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Editor

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RESEARCH ARTICLE

Evaluation of Nutritional and Phytochemical Constituents of *Cucurbita maxima Duchesne* and *Cucurbita moschata Lam* Seeds

Dhanalakshmi. A

P.G & Research Department of Microbiology, Sri Venkateshvara College of Arts and Science.

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Address for correspondence
Dhanalakshmi. A

P.G & Research Department of Microbiology,

Sri Venkateshvara College Of Arts and Science, Peravurani - 614 804, Thanjavur

Email ID: a.dhanam@ yahoo.co.in

ABSTRACT

The nutritional and chemical constituents of *Cucurbita maxima Duchesne* and *Cucurbita moschata Lam* have been evaluated adopting standard methodology and the results are presented in the present report. This investigation attempted to understand the nutritional importance of the seeds besides their phytochemical significance. The seeds of *Cucurbita maxima Duchesne* contain 8.62±1.50mg of carbohydrate, 34.93±0.42mg of protein, 20.6±0.62mg of amino acids, 4.03±0.04mg of chlorophyll and 1.15±0.96mg of carotenoids where as the seeds of *Cucurbita moschata Lam* appears to contain relatively more carbohydrate (8.86±2.60mg), protein (32.03±2.62mg), amino acids (18.6±0.26mg), chlorophyll (4.65±0.03mg) and carotenoids (0.26±0.006mg). However *Cucurbita maxima Duchesne* and *Cucurbita moschata Lam* contain more or less equal amounts of tannins, lignins, glycosides and serpentines. Further the level of calcium is found to be more in *Cucurbita moschata Lam* than in the *Cucurbita maxima Duchesne,* but the level of phosphorous, magnesium and potassium is more or less equal in both the seeds. Besides this, the antibacterial activity of both the seeds and their deficiencies has also been assessed using disk diffusion method by measuring the diameter of the growth inhibition zones and the results are reported here.

Keywords: Amino acids, pumpkin seeds, squash seeds, trace minerals.

INTRODUCTION

Until recent years, nutritionists have focused primarily on macro and micro nutrients in foods. Appreciation is now increasing that many food components of plant origin 'phytochemical' in particular has the potential to affect human biology. Phytochemical by definition are important components of food that may not be essential in the classical sense and may not even be required to sustain life as vitamins or minerals do, but are likely to contribute to optimal health [1]. Phytochemicals have been linked to many other positive health effects in human and animal studies, including coronary heart disease, cancer, diabetes, high blood pressure, inflammation, infection, psychotic disease, ulcers and macular degeneration. It is becoming evident that many phytochemicals may have multiple actions on human health [2]. Seeds contain high calories and protein and they are a good source of vitamin E and B as well as dietary fibre which is essential for regulating the bowel movements. Seeds may help to lower blood cholesterol levels because the fat content is largely unsaturated seeds and nutritional value to soups, salads and baked foods [3].

Phytosterols are compounds found in pumpkins seeds that have chemical structure very much similar to cholesterol and when present in the diet in sufficient amounts are believed to reduce blood levels of cholesterol enhance the immune response and decrease risk of certain cancers [4]. Pumpkin seeds are a balanced source of good proteins. They are nourishing and energizing. In additional to protein, they are an excellent source of iron, vitamin B, vitamin E, fibre, oil and minerals. Their popular medicinal uses have focused intense research on pumpkin for the post few decades using modern tools [5]. Squash fruits contain moisture, liquid, protein, ash and non polar lipids like glycolipids and phospholipids. The major fatty acid present in the lipid was reported to be linoleic acid. The leaf contain calcium, magnesium, iron, zinc and copper [6]. The aim of the present study is to evaluate the nutritional and chemical constituents of *Cucurbita maxima Duchesne* and *Cucurbita moschata Lam* adopting standard methodology.

MATERIALS AND METHODS

Collection of plant foods

The plant materials namely *Cucurbita maxima Duchesne* and *Cucurbita moschata Lam* seeds for the present investigation were obtained from Kumbakonam town, Thanjavur district, Tamilnadu, India during January 2008.

Analysis of phytoconstituents

Qualitative phytochemical analysis of carbohydrate, protein, amino acids, flavanoids, tannins, terpenoids, saponins, alkaloids, steroids and cardiac glucosides was carried out from the methanolic extract of Cucurbita maxima Duchesne and *Cucurbita moschata Lam* seeds using the standard procedure [7].

Secondary Metabolites

The secondary metabolites were quantitatively evaluated using HPLC by the method of Stahl Egon [8]. The mineral constituents of the seeds of the mentioned plants were quantitatively assayed using Atomic Absorption Spectra (AAS) by the method of Meyer and Keliher [9]. The macro minerals were analyzed using Flame Emission Spectra (FES) by the method Mohamed et al. [10].

Phytoconstituents

The carbohydrate was estimated by Anthrone method [11] where as protein by the method of Lowry et al. [12]**.** The amino acids were estimated adopting the method suggested by Moore and Stein [13].

Carotenoids

Absorbance of the 80% acetone extract was read at 473 nm and quantitative estimation done using an extinction coefficient of 2500 as an average value [14]. Chlorophyll a, b and total chlorophyll were estimated by the method of Arnon [15].

Carotenoids = 4 x OD at 473 x v /1000 x 10
v- Total volume of the sample

Antibacterial effect of *Cucurbita maxima Duchesne* and *Cucurbita moschata Lam*

The antimicrobial assay was performed by disk diffusion method [16] using *Staphylococcus aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

RESULT AND DISCUSSION

The nutrients obtained from food are vital to keep the body health and alive. Nutrients are required in order to build and repair cells and body tissues, maintain the organs and bone in optimum working conditions and to provide energy, fuel and warmth. Good nutrient is essential for good health and eating nutrients rich food can help to prevent common ailments, as well as more life threatening illness and disease [2]. The qualitative analysis of nutrients such as carbohydrate, protein and amino acids and phytochemical such as flavonoids, alkaloids, tannins, terpenoids and saponins in the *Cucurbita maxima Duchesne* and *Cucurbita moschata Lam* seeds showed positive results (**Table-1)**. The quantitative analysis of nutrients such as carbohydrate, protein, amino acids, chlorophyll and carotenoids in *Cucurbita maxima Duchesne* and *Cucurbita moschata Lam* seeds **(Table -2).** The carbohydrate levels range

from 7.11-10.0%. An analysis of variance shows that there is no significant difference between the *Cucurbita maxima Duchesne* and *Cucurbita moschata Lam* seeds. They help to prevent constipation and it can reduce blood cholesterol level [17]. The protein content ranges from 26.68 -40.49%. The test analysis of variance shows that the protein content of *Cucurbita maxima Duchesne was* significantly higher than that of *Cucurbita moschata Lam* seeds. Protein is a vital nutrient of the human body. These seeds are especially good for growing children, pregnant women, lactating mothers and old people [18]. The amino acid content of *Cucurbita maxima Duchesne is* relatively higher than *Cucurbita moschata Lam* seeds. Amino acids are the chemical units or "building blocks" of the body proteins. The chlorophyll content is present in trace amount in *Cucurbita maxima Duchesne and Cucurbita moschata Lam* seeds. Chlorophyll has protective properties such as prevention of coronary heart disease, certain cancers, diabetes and cataracts [19]. Carotenoids are pigments found in plant and micro organisms, but not synthesized in animals. It reduces cancers and cardiac vascular diseases [19]. The levels of secondary metabolites in *Cucurbita maxima Duchesne* and *Cucurbita moschata Lam* seeds are showed in **Table 3**. Flavonoids have been linked to their known medicinal properties as strong anti oxidants, free radical scavengers and metal chelators. Flavonoids have been reported to exert multiple biological effect including antibacterial, antiviral, and antitoxic and anti- inflammatory activities. Alkaloids act as reservoir of nitrogen, as well as protein synthesis. Tannins have been inhibitory effect on some Gram-negative bacteria [20]. Glucosides are useful in decreasing the heart rate, decreased sympathetic activity and decreasing systemic vascular resistance [21]. The level of macro mineral content (%/g) in *Cucurbita maxima Duchesne* and *Cucurbita moschata Lam* seeds showed in **Table-4**. Calcium builds strong bone and teeth, treats and prevents osteoporosis. Magnesium is necessary for calcium and vitamin C metabolism. It converts blood sugar into energy and improves energy production within the heart. The level of potassium found more or less equal in both the seeds. It helps to prevent strokes and works with sodium to control body water balance. A deficiency may lead to poor reflex cardiac arrest and muscle damage. Sodium is found mainly in blood plasma and in the fluids outside the body cells. It helps the muscle and heart to relax [22]. Quantitative analysis of micro minerals *Cucurbita maxima Duchesne* and *Cucurbita moschata Lam* seeds assayed both by colorimetry and FES and represented in **Table- 5**. The level of manganese is greater in *Cucurbita maxima Duchesne* than in *Cucurbita moschata Lam*. Manganese is needed for protein and fat metabolism, normal bone growth. Chromium maintains stable blood sugar levels through proper insulin utilization and it promotes a healthy circulation. Zinc is essential synthesis and collagen formation. A deficiency may lead to delayed sexual maturity [23]. Copper promote connective tissue formation and central nervous system function. Iron prevents anemia and fatigue and stimulates the immune system [24]. Antibacterial activity of *Cucurbita maxima Duchesne and Cucurbita moschata Lam* seeds was done by two different extracts and compared their activity using *Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae.* Thus both *Cucurbita maxima Duchesne and Cucurbita moschata Lam*. seeds show inhibitory actions against pathogens, but when compared to *Cucurbita maxima Duchesne and Cucurbita moschata Lam*. shows high degree of inhibitory action against *Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae.* Among the two extracts of *C.moschata*, methonolic extract shows high degree of activity against *Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae.* Comparing with the standard effect seemed to be less for both the plant extracts.

