *et al.,* 2010).In medical field this disease was mentioned as polycystic ovary syndrome (PCOS) based on ultrsonography. Polycystic ovary, sclerocystic ovary, non – ovulatory ovary associated with increased androgen and insulin resistance syndrome. These are the other name for PCOS (Rayan *et al.,* 1995; Speroff *et al.,* 1999). Presence of multiple cysts (> 10) in an ovary is called polycystic ovaries. Endocrine imbalance in the hypothalamus-pituitary-gonadal axis arrest the ovaries from the releasing an egg every month (Bhuvaneshwari *et al.,* 2015). And has an ateric fluid- filled with follicular structure along with thin granulose cell walls (De leo *et al.,* 2003).

Elevated lutenizing hormone (LH) and insulin levels mainly amplify the intrinsic abnormality of their steroidogenesis. Gonadotropin induced estrogen and progesterone synthesis in the follicle is altered by excess androgen activity (Wachs, 2008). With the help of P450 aromatase, testosterone and androstenedione are converted to estrodiol and estrone. P450 play a key role in the ovary's hormonal balaence. Down regulated activity of this enzyme result increased ovarian production and also produce PCO condition (Dunaif, 1997).*Pergularia daemia* (Aasclepiadaecae) is also called as Pergularia in English, veliparuthi in Tamil (Khare, 2007). *Pergularia daemia* is widely distributed in Asia and South Africa, Traditionally this plant is used to treat anthehelmintic, laxative, antipyretic, exporant and infantile diarrhea. This drug was used to treat malarial fever (Kirtikar and Basu, 1983).

MATERIALS AND METHODS

Experimental Animals

Albino wistar strain rats (*Rattus norvegicus*) were used as experimental animals for the present study.The body weights of the animal ranges from 120-180g were used. The animals were kept in a sterile and aerated area. They were fed with pellets were purchased from Sai Durga Enterprises, Chennai. Institutional animal Ethical committee approved the animal house of Holy Cross College, Trichirappalli, TamiNadu,India.

Experimental Design

The study was conducted on 36 female albino wistar rats, divided into five groups. The control group (Group I) that received only 1% aqueous solution of Carboxy Methyl Cellulose (CMC) (Sigma-aldrich, USA) once daily p.o. The four treatment group rats were administered with Letrozole (sigma-aldrich, USA) at a concentration of 1.0 mg/Kg p.o dissolved in 1% of CMC (2.0 ml/Kg) once daily. The treatment period was 21 days. Along with the normal control group, Letrozole induced PCOS rats (Group II) were divided into four treatment groups. The first PCOS induced rats (Group III) were administered with 2mg/100g Metformin (Sigma-aldrich, USA) for 15 days. The Group –IV, were administered with 0.5 ml of Methanolic leaf extract of *Pergularia daemia* for 7 days. Group –V were administered with a combination of Metformin & Methanolic leaf extract of the plant for 15 days. After the experiment the animals were sacrificed. The ovary was excised for histological studies

Stock Preparation

1mg/ kg Letrozole dissolved in 2.0ml/kg of 1% CMC (Carboxy Methyl Cellulose).

Preparation of Plant Extract

The leaves of *Pergularia daemia* were air-dried at room temperature (37°C) for 2 weeks, after which they were ground to a uniform powder of 40 mesh size. 100 g of air dried powder was extracted with methanol (40-60° C) in a Soxhlet extractor for 18-20 hours and solution was evaporated to dryness under reduced pressure and controlled temperature by using rotary evaporator. The extract was stored in a refrigerator at 4 °C in air-tight bottle until further use.

Preparation of Histological Sections

Ovaries were dissected out and fixed in 10% neutral formalin for light microscopic studies. Then the tissues were washed and processed through series of alcohol, for dehydration, cleared in Xylol and finally embedded in paraffin wax. Sections of 5µ thickness were cut using ultra microtome and stained with Haemotoxylin counterstained with Eosin. These sections were mounted in DPX and were studied and Photographed for further studies.

RESULTS AND DISCUSSION

Figure 1 depicts the photomicrograph section of transverse histological section of ovary of the control and experimental group. Control group showing healthy Corpora Lutea (figure 1 a) under 100 X and in 400X shows Corpora Lutea (CL) (figure 1 b). Letrozole induced PCOS group shows abnormal antral follicle (AF), Vascuolated Stroma (VS) (Figure 1 c) under (100 X) and 400X depicts the damaged blood vessels (BV) and more number of secondary follicles (SF) (Figure 1 d). Metformin treated group shows recovered Corpora lutea (CL) and follicle (F) and blood vessels (BV) (Figure 1 e) and in 400x shows regained follicle (F), Corpora Lutea (CL) and Blood Vessels (BV), (Figure 1 f). But it not like control group. Plant treated group recover the corpora lutea (CL) and antral follicle (AF) under 100 X and Thecal Layer (TL) in 400 X (Figure 2 a and b). Metformin combined with plant group showing recovered corpora lutea (CL), blood vessel (BV) under 100 X (Figure 2 c) and In 400 X Corpora Lutea (CL) and stroma (S) exhibit in Figure 2 d.