CONCLUSION

In conclusion the present investigation suggests that both the seeds contain a wide variety of phytochemicals, macro elements, microelements and amino acids essential for human nutrition. Further both of the seeds have been found to have strong antidiabetic, anticancer, antibacterial and antioxidant potential to attract the attention of nutritionist. The present study suggests that these seeds can further the processed and used with an advantage of enriched protein, amino acids, minerals and vitamins to combat malnutrition to enhance the nutrition of pre school children, lactating mothers. It is further suggested that those living in developing countries be encouraged to consume processed pumpkin seeds as it protects them against pathogenic organisms such as *Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae* which cause diseases.

Table-1 Qualitative analysis of nutrients in *Cucurbita maxima Duchesne* and *Cucurbita moschata Lam* seeds

S. No	Nutrients	*C.maxima*	*C.moschata*
1	Carbohydrates	+	+
2	Protein	+	+
3	Amino acids	+	+
4	Flavonoids	+	+
5	Tannins	+	+
6	Terpenoids	+	+
7	Saponins	+	+
8	Steroids	+	+
9	Cardiac glucosides	+	+
10	Alkaloids	+	+

(+) - Present

Table.2 Quantitative analysis of nutrients in *Cucurbita maxima Duchesne* and *Cucurbita moschata Lam*

S. No	Nutrients	*C.maxima* (mg/g)	*C.muschata*(mg/g)
1	Carbohydrates	8.62±1.50	8.86±2.60
2	Protein	34.93±0.42	32.03±2.62
3	Amino acids	20.6±0.62	18.06±0.26
4	Chlorophyll	4.035±0.04	4.685±0.03
5	Carotenoids	1.154±0.96	0.76±0.006

Note: Values are expressed as mean ± S.D

Table-3 Amount of secondary metabolites in *Cucurbita maxima Duchesne* and *Cucurbita moschata Lam* seeds determined by HPLC

S. No	Secondary metabolites	*C.maxima* (mg/Kg)	*C.muschata*(mg/Kg)
1	Total flavonoids	0.64	0.55
2	Total alkaloids	0.87	0.74
3	Tannins	0.09	0.08
4	Lignins	0.05	0.06
5	Glycosides	0.19	0.22
6	Sepertaines	0.23	0.19

Table-4: Quantitative analysis of macro minerals *Cucurbita maxima Duchesne* and *Cucurbita moschata Lam* seeds assayed both by colorimetry and FES.

S. No	Macro mineral	*C.maxima* (%)	*C.muschata* (%)
1	Calcium	10.67	12.36
2	Phosphorous	0.64	0.81
3	Magnesium	3.69	3.74
4	Potassium	6.34	6.97
5	Sodium	1.36	1.06
6	Sulphur	0.22	0.25
7	Ash	0.98	0.82
8	Nitrogen	4.52	5.19

Table-5: Quantitative analysis of micro minerals *Cucurbita maxima Duchesne* and *Cucurbita moschata Lam* seeds assayed both by colorimetry and FES.

S. No	Micro minerals	*C.maxima*(ppm/g)	*C.muschata*(ppm/g)
1	Manganese	12.36	10.26
2	Chromium	0.02	0.03
3	Zinc	3.48	3.19
4	Copper	1.06	1.25
5	Iron	45.36	55.26
6	Nickel	0.09	0.08

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RESEARCH ARTICLE

Groundwater Quality Assessment for Domestic Purpose in the Selected Locations of Arcot Region, Tamil Nadu, India

Rutharvel Murthy. K[1], Dhanakumar. S[2], Solaraj.G[2], Mohanraj. R[2], Jawahar Raj. N[3] and Kumaraswamy. K[1*]
[1] Department of Geography, School of Geosciences, Bharathidasan University, Tiruchirappalli – 620 024
[2] Department of Environmental Management, Bharathidasan University, Tiruchirappalli – 620 024
[3] Department of Geology, National College, Tiruchirappalli – 620 001

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[1*]Address for correspondence
Dr. Kumaraswamy. K
Professor and Head, Department of Geography, School of Geosciences,
Bharathidasan University, Tiruchirappalli – 620 024
Email ID: kkumargeo@gmail.com

ABSTRACT

Quality of groundwater is equally important to its quantity due to its suitability for various domestic uses. Water quality assessment is an important issue in groundwater studies. The present study was carried out in tannery dominant Arcot region of Vellore District, Tamil Nadu. Groundwater samples were collected during pre-monsoon and post-monsoon seasons of 2008. The results were compared with World Health Organization and Bureau of Indian Standards. It was found that the concentration of sodium and phosphate is higher than the permissible limits. The results of correlation and Paired *T- test* demonstrate the significant difference in the quality of groundwater between the sampling events.

Keywords: Groundwater, human health, tannery pollution, water quality

INTRODUCTION

Groundwater resources have been considered as a main source of water supply for drinking and other domestic purposes in the rural areas of India. The Indian Ministry of Water Resources has estimated the replenishing capacity of groundwater potential of the country as 431 km^3 per year. The water uses for drinking, industrial and other purposes (other than irrigation) is 16 per cent of the total potential and the remaining 84 per cent is used for irrigation purposes (360 km^3 per year). Undesirable changes in the groundwater quality caused by natural and anthropogenic sources may lead to human health consequences. As per the (WHO) World Health Organization's reports, about 80% of all the diseases in human beings are caused by water [1]. Since, groundwater resources are the main source for drinking, where the surface water is scarce [2]. In this connection, monitoring the groundwater quality remains to be a principal necessary and a most important challenge in rural India. Arcot region (parts of Arcot and Timiri blocks) is located in the eastern part of Vellore District in the state of Tamil Nadu, India. According to 2001 census, Arcot had a population of 3, 10,646. Geographically the region is located between Vellore city on the west and Palar River towards south, it lies between 12°37'37'' and 12°57'25'' North latitudes and 79°10'50'' and 79°28'41'' East longitudes. This region is surrounded by numerous leather tanning industries in western and northern directions. The present study attempted to assess the extent of groundwater quality in Arcot region with respect to domestic purposes.