The ovarian cortex shows the presence of healthy follicle in control group antral follicle, corpora lutea. According to Baravelle *et al.,* 2006; Kafali *et al.,* 2004, the histophathological analysis of PCOS induced rats showed the formation cysts in ovary. The cysts show the attenuated layer of granulosa cells and hyperplasia of thecal layer (Brawer, *et al.,* 1986). Atretic follicle exhibits massive degeneration and sloughing off of the central follicle into antrum. Formation of corpora lutea does not occur or number of corpora lutea is decreased in number that indicating that anovulation and the frequency of the estrous cycle is nearly nil in PCOS induced rats (Brawer *et al.,* 1986; Sasikala and Shamila, 2009). In present study PCOS induced group showing vasculated blood vessel, antral follicle, more than two secondary follicles. The ovarian cyst is formed from antral follicle by the process of apoptosis of oocyte and granulosa cells along with escape of epithelization basal layer of granulosa cells that escapees apoptosis (Salvetti *et al.,* 2003). In metformin treated group showing some recovered corpora lutea, blood vessels and follicles. Plant treated group showing the normalized structure of corpora lutea, follicles, antral follicle but it not clearly like control group. The metformin combined with plant group showing blood vessels, stroma and corpora lutea. Among the five groups, combined therapy of metformin and Pergularia daemia is better than metformin or plant alone.

CONCLUSION

In the current study, the histopathological analysis shows various outcomes from different treatment groups. The plant treated group showed normalcy of the corpora lutea and thecal layer. The metformin treated group recovered corpora lutea but cell debris was found. The regularization of the ovarian architecture was observed in all the groups but the combined therapy of metformin and the plant was more efficient to other groups. Ts it is understood from the

study that the combination of the metformin and the plant was more efficient in the treatment of PCOS rats induced by letrozole.

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100 X **400X**

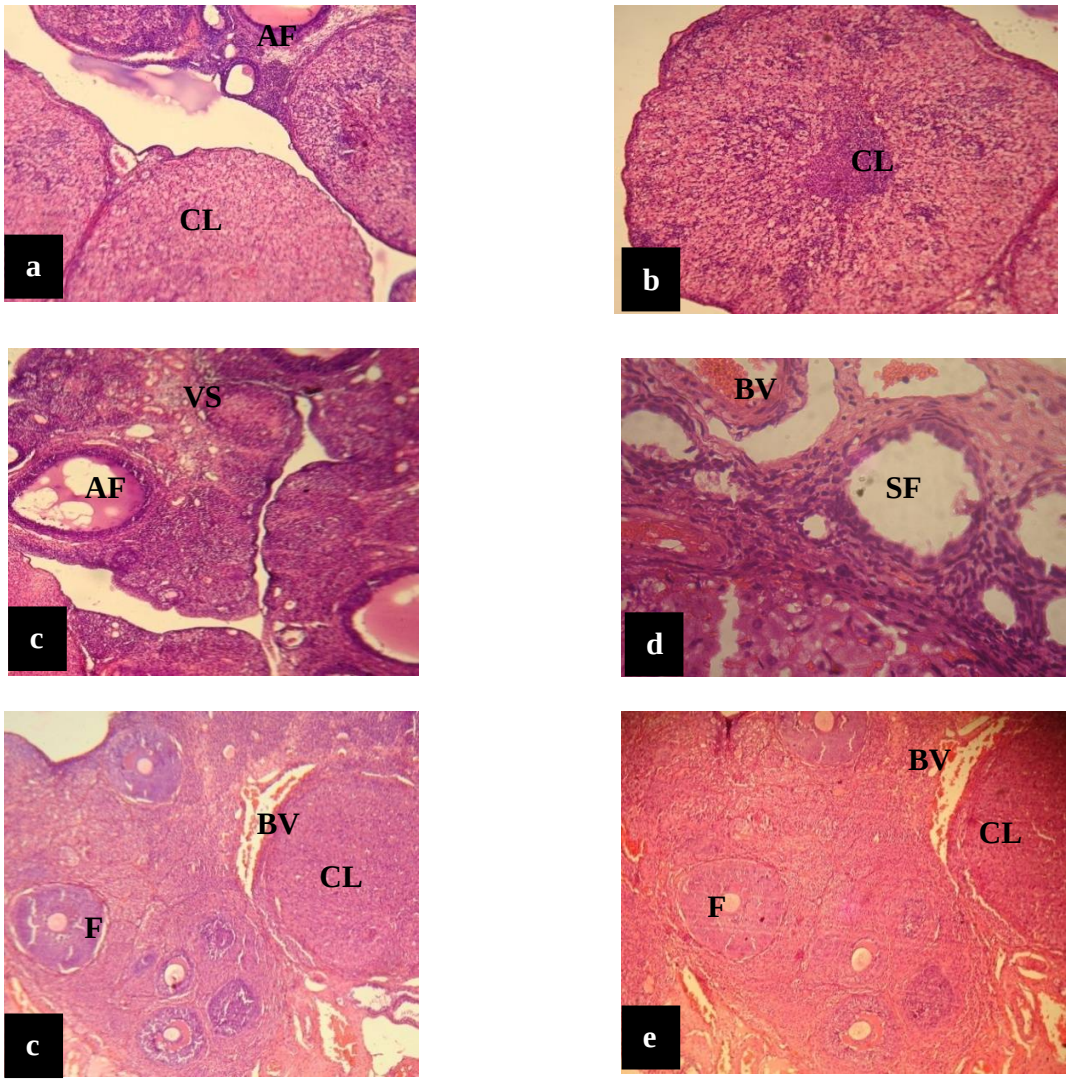

Fig. 1: Corpora Lutea (CL), Antral Follicle (AF), Vasculated Stroma (VS), Blood Vessels, (BV), Secodary Follicle (SF), Follicle (F)