MATERIALS AND METHODS

Geologically, Arcot region falls under sedimentary and hard rock formations. Sedimentary (or) quaternary alluviums are found along Palar River course and southern portions are covered by hard rock formations like gneiss and charnockites. Eight groundwater samples were collected from bore wells and open wells in the Arcot region during post-monsoon (January) and pre-monsoon seasons (July) in 2008. The locations of sampled wells are given in Figure 1. The pH, Electrical Conductivity (EC) and Total Dissolved Solids (TDS) were measured in the field using Water Analyzer (model: 371 make: Systronics). Carbonate, bicarbonate, Total Hardness (TH), calcium, magnesium, and chloride were estimated by titration method. Sodium and potassium was determined using Flame Photometer. Nitrate, fluoride, phosphate and sulphate were estimated as per standard spectrophotometer methods [3].

RESULTS AND DISCUSSION

The physico-chemical characteristics of groundwater were presented in the Table 1 and 2. The pH value of all stations lies between 6.65 and 8.07 irrespective of sampling events. All the samples were found within the maximum permissible limit recommended by standards of World Health Organization (6.5 to 9.2). Electrical Conductivity is known to be a measure of mineral content, was found varying from 0.98 to 3.83 mS/cm in post-monsoon and from 0.935 to 1.21 mS/cm during pre-monsoon season. The concentration of Total Dissolved Solids (TDS) differs from 364 to 1550 mg/l during pre-monsoon season and from 455 to 1850 mg/l during post-monsoon season. Higher level of total dissolved solids during both the seasons indicates sufficient input of ionic matter into groundwater samples. Maximum level of TDS was recorded at Sattampakkam in both the seasons may be contributed by highly polluted Palar River. Since, the sampling location is closely situated in the southern bank of River Palar, which has been contaminated by untreated effluents of tanneries and municipal sewages were probable contributors. Elevated level of TDS in groundwater is generally not harmful to human beings but high concentration of these may affect persons, who are suffering from kidney and heart diseases [4]. However, high solids may also cause laxative or constipation effects. Chloride content of the groundwater samples ranges from 113.44 to 397.04 mg/l in pre-monsoon season and from 106.35 to 659.37 mg/l in post-monsoon season. The slight decline in the chloride during the post-monsoon season suggested that the possible dilution effect on chloride concentration. Maximum level of chloride was recorded at Sattampakkam (659.37 mg/l) during pre-monsoon period. High chloride in groundwater samples may be due to the pollution from chloride rich effluents of sewage and municipal waste. In the both seasons chloride was recorded with in the limit of maximum permissible level of 1000 mg/l [5]. The carbonate-bicarbonate system in natural waters controls the pH and the natural buffer system. In the present study, the values of bi-carbonate ranges from 61 to 366 mg/l during pre-monsoon season and from 36.6 to 61 mg/l during post-monsoon season. The carbonate is absent in all the sample locations in the post-monsoon season. The result reveals that the alkaline nature of the groundwater is probably due to bi-carbonate and not because of carbonate alkalinity. The bi-carbonate content of all samples falls within the permissible limit (500 ppm) of World Health Organization. In the present investigation, the level of total hardness recorded between 230 and 690 mg/l during pre-monsoon season and from 120 to 315 mg/l during post-monsoon season. Desirable limit of total hardness is 300 mg/l; however in the absence alternate source it is permissible up to 600 mg/l [6]. Significant decline was observed in most of the post-monsoon samples and it may be due to the recharge of groundwater dominated by monsoonal rainfall. Sodium content of the groundwater samples ranges from 135.70 to 361.10mg/l in pre-monsoon season and from 32.20 to 177.10 mg/l in post-monsoon season. According to Srivastava [7], high level of sodium may adversely affect the cardiac, renal and circulatory functions. The potassium content in groundwater was found within the range of 0.34 to 6.57 mg/l in post-monsoon season and from 3.90 to 10.34 mg/l in pre-monsoon season. The concentration of phosphate varied between 0.01 - 0.21 mg/l and 0.04 - 0.51 mg/l during the period of pre-monsoon and post-monsoon seasons, respectively. The mean concentration of phosphate during post-monsoon season (0.18 mg/l) was fairly higher than pre-monsoon season (0.10 mg/l). Enrichment of phosphate in the groundwater was mainly because of domestic sewage, detergents and agricultural

effluents. Elevated level of phosphate leads to increase in the growth of algae and eutrophication. High concentration of phosphate is indicative of pollution and the major source of anthropogenic phosphorus is sewage, detergents, agricultural effluents, and fertilizers [8] [9]. The permissible limit of USPHS (United State Public Health Service) is 0.1 mg/l. The mean level of sulphate was found between 119.24 mg/l and 73.30 mg/l during pre-monsoon and post-monsoon periods, respectively. Significant reduction in the sulphate content during post-monsoon season was observed in all the sampling locations. Maximum level of sulphate was recorded at Sattampakkam in both sampling events. Baruah et al. [10] has reported that the high concentration of sulphate in the groundwater may be attributed by the untreated industrial and domestic waste effluents. The sulphate concentrations of all samples were found under the limit prescribed by BIS (Bureau of Indian Standards) in both the seasons. The level of nitrate lies between 0.09 - 17.41 and 0.44 - 17.72 mg/l during pre-monsoon and post-monsoon seasons, respectively. There were not any drastic changes found in the nitrate level in both the sampling events. Maximum level of nitrate was recorded in Varagurpattinam in both seasons. Since the sampling location was geographically located nearer to the River Palar. Infiltration of tannery and municipal sewage from the river bed may be probable contributor for the enhanced level of nitrate in this location. Galvez et al. [11] stated that the high concentration of nitrate can cause health problems in infants and animals, as well as eutrophication of water bodies. The prescribed desirable limit of nitrate is 45 mg/litre [12] The fluoride levels were recorded between 0.67 - 0.79 mg/l in post-monsoon season and 1.09 to 1.23 mg/l in pre-monsoon season. While comparing the post-monsoon samples mean fluoride content (0.73 mg/l) was higher during the pre-monsoon season (1.16 mg/l), it may be because of dilution effect. Chronic exposure of high level of fluoride may lead to thyroid functional disorders and neurological effects [13]. In the present study, fluoride content in all groundwater samples were within the optimum concentration of 1.5 mg/l, as recommended by WHO [14]. The correlation matrices for pre-monsoon and post-monsoon samples were given in the table 3 and 4. The total hardness with EC and TDS, calcium with sulphate were strongly correlated in the both seasons. Ca and Mg also positively correlate with total hardness in the both sampling events. EC, TDS, TH and Ca constituents have shown their good correlation with chloride, suggesting the probable contribution from anthropogenic sources like leaching or salt present in industrial effluents. The same trend of observation was also reported by Mehlknecht et al. [15]. Nitrate showed moderate correlation with bicarbonate during pre-monsoon, may be due to the ion-exchange process in the groundwater aquifer system. The concentration of Ca, Mg and SO_4 in the both the seasons were shown in figure 2 and 3. Paired *T test* results indicates that pH, total hardness, sulphate, nitrate, bicarbonate, fluoride, potassium and sodium shows statistically significant difference between the sampling events at 95% of confident level .

CONCLUSION

The present investigation reveals that the groundwater quality in the study area did not fall under polluted category except the sodium and phosphate elements. High concentration of sodium and phosphate in the groundwater may leads to deterioration of human and environmental health. Suitable land use plan and sewage treatment systems are highly essential to maintain the quality of groundwater in Arcot region.