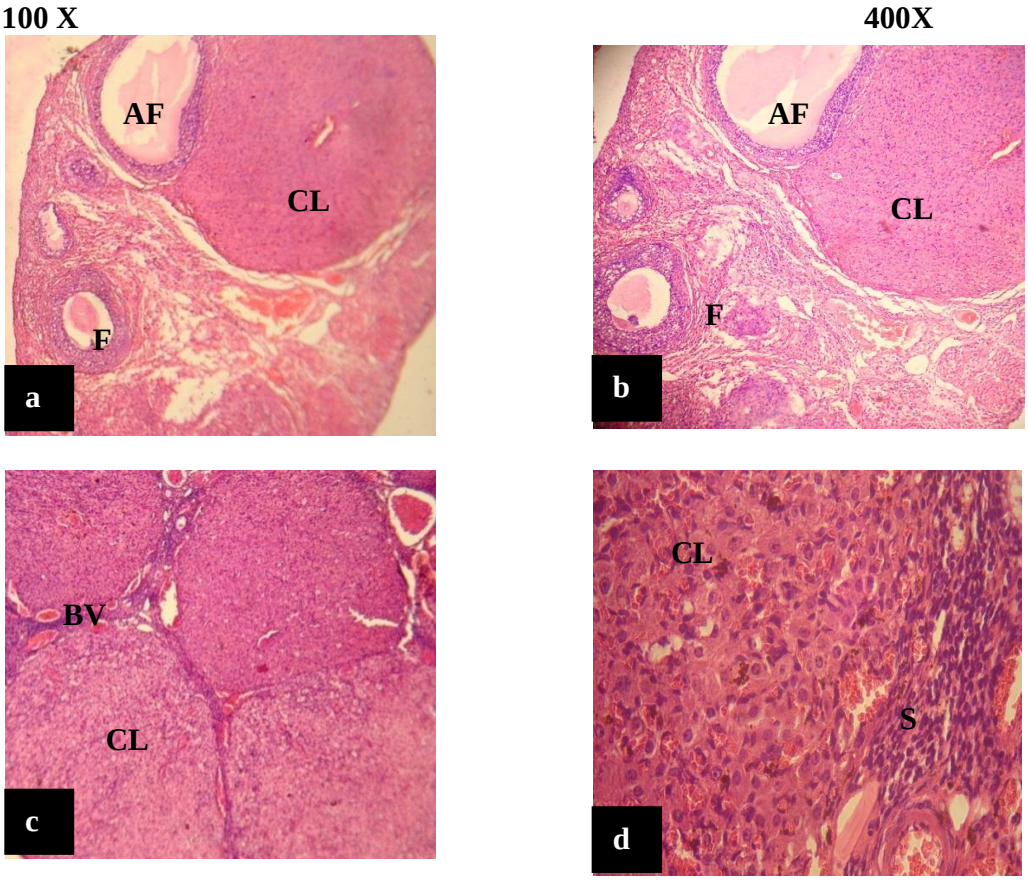

Fig. 2: Antral Follicle (AF), Corpora Lutea (CL), Stroma (S), Blood Vessel (BV),

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RESEARCH ARTICLE

Antimicrobial Activity of Leaves and Stem of Different Extracts of *Tarenna asiatica*

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ABSTRACT

In the present study, we evaluated the antibacterial and antifungal activity of the medicinal plant (leaves &stem) of *Tarenna asiatica* against pathogenic bacteria and fungi by appropriate methods.The results shows that the plant parts posses both antibacterial and antifungal activity.

Keywords: *Tarenna asiatica*, Antibacterial, Antifungal, pathogenic bacteria.

INTRODUCTION

Medicinal plants have been abundantly used for the treatment of various ailments in different countries [1]. Medicinal plants contain many compounds which play very significant role in preventing and treating the various ailments caused by microorganisms [2,3].The plant based medicines are cost effective, natural and less toxic than synthetic or chemical drugs [4,5]. There are many plants that are used as antibiotics against the bacteria and fungi [6, 7]. So in the present study, *Tarenna asiatica* (L) *Kuntze ex Schumann* (Rubiaceae) used for suppuration in boils and skin diseases, injuries etc.,) is carried out for screening *Tarenna asiatica* leaves and stems against pathogenic bacteria and fungi is done in order to detect new origin of antimicrobial agents.

MATERIALS AND METHODS

General laboratory techniques were followed for the preparation of media, inoculation and maintenance of cultures recommended by many methods [8&9].

Cleaning of glassware

All the glassware (Borosil or Corning) were immersed in cleaning solution for a few hours. Then the glassware were washed thoroughly with tap water, followed by detergent solution and finally rinsed with distilled water.The cleaned glassware were dried in hot air oven and stored.Cleaning solution (Mahadevan and Sridhar, 1996).

Potassium dichromate	-	60 g
Conc. H_2SO_4	-	60 ml
Distilled water	-	1000 ml

Potassium dichromate was dissolved in warm water, cooled and sulphuric acid was added slowly. It was mixed thoroughly and used for cleaning glassware.

Sterilization

Dried glassware and media were sterilized in an autoclave for 15 min at 15 lb/sq inch pressure. Soils for pot experiment were sterilized in an autoclave at 20 lb pressure for 2 h on 3 consecutive days.