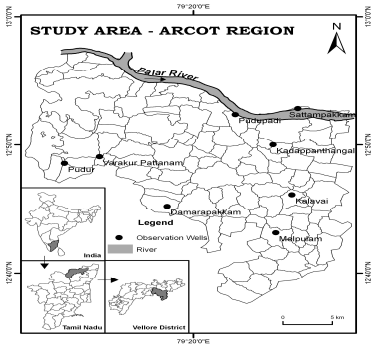

Figure 1 – Study Area: Arcot Region

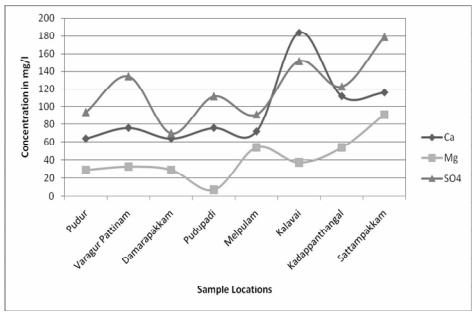

Figure 2 – Concentration of Ca, Mg and SO_4 in pre-monsoon season

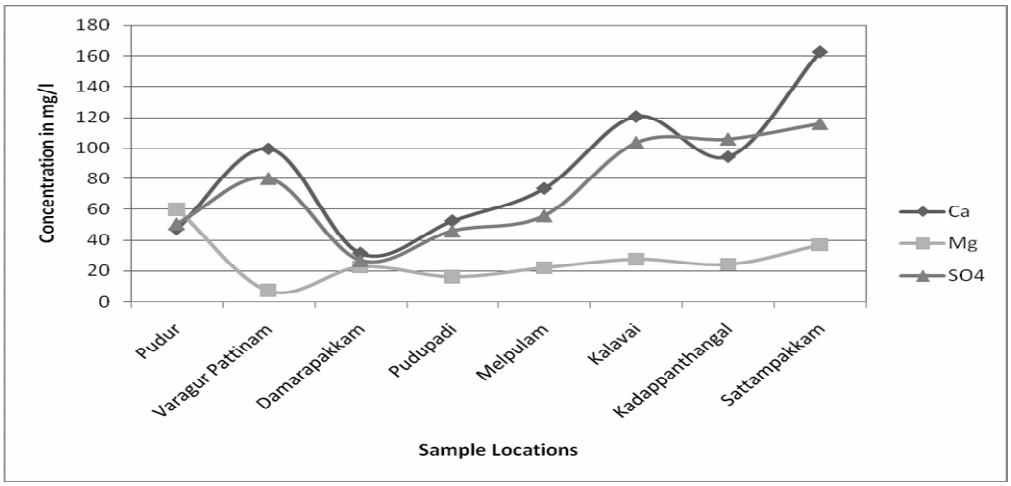

Figure 3 – Concentration of Ca, Mg and SO_4 in post-monsoon season

Table 1 - Physico-chemical characteristics of groundwater during pre-monsoon season in the Arcot Region. All parameters were reported in mg/l except pH and Electrical Conductivity (mS/cm^{-1}).

Parameters	Pre-monsoon							
	Pudur	Varagur pattinam	Damaraipakkam	Pudupadi	Melpulam	Kalavai	Kadappanthangal	Sattampakkam
pH	7.14	7.58	7.23	8.07	7.52	7.31	7.32	7.24
EC	879	1.66	935	1.21	1.78	2.73	1.55	3.69
TDS	364	680	397	498	740	1140	660	1550
Ca	64	76	64	76	72	184	112	116
Mg	29.2	32.8	29.2	6.8	53.6	37.2	53.6	90.8
TH	330	360	330	230	460	490	500	690
Na	166.75	361.1	135.7	179.4	140.3	217.35	138	246.1
K	6.24	4.875	3.9	7.02	6.24	10.335	5.46	4.095
PO_4	0.03	0.09	0.01	0.13	0.21	0.1	0.08	0.16
F	1.16	1.09	1.19	1.14	1.09	1.23	1.22	1.17
Cl	368.68	397.04	113.44	382.86	134.71	354.5	354.5	177.25
HCO_3	61	366	122	91.5	122	183	91.5	122
CO_3	30	30	0	15	0	0	0	0
Nitrate	17.12	17.41	0.09	1.77	0.89	7.97	2.44	1.11
SO_4	93.92	134.21	70.05	111.61	91.25	151.48	122.70	178.72

Table 2 - Physico-chemical characteristics of groundwater during post-monsoon season in the Arcot Region. All parameters were reported in mg/l except pH and Electrical Conductivity (mS/cm^{-1}).

Parameters	Post-monsoon							
	Pudur	Varagur pattinam	Damaraipakkam	Pudupadi	Melpulam	Kalavai	Kadappanthangal	Sattampakkam
pH	6.65	7.41	7.18	7.09	7.41	6.94	7.15	7.26
EC	2.11	1.63	0.98	1.11	1.08	2.95	1.39	3.83
TDS	990.00	760.00	455.00	525.00	519.00	1410.00	670.00	1850.00
Ca	47.25	99.75	31.50	52.50	73.50	120.75	94.50	162.75
Mg	60.20	7.35	22.72	16.40	22.23	27.76	24.42	37.00
TH	295.00	130.00	125.00	120.00	165.00	235.00	195.00	315.00
Na	69.00	32.20	50.60	73.60	73.70	177.10	117.30	64.40
K	4.68	1.37	1.66	3.12	0.68	6.57	0.34	3.76
PO_4	0.28	0.16	0.04	0.13	0.12	0.09	0.51	0.15
F	0.79	0.72	0.74	0.72	0.68	0.79	0.76	0.67
Cl	283.60	163.07	141.80	120.53	106.35	389.95	141.80	659.37
HCO_3	36.60	42.70	48.80	48.80	36.60	36.60	36.60	61.00
CO_3	0	0	0	0	0	0	0	0
Nitrate	17.72	17.72	0.44	1.77	0.89	8.86	2.66	1.33
SO_4	50.68	80.16	26.81	46.05	56.02	104.03	106.28	116.38

Table 3 - Correlation matrix of pre-monsoon season samples

Pre-monsoon														
	pH	EC	TDS	TH	Ca	Mg	PO_4	Cl	Na	K	F	HCO_3	NO_3	SO_4
pH	1.000													
EC	-0.200	1.000												
TDS	-0.211	1.00**	1.000											
TH	-0.210	0.680	0.682	1.000										
Ca	-0.510	0.774*	0.782*	0.303	1.000									
Mg	-0.508	0.873**	0.880**	0.554	0.961**	1.000								
PO_4	0.149	0.379	0.365	0.144	0.093	0.123	1.000							
Cl	0.176	0.124	0.118	0.661	-0.333	-0.100	-0.062	1.000						
Na	0.413	0.549	0.543	0.157	0.444	0.433	0.095	0.145	1.000					
K	-0.472	0.185	0.199	0.645	0.109	0.282	-0.373	0.302	-0.448	1.000				
F	0.318	-0.191	-0.199	0.210	-0.469	-0.350	0.387	0.452	-0.234	0.079	1.000			
HCO_3	0.156	0.160	0.147	0.096	-0.058	-0.023	0.874**	-0.031	0.029	-0.400	0.262	1.000		
NO_3	-0.158	-0.217	-0.228	-0.080	-0.292	-0.279	0.578	0.157	-0.380	-0.243	0.646	0.498	1.000	
SO_4	-0.048	0.886**	0.883**	0.689	0.578	0.704	0.596	0.178	0.385	0.202	0.252	0.307	0.064	1.000
* - Correlation is significant at the 0.05 level (2-tailed)														
** - Correlation is significant at the 0.01 level (2-tailed)														

Table 4 - Correlation matrix of post-monsoon season samples

Post-monsoon														
	pH	EC	TDS	TH	Ca	Mg	PO_4	Cl	Na	K	F	HCO_3	NO_3	SO_4
pH	1.000													
EC	-0.196	1.000												
TDS	-0.186	1.000**	1.000											
TH	-0.468	0.833*	0.833*	1.000										
Ca	0.300	0.820*	0.828*	0.536	1.000									
Mg	-0.752*	0.441	0.435	0.828*	-0.030	1.000								
PO_4	-0.195	-0.072	-0.067	0.242	0.076	0.238	1.000							
Cl	-0.168	0.978**	0.979**	0.825*	0.772*	0.463	-0.127	1.000						
Na	-0.368	0.311	0.316	0.274	0.302	0.122	0.182	0.195	1.000					
K	-0.677	0.669	0.660	0.570	0.254	0.503	-0.307	0.607	0.517	1.000				
F	-0.496	-0.343	-0.357	-0.340	-0.469	-0.092	0.208	-0.426	0.179	0.161	1.000			
HCO_3	0.227	0.398	0.403	0.130	0.341	-0.072	-0.374	0.547	-0.436	0.049	-0.370	1.000		
NO_3	-0.323	0.144	0.124	0.203	-0.031	0.261	0.129	0.011	-0.105	0.313	0.440	-0.399	1.000	
SO_4	0.148	0.719*	0.727*	0.533	0.928**	0.014	0.389	0.633	0.487	0.210	-0.241	0.094	0.032	1.000
* - Correlation is significant at the 0.05 level (2-tailed)														
** - Correlation is significant at the 0.01 level (2-tailed)														

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RESEARCH ARTICLE

Screening of Phytochemicals in *Nigella sativa L.* by using GC-MS Technique

Ramya. B* and Nagaraju Perumandla

Department of Biochemistry, J.J College of Arts and Science, Pudukkottai.

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***Address for correspondence**
B. Ramya

Department of Biochemistry, J.J College of Arts and Science,

Pudukkottai, TamilNadu, Email ID: rammyaa_79@rediffmail.com

ABSTRACT

The seeds of *Nigella sativa L*. (Ranunculaceae) commonly known as black seed (or) black cumin, are used in herbal medicine all over the world for the treatment and prevention of a number of disease. The seeds contain both fixed and essential oils, proteins, alkaloids and saponin. Much of the biological activity of the seeds has been shown to be due to thymoquinone, the major component of the essential oil which is also present in the fixed oil. The present study deals with the analysis of phytochemicals by qualitative and quantitative procedures using the ethanolic extracts of seeds of *N.sativa L*. by using soxhlet apparatus. Phytochemical constituents like alkaloids, flavanoids, saponins, thymoquinone, glycosides in the ethanolic extract of *Nigella sativa L*, Thymoquinone and Flavonoid were also identified by GC-MS technique.

Key words: *Nigella sativa L.*, Thymoquinone (TQ), GC-MS.

INTRODUCTION

Plant medicines are the most widely used medicines in the world today. A full eighty-five percent (85%) of the world's population employs herbs as their primary medicines [1]. Plants are the original source materials for as many as 40% of the pharmaceuticals. Natural plant-based remedies are used for both acute and chronic health problems. As late as the early 1950's, many of the larger pharmaceutical companies still offered a broad variety of plant-based drugs in tablet, liquid and ointment forms [2]. Plant medicines are far and away safer, gentler and better for human health than synthetic drugs. This is so because human beings have co-evolved with plants over the past few million years. The results of synthetic drug explosion have been unfortunate [3]. Synthetic drugs often act in the body as irritants and toxins, upsetting the balance of whole systems, producing side effects that can be lethal. The World Health Organization (WHO) has shown great interest in documenting the use of medicinal plants by tribal in different parts of the world (1977). *Nigella sativa L* belongs to family Ranunculaceae, native to Southern Europe, North Africa and South Asia. Common names applied to members of this genus are Devil-in-a-bush (or) Love in a mist [4]. It is known as Kalonji in Hindi and Karanjiragam in Malayalam, Karunjeerakam in Tamil and Nallajirakara in Telugu. Prophet Mohammed [2] once states that – black seed can cure every disease-except death [5]. *Nigella sativa L*. is used as an Emmenagogue, Lactogogue, Diuretic, antihelmintic, antimicrobial, antioxidant, antitumour, immunomodulatory, and anticonvulsant and used in treatment of Opioid dependence persons [6].

Biochemical Constituents present in *Nigella sativa L.*

Alanine, arginine, ascorbic-acid, asparagine, campesterol, carvone, cymene, cystine, dehydroascorbic-acid, eicosadienoic-acid, glucose, glutamic-acid, glycine, iron, isoleucine, leucine, d-limonene, linoleic-acid, linolenic-acid, lipase, lysine, methionine, myristic-acid, nigellin, nigellone, oleic-acid, palmitic-acid, phenylalanine, phytosterols, potassium, beta-sitosterol, alpha-spinasterol, stearic-acid, stigmasterol, tannin, threonine, thymohydroquinone, thymoquinone, tryptophan, tyrosine[7]. Primary substance like carbohydrates, proteins, fats, thiamin, riboflavin, pyridoxine, niacin, folacine, iron, calcium, phosphorus, zinc, copper, mono and poly unsaturated fatty acids, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid [8]. Secondary substances like Alkaloids, Flavanoids, Saponins, Thymoquinone, 4-terpinol, and carvacrol [9].

MATERIALS AND METHODS

Preparation of plant extract

The seeds of *Nigella sativa L* were purchased from local market and were fine powdered. The powdered seeds were extracted in a Soxhlet apparatus with ethanol. Powdered plant seeds material (100gm) was packed in to Soxhlet apparatus (2L) and extracted exhaustively with 500ml of ethanol for 6 hours. The Ethanol was evaporated using a water bath and then left at laboratory temperature for 2 days for evaporation of the remaining ethanol. The obtained extract was dried in hot air oven for 2 hours and dried powder was taken and was stored for analysis.

Preliminary Phytochemical Analysis

Phytochemical screening was carried out to assess the qualitative chemical composition of crude extracts using commonly employed precipitation and coloration reaction to identify the major natural chemical groups such as alkaloids, saponins, tannins, glycosides, flavanoids, steroids, phenols, proteins, carbohydrates, fats, and amino acids vitamins and minerals. General reactions in this analysis revealed the presence (or) absence of these compounds in the crude extract tested.

Quantitative Analysis and GC-MS Screening

Biochemical procedures are employed to analyze quantitatively the amount of alkaloids [10], tannins [11], saponins [12], Flavonoids [13] and total phenol [12]. Secondary metabolites are screened by using GC-MS technique [14].

GC - MS Analysis

Gas chromatography-Mass Spectrometry (GC-MS) was performed using an auto HRGC/ MS Carlo Erba Instrument using a SPB 5 (30 m / 0.25mm / 0.5μm) capillary column. 1mg / ml of extract powder was dissolved in methanol was injected in the following conditions [13].

Temperature : 28°C
Carrier gas : Helium
Pressure : 150 kPa
Ion voltage : 60 eV
Temperature gradient : 20°C / min (from 100 – 315°C)

RESULTS AND DISCUSSION

Seeds of *Nigella sativa L*. were analyzed by proximate amount (Table 1) in the crude extract. Fat was high when comparing to other protein and fibers. Qualitative test proved that Thymoquinone, saturated and unsaturated fatty acids are very much abundant and Flavonoids in Secondary stage (Table 2). Vitamins and Minerals are also analyzed and in this Thiamine is rich in source of minerals and Cu is as same as rich of minerals occurrence (Table 3) [15]. Secondary metabolites of *Nigella sativa L* also were analyzed quantitatively by using GC-MS technique. The GC-MS method conform that *N. sativa L*. contain all the classes of secondary metabolites especially the Thymoquinone and Flavonoids was given by Mass- Spectroscopy coupled with GC [16]. The GC-MS analysis of the *N. sativa L* volatile oil showed 31 compounds such as (in %) Alpha -Thujene- 2.4, p-Cymene-8-ol - 0.4, 3-Methyl Nonane - 0.6, Nerol - 1.3, alpha –Pinene - 1.2 Estragole - 1.9, Sabinene - 1.4, Dihydrocarvone - 0.3, beta -Pinene- 1.3, Carvone - 2.0, Myrcene - 0.6, Thymoquinone - 11.8, n-Decane - 0.4, Anisaldehyde - 1.7, alpha -Phellandrene - 0.6, Trans-Anethole - 27.1, p-Cymene - 9.0, Carvacrol - 3.7, Limonene –

4.3, alpha -Longipinene - 0.3, 1-Methyl-3-propyl benzene - 0.7, n-Tetradane - 0.2, gamma –Terpinene - 0.5, Longifolene - 5.7, 1-Ethyl-2,3-dimethyl benzene - 0.2 , Uvidine- 1.3, 2(1H)-Naphthalenone - 2.6, Myristicin - 1.4, Fenchone 1.1, n Hexadecane - 0.2, Terpinen-4-ol - 0.7 and Apiole - 1.0 which included two new chemical compounds viz. 2(1H)-Naphthalenone ($C_{11}H_{18}O$) and Uvdin ($C_{15}H_{24}O_3$) [17].