Chemicals

Analytical grade chemicals supplied by Loba, Hi-Media, S.D.Fine chemicals, E.Merck, Qualigens and sigma Chemicals (U.S.A) were used

Collection of plant materials

The plant specimens were collected from the wild and subsequently, the identification was confirmed by Botanical Survey of India at Coimbatore. Fresh aerial parts of *Tarenna asiatica* (L.) Kuntze ex K.Schum. – Rubiaceae was collected between January to February 2014 from Shervarayan hills at Salem. Leaves and node parts were collected for tissue culture work. Science the explants were collected from the field, they were subjected to sterilization process with suitable sterilants. Subsequently, the aerial parts were collected and washed thoroughly in running tap water followed by distilled water and then shade dried. The dried plants were powdered using mechanical pulveriser and subjected for extraction.

Preparation of crude extract

Leaf samples of *T.asiatica* were air and shade dried for two weeks and pulverized to powder using mortar. This powder (50g) was extracted successively with 200 ml petroleum Ether, Ethyl Acetate, Ethanol, Acetone and Aqueous by using Soxhlet extractor for 48 h at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whattman filter paper (No.1) and then concentrated in vacuum at 40°C using Rotary evaporator. The residues obtained were stored in a freezer - 70°C for future experiments (10). These extracts were dissolved in dimethyl sulphoxide (100 mg/ml) to make the final concentrations.

Evaluation of Antimicrobial activities

The crude extracts were used for bioassay against both bacteria and fungi. The agar plates were prepared and the inoculated. The disc diffusion method was used for the antimicrobial evaluations. Disc of 10mm diameter were placed in to the sterile medium with the test organisms and soaked in different concentration of plant extracts. The plates were incubated at 37°C for 18 to 24h. Antimicrobial activity was evaluated by measuring the inhibition zone in mm in diameter and tabulated.

RESULTS AND DISCUSSION

Antibacterial activity-Diffusion method in *T. asiatica*

Antimicrobial activity showed the zone of inhibition in different part of plant, -ethanol extract, which were extracted by using acetone and ethanol. Leaf acetone extract were showed higher zone of inhibition when compared with leaf ethanol extract. Whereas stem acetone extract showed greatest zone of inhibition than stem-ethanol extract. Here DMSO (Dimethyl sulfoxide) used as a control plate. In this result *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella oxytoca* and *Enterococcus faecalis* indicate highest zone of inhibition in leaf acetone extract. The results were shown in the table 1.In the present investigation, antimicrobial activity of leaf and stem extracts against bacterial and fungal pathogens were recorded considerable antimicrobial activity in different solvent extracts. Hence this extracts centre used for the treatment of various diseases.

Antifungal activity-Diffusion method in *T. asiatica*

The methanol and crude water extracts of *T. asiatica* was very effective to the fungal plant pathogens and the results were shown in table 2. It is a fairly well known fact that medicinal plants serve as significant therapeutic aids for ever so many aliments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century [11].To determine the potential and promote the use of herbal medicine, it is essential to intensify the study of medicinal plants that find place in folklore[12].In the present investigation, antimicrobial activity of leaf and stem extracts against bacterial and fungal pathogens were recorded considerable antimicrobial activity in different solvent extracts. Hence this extracts centre used for the treatment of various diseases.

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Table 1: Antibacterial activity – Diffusion method in *T. asiatica*

Test Organism	Control (DMSO)	L-Ace (mm)	L-EtOH (mm)	St-Ace (mm)	St-EtOH (mm)
Staphylococcus aureus	6.0	12.0	12.0	10.0	7.0
Escherichia coli	6.0	9.0	7.0	10.0	7.0
Klebsiella oxytoca	6.0	10.0	9.0	8.0	7.0
Enterococcus faecalis	6.0	15.0	9.0	10.0	7.0

Table 2: Antifungal activity – Diffusion method in *T. asiatica*

S. No.	Fungi	Methanol (zone in mm)		Water (zone in mm)	
		50 mg	100 mg	50 mg	100 mg
1	*Aspergillus niger*	13	25	13	26
2	*A Flavus*	13	20	12	24
3	*Penicilium sp*	11	23	24	40
4	*Rhizopus sp*	10	16	10	19

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RESEARCH ARTICLE

Phytochemical Screening and Antimicrobial Activity of *Phyllanthus virgatus*

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ABSTRACT

The present study was carried out to determine the presence of phytochemicals present in leaves and stems of *Phyllanthus virgatus* and also the antibacterial and antifungal properties of the plant. Among the various extracts, ethanol and acetone extracts showed the presence of many phytochemical compounds. This study also showed that the antimicrobial activity were also dose dependent.

Key words: Phytochemical screening, *Phyllanthus virgatus*, Antimicrobial, Antibacterial, Antifungal.

INTRODUCTION

India is extensively identified as the botanical garden of the earth as it is the largest manufacturer of medicinal herbs (Shariff *et al*., 2006). Medicinal plants act as a native source of new compounds possessing remedial value and can also be used in drug development. Most of the human population of the developed and developing countries rely on the herbal medicines (Vines, 2004). Due to the abundance use of medicinal plants, the demand for the same has been increased all over the world. Medicinal plants are used because of its least toxicity, cost effective, pharmacologically active and provide easy cure for many human diseases when compared to synthetic drugs (Saet *et al*., 2007).In current scenario the world has witnessed a gradual increase in herbal medicine usage and a decrease in chemical or synthesized antibiotics. Medicinal plants contain abundant active principles which are investigated and isolated for drug development. The drugs are then standardized to ensure its quality.