CONCLUSION

These results suggest *Nigella sativa L*. is a source of alkaloids, flavonoids, saponins, Thymoquinone, Glycosides etc. In ethanolic extracts of *Nigella sativa L.* GC-MS screening of Thymoquinone and Flavonoids of *Nigella sativa L.* was recorded and which confirm the presence of all classes of Thymoquinone and Flavonoids. Black cumin holds nutraceutical potential against various physiological threats owing to its rich phytochemistry especially due to presence of Thymoquinone and Tocopherol. Finally fixed and essential oil supplement in food products especially bakery items is feasible and can be employed to achieve the allied health charging.

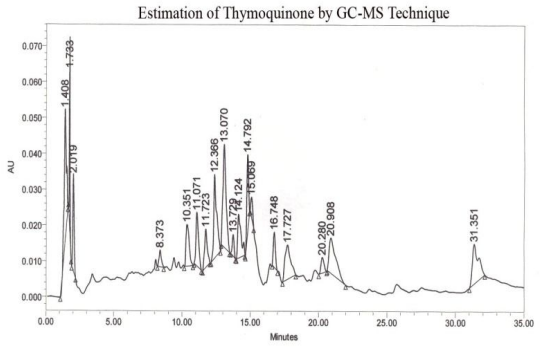

Figure 1: Estimation of Thymoquinone (TQ) by GC-MS technique

Table.1: Proximate composition of Black cumin seeds.

S.NO	Proximate composition	Percentage
1.	Moisture	6.46±0.17
2.	Crude protein	22.80±0.60
3.	Crude Fat	31.16±0.82
4.	Crude Fiber	6.03±0.16
5.	Ash	4.20±0.11
6.	Nitrogen free Extract(NFE)	29.36±0.78

Table.2: Preliminary Phytochemical Screening of Nigella sativa L.

S.NO	Phytochemicals	Observation	Percentage
1.	Carbohydrates	++	**35 %**
2.	Proteins	+	**21 %**
3.	Fats	++	**38 %**
4.	Saturated&Unsaturated fatty acids	+++	**37 %**
5.	Free Fatty acids	+	**19 %**
6.	Alkaloids	+	**2.5 %**
7.	Flavonoids	++	**24 %**
8.	Saponins	+	**2.8 %**
9.	Thymoquinone	+++	**57.1 %**
10.	Carvacrol	+	**11.6 %**
11.	4-Terpinol	+	**6.6 %**
12.	Tanins	+	**1.6 %**
13.	Glycosides	+	**10.5 %**
14.	Pholbatanins	-	-

Table.3: Vitamins and Minerals Composition of *Nigella sativa L.*

S.No	Vitamins and Minerals	Percentage
1.	Thiamine	15ug/g
2.	Riboflavin	1 ug/g
3.	Pyridoxine	5 ug/g
4.	Niacin	57 ug/g
5.	Folacin	610 IU/g
6.	Potassium	1.74 mg/g
7.	Calcium	1.859 mg/g
8.	Phosphorous	5.265 mg/g
9.	Magnesium	4.21 mg/g
10.	Sodium	2.8 mg/g
11.	Iron	10.5 ug/g
12.	Manganese	70 ug/g
13.	Zinc	60 ug/g
14.	Copper	18 ug/g

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RESEARCH ARTICLE

Effect of *Dolichos biflorus Linn* Seed decoction on ammonium oxalate induced Urolithiasis in rats

Nithya Devi. M

Department of CARISM, SASTRA University, Thanjavur – 613 402.

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***Address for correspondence**

Nithya Devi. M

Department of CARISM, SASTRA University,

Thanjavur – 613 402. E-mail ID: nithyadevi77 @gmail.com

ABSTRACT

In India, horse gram (*Macrotyloma uniflorum* Lam (*Dolichos biflorus Linn*) is commonly used as a phytotherapeutic agent. The effect of oral administration of seed decoction of *Dolichos biflorus Linn* on Ammonium oxalate urolithiasis has been studied in male Wister albino rats. Ammonium oxalate feeding resulted in hyperoxaluria as well as increased renal excretion of calcium, oxalate and phosphate. Supplementation with seed decoction of *Dolichos biflorus Linn* significantly reduced the elevated urinary oxalate showing a regulatory action on endogenous oxalate synthesis. The increased deposition of stone forming constituents in the kidneys of calculogenic rats was also significantly lowered by curative and preventive treatment using seed decoction. The results indicate that the seed decoction of *Dolichos biflorus Linn* is endowed with antiurolithiatic activity.

Key words: *Dolichos biflorus Linn*, Hyperoxaluria, Urolithiasis,

INTRODUCTION

Macrotyloma uniflorum Lam (Dolichos biflorus Linn) (family leguminoseae) commonly known as "horse gram" is grown mostly under dry land agriculture. It occurs all over India up to an altitude of 5000 feet. It is used medicinally for high cough, eye trouble, piles, enlargement of spleen, improves the complexion, lowers cholesterol, dissolve phosphate kidney stones parturient women to promote the discharge of Lochia and in leucorohoea and in menstrual dearrangements [1-3]. The seed decoction of *Dolichos biflorus Linn* contains alkaloids, flavonoids, steroids, tannins and glycosides. Decoction contains Protein 22%, Fat 0.5%, Minerals 3.1%, Calcium 0.28%, Carbohydrates 57.3%, Iron 0.0076 %, Nicotinic acid 0.0015%, Carotene 119 Iu/100gm, Arginine 6-7% of total nitrogen, Tyrosine 6.68% of total nitrogen, Lysine 7-6% of total nitrogen [4]. In recent decades the extracts and seed decoction of *Dolichos biflorus Linn* was used in many purposes. It is used in spontaneous and induced mutations at endogenous and transgenic loci. In glycoconjugates in human kidney, in tumour, anticalcifying factor which acts as inhibitor of crystallization [5]**,** in ayurvedic medicine and renal calculi [6]. Urinary stone disease has afflicted humankind since antiquity and can persist, with serious medical consequences throughout a patient lifetime. In addition, the incidence of kidney stones has been increased in western societies in the last five decades, in association with economic development. Most calculi in the urinary system arises from a common component of urine, e.g. calcium oxalate (ca ox), representing up to 80 % of analyzed stones [7-9]. Currently, open renal surgery for nephrolithiasis is unusual and used only rarely since the introduction of extracorporeal shock wave lithotripsy (ESWL), which has revolutionized urological practice and almost become the standard procedure for eliminating kidney stones. However, in addition to the traumatic efforts of shock wave, persistent residual stone fragments and the possibility of infection suggest that ESWL may

cause acute renal injury, a decrease in renal function and an increase in stone recurrence [10-11]. A number of vegetable drugs have been used in India and elsewhere, it claims efficient cure of urinary stones [12]. In the indigenous system of medicine, the seed decoction of *Dolichos biflorus Linn* is reported to be useful in the treatment of urinary stones [13]. However, so far no systematic study has been reported regarding the antiurolithiatic property of decoction of *Dolichos biflorus Linn* seeds. In the present study, an effort has been made to establish the scientific validity for the antiurolithiatic property of *Dolichos biflorus Linn* seed decoction using ammonium oxalate induced hyperoxaluria model in rats.

MATERIALS AND METHODS

Plant material

The dry seeds of *Dolichos biflorus Linn* were collected from local market of Coimbatore. The seeds were authenticated at the seed culture department in Tamilnadu Agricultural University (TNAU). The seeds cleaned and kept in airtight container.

Preparation of extract

20% W/V decoction of *Dolichos biflorus Linn* seeds in water was prepared and checked for the pH 7.4. The prepared decoction was then administrated to rats at a rate of 1ml/rat/day for 15 days, by gastric intubation tube of oral administration.

Pharmacological screening for antiurolithiatic activity

Animal selection

For acute toxicity studies, healthy adult male Wister albino rats weighing between 150 and 200g were selected for the antiurolithiatic activity. The animals were acclimatized to standard laboratory conditions (temperature: 25 $\pm$ 2^{o}C) and maintained on 12-hr light: 12-hr dark cycle. They were provided with Gold Mohar commercial feed manufactured by Hindustan lever ltd, Bangalore and drinking water *ad libitum*.

Ammonium oxalate induced urolithiasis model

Ammonium oxalate induced hyperoxaluria model [14] was used to access the antilithiatic activity in albino rats. Animals were divided in to three groups containing six animals in each group. Group I served as control and received normal pelleted diet and drinking water *ad libitum*. Group II received normal pelleted diet + 2% ammonium oxalate (1ml/rat/day) for 15 days. Group III received 2% ammonium oxalate and *Dolichos biflorus Linn* seed decoction (1ml/rat/day) from 15th day till 30th day. Decoctions were given once daily by oral route.

Assessment of antiurolithiatic activity

Collection and analysis of urine

All animals were kept in individual metabolic cages and urine samples of 24h were collected on 15th day. Animals had free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to urine before being stored at 4^{o}C. the urine was analyzed for Oxalate [15], Calcium [16], Magnesium [17], Phosphorus [18] Uric acid [19] Urea [20], Protein [21], Mucoprotein [22], Creatinine [23]**.** A portion of the urine was dialyzed against distilled water for 3hrs at 4^{o}c. The dialysate was then used for the estimation of protein and mucoprotein. An aliquot of urine sample was treated with 10% TCA. The suspension was centrifuged at 1500xg for 10 minutes and the supernatant was used for estimation of citric acid.

Statistical analysis

Results were expressed as mean $\pm$ SD. Differences among data were determined using one-way ANOVA followed by 't' test. Differences between the data were considered significant at $p < 0.05$. Results were given in Table: 1.

RESULTS

In the present study, chronic administration of ammonium oxalate 2% (v/v) solution to male wistar rats resulted in hyperoxaluria. Oxalate, calcium and phosphate excretion were grossly increased in calculi induced animals (Group II). However, supplementation with seed decoction of *Dolichos biflorus Linn* significantly ($P<0.001$) lowered the elevated levels of oxalate, calcium and phosphorus in urine as compared to treated animals (Group III).Magnesium level was found to be significantly reduced in the ammonium oxalate induced hyperoxaluric, when compared to

controls with seed decoction treatment, a sight elevation was seen in treated group. Urinary organic acids such as uric acid, oxalic acid and citric acid were found to be increased in calculi induced compared to control. Seed decoction administration group resulted in decrease in organic acid level. Protein and mucoprotein was found to be increased in ammonium oxalate fed rats compared with control; administration of seed decoction restored the urinary mucoprotein level to that of normal. In urinary excretion, creatinine was found to be significantly decreased in calculi induced and treatment with seed decoction restored the normal level in treated animals. In pH and volume of the urine found to be decreased in group II towards pH range compared with that of group I and the treatment restored to normal by elevating the pH towards normal and increased the urinary volume.

DISCUSSION

In the present study, male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans [24], the amount of stone deposition in female rats was found to be significantly less with that of male [25]. Urinary super saturation with respect to stone forming constituents is generally considered to be one of the causative factors in calculogenesis. Stone formation in ammonium oxalate fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate [26]. Similar results have been obtained when rats are treated with ethylene glycol [27-28]. In the present study oxalate and calcium excretion are progressively increased in group II. Since it is accepted that hyperoxaluria is an important risk factor in the pathogenesis of renal stones than hypercalcuria [29]. However the seed decoction of *Dolichos biflorus Linn* lowers the levels of oxalate as well as calcium excretion. An increase in urinary phosphate is observed in group II. It shows that an environment is appropriate for stone formation by forming calcium phosphate crystals, which epitoxically induces calcium oxalate deposition [30-31]. Treatment of seed decoction restores phosphate level, thus reducing the risk of stone formation. The Glomerular Filtration Rate (GFR) decreases due to the obstruction to the outflow of urine by stones in urinary system. Due to this, waste products, particularly nitrogenous substances such as urea, Creatinine, uric acid get accumulated in the blood [32]. So the level decreased in urine and proteins, mucoprotein which are not observed get released in the urine found to be increased (group II). The treatment restores the normal level [33-34].

CONCLUSION

In conclusion, the presented data indicate that administration of seed decoction of *Dolichos biflorus Linn* to rats with ammonium oxalate induced lithiasis, reduced and prevented the growth of urinary stones, supporting folk information regarding antiurolithiatic activity of the seed. The mechanism underlying this effect is still unknown, but is apparently related to increased diuresis and lowering of urinary concentrations of stone forming constituents. These effects could conclude the antiurolithiatic property of *Dolichos biflorus* Linn.

Table 1: Effect of *Dolichos biflorus* Linn. *seed* decoction on urine parameters in control and experimental animals.

Urine Sample (mg/dl)	Group I Control	Group II Calculi induced	Group III Calculi treated
Calcium (mg/24hr)	0.36±0.04	0.75±0.04 $a^{\#}$	0.86±0.10 $b^{\#}$ c^{*}
Magnesium (mg/24hr)	4.87±0.50	2.83±0.22 $a^{\#}$	4.65±0.33 $c^{\#}$
Phosphorus	2.85±0.10	3.32±0.09 $a^{\#}$	3.21±0.14 b^{*} $c^{\#}$
Uric acid	1.17±0.10	2.48±0.29$a^{\#}$	1.20±0.14 $b^{\#}$ $c^{\#}$
Oxalic acid	0.52±0.03	0.99±0.06 $a^{\#}$	0.78±0.12 $b^{\#}$ $c^{\#}$
Urea	3.79±0.51	1.66±0.05 $a^{\#}$	1.93±0.11 $b^{\#}$ $c^{\#}$
Protein	12.35±1.45	25.19±3.13 $a^{\#}$	13.00±1.75 $c^{\#}$
Mucoprotein	6.17±0.39	8.22±0.07 $a^{\#}$	6.68±0.50 $c^{\#}$
Creatinine (mg/24hr)	8.36±0.31	6.29±0.50 $a^{\#}$	8.10±0.14 $a^{\#}$
Urine Volume (ml)	2.78±0.30	2.25±0.75	2.60±0.34
Urine pH	7.05±0.21	4.58±0.26 $a^{\#}$	6.400±0.12 $c^{\#}$

Values of urine parameters are answered in 24 h urine sample.

All values are expressed as mean ± SD for six animals in each group.

a. Comparison are made between Group I and II
b. Comparison are made between Group I and III
c. Comparison are made between Group II and III
* Stastiscally significant at p< 0.05
\# Stastiscally significant at p< 0.001

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RESEARCH ARTICLE

Biofarming techniques for Augmenting the Productivity of Glori lily *Gloriosa superba* L.

Deivasigamani. S*, Thanunathan. K and Ravichandran. M[-1]

Department of Agronomy, Department of Soil Science and Agricultural chemistry[-1]

Faculty of Agriculture, Annamalai University

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***Address for correspondence**

Deivasigamani. S

Department of Agronomy, Faculty of Agriculture, Annamalai University Annamalai Nagar.

Tamil Nadu – 608 002, E-mail ID: agrisiga2007@gmail.com

ABSTRACT

Field experiment was conducted at the farmer's field at Devanur village of Ariyalur District, Tamilnadu during July 2007 - December 2008 to study the effect of biofarming practices on the yield performance of glory lily (*Gloriosa superba L*) and soil fertility status. The experiment was conducted in RBD design with three replications. The treatments consisted of different organic manures *viz.,* T_1-control, T_2-FYM@12.5 t ha^{-1}, T_3- EFYM @ 750 kg ha^{-1} and T_4-Pressmud compost @5t ha^{-1}. Among the organic manures, application of EFYM@750 kg ha^{-1} (T_3) recorded the highest yield attributes like number of pods $plant^{-1}$, number of seeds pod^{-1}, test weight (g), seed yield and tuber yield.

Key words: *Gloriosa superba L*, Biofarming techniques, Yield performance, Soil fertility.