Phyllanthus virgatus F (Eurphobacea) is commonly known as Bhuiamla, is been used traditionally for treatment of many diseases like intestinal disorders, liver, kidney and bladder disorders. Literature review states that this plant contains immense antioxidant and antihyperglycemic activity (Kirtikar and Basu, 1933; Calixto *et al*., 1998; Kumaran and Joel Karunakaran, 2007; Shabeer *et al*., 2009; Hashim *et al*., 2013). Phyllanthus virgatus contains neither phytoconstituents like nor lignin compounds *(*Huang *et al*., 1998).Though there are wide researches on medicinal plants, many plants have not been explored still and one among them is *Phyllanthus virgatus* F. So an attempt has been made to find the phytochemical screening and antimicrobial activity of the leaves and stem of *Phyllanthus virgatus*.

MATERIALS AND METHODS

Collection of Fresh Plant

Fresh plant materials like leaves, stem, and roots of *Phyllanthus virgatus* were collected in around Namakkal district. The fresh fully-grown plant having sufficient leaves is selected. Collected plant materials were cleaned to remove mud and other adhering weed plants. Fresh plants materials were dried at the room temperature first and then shade dried for 2-3 days.

Preparation of Extract

The coarsely powdered dried leaves and stem of *Phyllanthus virgatus* (50 g) was extracted with petroleum ether, Benzene, Chloroform, Acetone, Ethanol and water by hot extraction process (soxhlation) for 4 hours. After completion of extraction the solvent was removed by distillation and concentrated *in vacuo*. These extracts were used for phytochemical studies.

Preliminary Phytochemical Studies on Extract of *Phyllanthus virgatus* leaves, and stem

The extracts was subjected to qualitative chemical tests for the detection of various plant constituents like alkaloids, steroids, carbohydrates, fixed oils, fats, tannins, phenolic compounds, proteins, amino acids, saponins, flavonoids etc.

Detection of Carbohydrates

A minimum amount of extract was suspended in 5ml of distilled water. The suspension was subjected to the following chemical tests.

a) Molisch's Test

The extract was treated with 2-3 drops of 1 % alcoholic α-naphthol and 2 ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of purple ring between two layers indicates the presence of carbohydrates.

b) Fehling's Test

The extract was treated with mixture of Fehling's A (copper sulphate) and Fehling's B (sodium potassium tartarate) solutions and heated for few minutes. Formation of red precipitate shows the presence of reducing sugar.

c) Benedict's Test

The extract was treated with Benedict's reagent (10 % sodium carbonate, 17.3 % sodium citrate and 1.73 % copper (II) sulphate) and heated for few minutes. Formation of red precipitate shows the presence of reducing sugar.

Detection of Glycosides

A minimum quantity of the extract was hydrolysed with hydrochloric acid for few minutes on a water bath and the hydrolysate was subjected to the following tests.

a) Legal's Test

To the hydrolysate 1 ml of the pyridine and a few drops of sodium nitroprusside solution were added and then it

was made alkaline with sodium hydroxide solution. Appearance of pink to red colour confirms the presence of glycosides.

Detection of Proteins and Amino Acids

A small quantity of extract was dissolved in few ml of water and it was subjected to the following tests.

a) Millon's Test

The extract was treated with Millon's reagent (mercuric nitrate in nitric acid containing a trace of nitrous acid) and boiled in a water bath for 5 minutes. Appearance of pink to red colour indicates the presence of proteins.

b) Ninhydrin Test

The extract was treated with ninhydrin reagent. Appearance of purple colour confirms the presence of amino acids.

c) Biuret Test

To the extract equal volume of 5 % sodium hydroxide solution and 1 % copper- sulphate solution was added. Appearance of violet colour shows the presence of amino acids.

Detection of Fixed Oils and Fats

a) Spot Test

Small quantity of the extract was placed between two filter papers. Formation of oil stain with extract shows the presence of fats and fixed oils.

b) Saponification Test

A few drops of 0.5 N alcoholic potassium hydroxide was added to the extract with a few drops of phenolphthalein solution. Later the mixture was heated on a water bath for about 1-2 hours. Soap formation indicates the presence of fat and fixed oils in the extract.

Detection of Alkaloids

A small quantity of the extract was treated with a few drops of dilute hydrochloric acid and filtered. The filtrate was treated with various alkaloidal reagents to perform the following test.

a) Mayer's Test

The extract was treated with Mayer's reagent (potassium mercuric iodide solution). Formation of cream precipitate shows the presence of alkaloids.

b) Dragendorff's Test

The extract was treated with Dragendorff's reagent (potassium bismuth iodide solution). Appearance of orange brown precipitate shows the presence of alkaloids.

c) Hager's Test

The extract was treated with Hager's reagent (picric acid solution). Formation of yellow precipitate shows the presence of alkaloids.

d) Wagner's Test

The extract was treated with Wagner's reagent (solution of iodine in potassium iodide). Formation of reddish brown precipitate indicates the presence of alkaloids.

Detection of Phytosterols

A small quantity of extract was suspended in 5 ml of chloroform separately. The chloroform layer was subjected to Libermann's test and Salkowski test.

a) Libermann Burchard Test

The chloroform layer of extract was treated with a few drops of concentrated sulphuric acid followed by 1 ml of acetic anhydride solution. Appearance of bluish green colour solution in the extract shows the presence of phytosterols.

b) Salkowski Test

To the 1 ml of chloroform layer, few drops of concentrated sulphuric acid were added. Appearance of brown ring with the extract indicates the presence of phytosterols.

Detection of Flavonoids

a) Shibata's Test

A small quantity of the extract was dissolved in alcohol. To that magnesium metal and concentrated hydrochloric acid was added. Appearance of orange pink colour shows the presence of flavonoids.

Detection of Tannins and Phenolic Compounds

The methanol extract was dissolved separately in minimum amount of water and filtered. The filtrate was subjected to the following tests.

a) Ferric Chloride Test

To the filtrate a few drops of ferric chloride solution was added. Formation of violet colour precipitate shows the presence of phenolic compound in the extract.

b) Lead Acetate Test

To the filtrate a few drops of lead acetate solution was added. Formation of white colour precipitate shows the presence of tannins in the extract.

c) Gelatin Test

To the extract, 1 ml of 1 % solution of gelatin was added. Formation of white colour precipitate shows the presence of tannins in the extract.

Detection of Saponins

a) Foam Test

The extract was diluted with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponin.

b) Haemolysis Test

About 2 ml of human blood was taken in the test tube. To the test tube, an equal quantity of extract was added. Formation of clear red liquid in the test tube indicates that the red blood corpuscles are haemolysed.

Detection of Triterpenoids

A small quantity of the extract was added with little amount of tin and thionyl chloride. Formation of light red colour solution with the extract shows the presence of triterpenoids.

Antimicrobial Activity

The four bacterial strains and the three fungal strains used in the present study.The bacteria used were Escherichia *coli, Staphylococcus aureus, Klebsiella pneumonia* and *Salmonella typhi*.The fungal strains used were *Colletotrichum falcatum, Aspergillus niger* and *Aspergillus flavus*.The screening of ethanol and acetone extracts of leaves, stem and root for antibacterial activity was determined by agar disc diffusion method. The molten Mueller Hinton Agar No. 2 media (Hi-Media) was inoculated with 200 µl of the inoculum (1x108 cfu/ml) when the temperature of media reached 40-42°C and then poured into the Petri plate (Hi-Media). Sterile disc (7 mm) (Hi-Media) was saturated with 100 µl of the extract and allowed to dry.The disc was then introduced on the upper layer of the seeded agar plate. For each bacterial strain controls were maintained where pure solvents were used instead of the extract. The plates were incubated at 37°C for 24 h. The result of antibacterial activity was obtained by measuring the diameter of the zone of inhibition. The experiment was performed under strict aseptic conditions for three times to minimize error.

Fungal strains used

The investigated fungal strains are identified strains and were obtained from the National Chemical Laboratory (NCL), Pune, India. The test fungal strains include *Colletotrichum falcatum, Aspergillus niger* and *Aspergillus flavus.* The fungal strains were grown on sabouraud broth and maintained on MGYP slants (yeast) and potato dextrose agar slants (mould) at 4°C.

Assay for antifungal activity

Preparation of inoculum

The test fungal strains were inoculated into sabouraud dextrose broth and incubated at 28°C on a rotary shaker. The inoculum size waas maintained as per the 0.5 McFarland standards (1x108 cfu/ml). The activated inoculum was used for antifungal assay.

Preparation of test compound

The ethanol and acetone extracts of the leaves, stem and roots were diluted in 100 % dimethylsulphoxide (DMSO). The antifungal activity was evaluated at three different concentrations viz. of 2.5 µg/disc,5 µg/disc, 10 µg/disc and 20 µg/disc and allowed to dry.

The disc was then introduced on the upper layer of the seeded agar plate. For each fungal strain, controls were maintained where pure solvents were used instead of the extract. The plates were incubated at 28°C for 48 hours. The result of antifungal activity was obtained by measuring the diameter of the zone of inhibition. The experiment was performed under strict aseptic conditions for three times to minimize error.

RESULTS

PHYTOCHEMICAL STUDIES

The preliminary phytochemical screening tests were carried out in leaves and stem of *Phyllanthus virgatus* and the results of phytochemical analysis are showed in Table 1 and 2.This study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as terpenoids, reducing sugar, flavonoids, alkaloids were present in the samples. In the present study, the acetone and ethanol extracts showed positive results for many phytochemical tests. They showed positive results for carbohydrates, alkaloids, flavonoids and triterpenoids. The pet ether and hexane extracts showed the presence of phytosterols.

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. The secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value.Among the six extracts used for the study, ethanol and acetone showed the presence of many phytochemical components. So the acetone and ethanol extracts were used for antimicrobial activity and ethanol extract for *in vitro* antioxidant activity.

ANTIMICROBIAL STUDIES

Antibacterial Activity

Leaf extracts

The ethanol extract showed highest antibacterial activity against *Klebsiella pneumoniae* (35 mm) followed by *S. aureus* and *S. typhi* (32 mm) and *Escherichia coli* (31 mm). The maximum zone of inhibition was observed at the concentration of 20 mg/mL. Varying degree of antibacterial activity by leaf extracts of *Phyllanthus Virgatus* against various tested bacterial species has been shown in the table 3.On the other hand, the acetone extract exhibited a much better antibacterial activity as compared to ethanol extract against *Klebsiellap neumoniae* (33 mm) followed by *S. aureus* and *S. typhi* (34 mm) and *Escherichia coli* (33 mm). The acetone extract was most potent against *S. aureus*, showing the maximum zone of inhibition at the concentration of 20 mg/mL.

Stem extracts

The ethanol extract showed highest antibacterial activity against *Klebsiella pneumoniae* (33 mm) followed by *S. aureus* and *S. typhi* (35 mm) and *Escherichia coli* (30 mm). The maximum zone of inhibition was observed at the concentration of 20 mg/mL. Varying degree of antibacterial activity by stem extracts of *Phyllanthus Virgatus* against various tested bacterial species has been shown in the table 4 .On the other hand, the acetone extract exhibited a much better antibacterial activity as compared to ethanol extract against *Klebsiellap neumoniae* (34 mm) followed by *S. aureus* (35 mm) and *S. typhi* (32 mm) and *Escherichia coli* (33 mm). The acetone extract was most potent against *S. aureus*, showing the maximum zone of inhibition at the concentration of 20 mg/mL

Antifungal Activity

Leaf extracts

The acetone extract showed highest antifungal activity against *Colletotrichum falcatum* (18 mm) followed by *Aspergillus niger* (22 mm) and *Aspergillus flavus* (27 mm). The maximum zone of inhibition was observed at the concentration of 20 mg/mL. Varying degree of antifungal activity by leaf extracts of *Phyllanthus Virgatus* against various tested fungal species has been shown in the table 5.On the other hand, the ethanol extract exhibited a much better antifungal activity as compared to ethanol extract against *Colletotrichum falcatum* (19 mm) followed by *Aspergillus niger* (24 mm) and *Aspergillus flavus* 18 mm). The ethanol extract was most potent against *Aspergillus niger* showing the maximum zone of inhibition at the concentration of 20 mg/mL.

Stem extracts

The acetone extract showed highest antifungal activity against *Colletotrichum falcatum* (24 mm) followed by *Aspergillus niger* (23 mm) and *Aspergillus flavus* (25 mm). The maximum zone of inhibition was observed at the concentration of 20 mg/mL. Varying degree of antifungal activity by leaf extracts of *Phyllanthus Virgatus* against various tested fungal species has been shown in the table 6.On the other hand, the ethanol extract exhibited a much better antifungal activity as compared to ethanol extract against *Colletotrichum falcatum* (19 mm) followed by *Aspergillus niger* (25 mm) and *Aspergillus flavus* (25 mm). The ethanol extract was most potent against *Aspergillus niger* showing the maximum zone of inhibition at the concentration of 20 mg/mL.

DISCUSSION

Medicinal plants posses many constituents and therefore it is of great concern to carry out the screening of those plants which may possess a new antimicrobial agents. They may also posse's effective curative agents against the microorganisms and can act as excellent antimicrobial agents (Mehta *et al*., 2010; Okwu, 2001).Phytocompounds such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that defend us against the attack of many microorganisms, insects and other herbivores. In this study, the different extracts of leaves and stem showed the presence of alkaloids, flavonoids, glycosides, steroids, phenols and tannins. The alkaloids have been investigated for many pharmacological properties including antiprotozoal, cytotoxic, antidiabetic and anti-inflammatory properties (Akindele and Adeyemi, 2007). Flavonoids have been reported to possess many useful properties, including anti-inflammatory, antiallergic, antioxidant, vascular and antitumour activity (Harborne and Williams, 2000). Glycosides are known to lower the blood pressure according to many reports (Nyarko and Addy, 1990) Glycosides were reported to exhibit antidiabetic characteristics (Ogbonnnia *et al*., 2008).Plant steroids are known to be important for their cardiotonic activities, possession of insecticidal, anti-inflammatory, analgesic properties, central nervous system activities and antimicrobial properties (Argal and Pathak, 2006).

The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al*., 2007). Phenols and phenolic compounds are greatly used in skin infections and other wounds treatment and also for healing, when compared to other bactericides (Okwu, 2001).Tannins bind to proline rich protein and interfere with protein synthesis (Argal and Pathak, 2006). Tannins decrease the bacterial proliferation by blocking key enzymes in microbial metabolism. Tannins act as potent antioxidant (Dharmananda Subhuti, 2003).

In the present investigation, the antimicrobial activity of various test samples was determined and found to proceed in a dose-dependent manner for different bacterial strains. *S. aureus* is known to cause serious diseases such as pneumonia, meningitis etc., in hospital patients (Curran and Al-Salihi, 1980). *E. coli* cause the urinary tract infections (UTI), pulmonary tract infections, burns, wounds, dysentery-like diarrhoea and other blood infections and similar also true for *K.pneumonia*, *S. typhii* and *S. epidermidis* (Ryan and Ray, 2004). Moreover, the use of *Phyllanthus virgatus* as an antimicrobial agent is still unexplored in scientific research. This study pioneered research work regarding the antimicrobial properties of these plant extracts. Due to the reported development of resistance by bacteria to various commercially available antimicrobial agents, the leaf extracts of these plants are potential sources of new compounds which may be developed as effective drugs against microorganisms if specific chemical components can be isolated and purified.

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Table. 1. Phytochemical screening of leaves of *Phyllanthus virgatus*

S.No.	Phytochemical test	*Phyllanthus Virgatus* (leaves)					
		Pet.	Benzene	Chloroform	Acetone	Ethanol	Aqueous
1	**CARBOHYDRATES**						
	1. Molisch's Test	+	+	+	++	+++	+
	2. Fehling's test	+	+	+	+	+++	+
	3. Benedict's Test	+	+	+	+	+++	+
2	**GLYCOSIDES**						
	1. Legal's Test	+	+	+	+	+	+
3	**PROTEINS AND AMINOACIDS**						
	1. Millon's Test	-	-	-	+	+++	+
	2. Ninhydrin test	-	-	-	+	+++	+
	3. Biuret Test	-	-	-	+	+++	+
4	**FIXED OILS AND FATS**						
	1. Spot Test	+	+	-	-	-	-
	2. Saponification Test	+	+	-	-	-	-
5	**ALKALOIDS**						
	1. Mayer's Test	-	-	+	++	+++	+
	2. Dragandorff's Test	-	-	+	++	+++	+

	3. Hager's Test	-	-	+	++	+++	+
	4. Wagner's Test	-	-	+	++	+++	+
6	**PHYTOSTEROLS**						
	1. Liebermann burchard	+	+	-	-	-	-
	2. Salowski Test	+	+	-	-	-	-
7	**FLAVONOIDS**						
	1. Shibata's Test	-	-	+++	+++	+++	+++
8	**TANNINS &PHENOLIC COMPOUNDS**						
	1. Ferric Chloride Test	-	-	+	++	+++	+
	2. Lead acetate test	-	-	+	++	+++	+
	3. Gelatin Test	-	-	+	++	+++	+
9	**SAPONINS**						
	1. Foam Test	-	-	+	++	+++	+
	2. Haemolysis Test	-	-	+	++	+++	+
10	**TRITERPENOIDS**	-	-	+	++	+++	+

Table. 2. Phytochemical screening of stem of *Phyllanthus virgatus*

S.No.	Phytochemical test	*Phyllanthus Virgatus* (stem)					
		Pet.	Benzene	Chloroform	Acetone	Ethanol	Aqueous
1	**CARBOHYDRATES**						
	1. Molisch's Test	+	+	+	++	+++	-
	2. Fehling's test	+	+	+	+	+++	-
	3. Benedict's Test	+	+	+	+	+++	-
2	**GLYCOSIDES**						
	1. Legal's Test	+	+	+	+	+	-
3	**PROTEINS AND AMINOACIDS**						
	1. Millon's Test	-	-	-	+	+++	-
	2. Ninhydrin test	-	-	-	+	+++	-
	3. Biuret Test	-	-	-	+	+++	-
4	**FIXED OILS AND FATS**						
	1. Spot Test	+	+	-	-	-	-
	2. Saponification Test	+	+	-	-	-	-
5	**ALKALOIDS**						
	1. Mayer's Test	-	-	+	++	++	+
	2. Dragandorff's Test	-	-	+	++	++	+
	3. Hager's Test	-	-	+	++	++	+
	4. Wagner's Test	-	-	+	++	++	+
6	**PHYTOSTEROLS**						
	1. Liebermann burchard	+	+	-	-	-	-
	2. Salowski Test	+	+	-	-	-	-
7	**FLAVONOIDS**						
	1. Shibata's Test	-	-	++	++	+++	+++
8	**TANNINS &PHENOLIC COMPOUNDS**						
	1. Ferric Chloride Test	-	-	+	++	+++	+
	2. Lead acetate test	-	-	+	++	+++	+
	3. Gelatin Test	-	-	+	++	+++	+

9	**SAPONINS**						
	1. Foam Test	-	-	+	+	++	+
	2. Haemolysis Test	-	-	+	++	+++	+
10	**TRITERPENOIDS**	-	-	+	+	++	+

Table 3. Antibacterial activities of leaves of *Phyllanthus virgatus*

Zone of inhibition in mm					
		Escherichia coli	***Klebsiella pneumoniae***	***Salmonella typhi***	***Staphylococcus aureus***
Acetone Extract	**2.5 mg**	24	24	23	22
	5 mg	27	28	27	25
	10 mg	29	30	30	29
	20 mg	33	33	34	34
Ethanol Extract	**2.5 mg**	22	23	21	22
	5 mg	26	26	27	26
	10 mg	28	30	30	29
	20 mg	31	35	32	32

Table 4 Antibacterial activities of stem of *Phyllanthus virgatus*

Zone of inhibition in mm					
		Escherichia coli	***Klebsiella pneumoniae***	***Salmonella typhi***	***Staphylococcus aureus***
Acetone Extract	**2.5 mg**	24	23	25	22
	5 mg	26	26	28	24
	10 mg	29	30	31	28
	20 mg	33	34	35	32
Ethanol Extract	**2.5 mg**	22	25	24	26
	5 mg	25	27	27	28
	10 mg	28	30	30	31
	20 mg	30	33	35	35

Table 5. Antifungal activities of leaves of *Phyllanthus virgatus*

Zone of inhibition in mm				
		Colletotrichum falcatum	***Aspergillus niger***	***Aspergillus flavus***
Acetone Extract	**2.5 mg**	12	-	14
	5 mg	14	14	18
	10 mg	16	18	22
	20 mg	18	22	27
Ethanol Extract	**2.5 mg**	12	-	-
	5 mg	15	13	12
	10 mg	17	18	15
	20 mg	19	24	18

Table 6. Antifungal activities of stem of *Phyllanthus virgatus*

Zone of inhibition in mm				
		Colletotrichum falcatum	***Aspergillus niger***	***Aspergillus flavus***
Acetone Extract	**2.5 mg**	18	14	11
	5 mg	20	16	15
	10 mg	22	18	19
	20 mg	24	23	25
Ethanol Extract	**2.5 mg**	12	-	13
	5 mg	15	14	16
	10 mg	17	19	18
	20 mg	19	25	25