INTRODUCTION

India is endowed with a rich wealth of medicinal plants, which have contributed to the development of ancient Indian materia medica. In one of the earliest treatises on Indian medicine, the charak, samhita (1000 B.C.) records the use of over 840 drugs of plant origin. Glori lily *Gloriosa superba* L. is an important medicinal crop. It belongs to the family Liliaceae, being locally called as "Kanvali kizhangu" or Karthigai kizhangu" or Kalappai kizhangu or "Kanthazh" in Tamil. In India, it is cultivated in an area of 2000 ha and in Tamilnadu around 1000 ha with an annual production of 650 metric tonne of seeds and tubers. It resumes new growth by July-September and produces flowers during October-November [1]. It is a native to tropical Asia and Africa and found growing throughout tropical India up to an altitude of 2500 m [2]. Commercial cultivation of this crop is mostly confined to the Southern states of India [3]. The drug extracted from the seed is bitter, pungent and astringent in taste. In Ayurvedic system of medicine, the underground rhizomes are used since ancient times as an anthelosintic, anti-inflammatory and anti-leprotic. The drug from the tuber is a gastro-intestinal irritant and may cause vomiting or purging. It is useful in demotosis, piles, chronic ulcers, colic pain and as a cataplasm in neuralgic pains. The white starchy powder made from the tubers after washing with water is used in the treatment of gonorrhoea and the leaf juice is used for killing head lice. The underground stocks have been used as an abortifacient and hence the name "Garbhaghatini." The tubers are administered to cattle for expulsion of worms and also used as an adulterate of Aconite [4].The seed of glori lily was found to be good source of colchicines 3-Dimethylcolchicine and colicoside [5]. The root pieces after certain pre-treatments and preservation are administered as an antidote in cobra poisoning. In scorpion and centipede sting, on application of the paste of the root, relief is obtained [6]. Application of organic manures recorded highest yield in Potato [7]. Application of Pressmud at 20 t ha^{-1} significantly increased the plant height, grain and straw yield of ragi

[8]. The bulky organic manure requires improvement in quality with reference to its nutrient content through enrichment, Shailendranath and Rao [9] reported pre-treatment of FYM with urea and phosphate fertilizer had significantly increased growth and yield on medicinal plants. Nayak [10] reported that application of one tone of EFYM ha^{-1} was on par with six tons of conventional FYM ha^{-1} in increasing yield on finger millets.

MATERIALS AND METHODS

Field experiment was conducted during July-2007 -Dec-2008 under rain fed conditions in a farmer's field at Devanur village of Ariyalur district in Tamilnadu, to study the effect of biofarming practices on the seed and tuber yield of glori lily. The experimental field was red loamy soil with pH 6.5, available nitrogen (231 kg ha^{-1}), available phosphorus (16 kg ha^{-1}) and available potassium (293 kg ha^{-1}). The experiment was laid out in Randomised Block Design (RBD) with three replications. Different organic manures *Viz.,* T_1 – Control, T_2 – FYM @ 12.5 t ha^{-1}, T_3 – EFYM @ 750 kg ha^{-1}, T_4 – Press mud compost @ 5t ha^{-1} were tried. The nutrient content of FYM is N- 1.5%, P_2O_5 – 0.61% and K_2O – 0.81% where as nutrient content of EFYM is N- 1.39%, P_2O_5 – 0.24% and K_2O – 3.5%. The nutrient content of Press mud compost is N- 1.51%, P_2O_5 – 0.67% and K_2O – 0.81%

RESULTS AND DISCUSSION

Yield attributes and yield

All the organic manure treatments had significant influence on yield attributes, seed yield and tuber yield (Table1and 2). Application of EFYM @ 750 kg ha^{-1} (T_3) recorded significantly higher yield attributes viz., number of pods $plant^{-1}$ of 20.36 ,number of seeds pod^{-1} of 44.34, test weight of 2.31g, seed yield of 686.34 kg ha^{-1} and tuber yield of 2147.85 kg ha^{-1}. Seed and tuber yield were significantly increased by the use of organic manure sources. Similar findings of increased tuber and seed yield due to use of organic nutrient source was reported by Warade et al. [11] in onion.

Soil fertility

Effect of different biofarming techniques on post harvest soil fertility status are given in (Table 3). Among the treatments, application of FYM @ 12.5 t ha^{-1} (T_2) recorded the highest available soil nitrogen, phosphorus and potash of 221.21, 17.50 and 286.26 kg $ha^{-1}$and the least post harvest available soil NPK of 217.87, 16.17 and 283.33 kg ha^{-1} were recorded in EFYM@750 kg ha^{-1}(T_3). Similar finding of increased post harvest NPK status due to use of organic nutrient source was reported by Nayak [10] in ragi. Based on the above results, it can be concluded the use of biofarming with EFYM @ 750 kg ha^{-1} is found to be an appropriate agro-technique for augmenting the productivity of glori lily.

Table1: Effect of biofarming on number of pods $plant^{-1}$ and number of seeds pod^{-1} of Glori lily

S. No	TREATMENTS	No. of Pods $plant^{-1}$	No. of Seeds pod^{-1}
1	T_1 - Control (Untreated)	17.97	41.30
2	T_2- Farmyard Manure (FYM) @ 12.5 t ha^{-1}	18.59	43.43
3	T_3- Enriched Farmyard Manure (EFYM) @ 750 kg ha^{-1}	20.36	44.34
4	T_4- Pressmud Compost @ 5 t ha^{-1}	18.80	43.84
MEAN		18.83	43.23
SED **CD (P=0.05)**		**0.44** **0.63**	**0.28** **0.40**

Table2: Effect of biofarming on hundred seed weight (gram), seed and tuber yield (kg ha^{-1}) of glori lily

S. No	TREATMENTS	Hundred seed wt (g)	Seed yield (kg ha^{-1})	Tuber yield (kg ha^{-1})
1	T_1 – Control (Untreated)	2.14	529.25	1872.86
2	T_2- Farmyard Manure (FYM) @ 12.5 t ha^{-1}	2.21	573.61	1949.37
3	T_3- Enriched FarmYard Manure (EFYM) @ 750 kg ha^{-1}	2.31	686.34	2147.85
4	T_4- Pressmud Compost @ 5 t ha^{-1}	2.20	589.26	1968.91
MEAN		2.22	594.62	1984.75
SED **CD (P=0.05)**		**0.04** **0.06**	**0.74** **1.81**	**2.07** **5.08**

Table 3: Effect of biofarming on Post harvest soil available nitrogen, phosphorus and potassium (kg ha^{-1}

S. No	TREATMENTS	Nitrogen (kg ha^{-1})	Phosphorus (kg ha^{-1})	Potassium (kg ha^{-1})
1	T_1 – Control (Untreated)	220.93	16.17	279.43
2	T_2- Farmyard Manure (FYM) @ 12.5 t ha^{-1}	221.20	17.50	286.26
3	T_3- Enriched FarmYard Manure (EFYM) @ 750 kg ha^{-1}	217.87	16.30	283.33
4	T_4- Pressmud Compost @ 5 t ha^{-1}	221.07	16.24	283.08
MEAN		220.42	16.60	283.02
SED		**0.06** **0.12**	**0.46** **1.12**	**1.92** **2.10**

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RESEARCH ARTICLE

Air pollution tolerance index of selected plants in Kuvempu university campus, Shankarghatta, Karnataka state, India

Adamsab .M. Patel[1]*, Hina Kousar*, Shwetha D.S*, Sirajuddin .M. Horginamani** and Ravichandran .M **

***Department of Post Graduate Studies and Research in Environmental Science, Kuvempu University, Shivamogga District, Karnataka, India.

****Department of Environmental Management, School of Environmental Sciences, Bharathidasan University, Tiruchirappalli - 620 024, Tamil Nadu. India.

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[1]***Address for correspondence**
Adamsab .M. Patel,
Department of Post Graduate Studies and Research in Environmental Science, Kuvempu University, Shankaraghatta-577451, Shivamogga District, Karnataka, India.
E-mail ID: ecoadams@yahoo.com

ABSTRACT

Air pollution tolerance index of 14 different selected plant species growing in Kuvempu University Campus, Shankarghatta of Shivamogga District, Karnataka, were determined by calculating ascorbic acid, total chlorophyll, leaf extract pH and relative water content of leaf tissues. It is found that *Azadirachta indica A Juss* (29.46), *Eucalyptus mysoresins* (22.63), *Mangifera indica L* (20.7), *Carica papaya L*(19.96), *Polyalthia longifolia BENTH. & HOOK. F.* (16.65), *Ricinus communis L* (15.84), *Calotropis gigantea L* (14.11), *Nerium indicum Mill* (13.4), *Psidium guajava L* (12.69), *Parthenium hysterophorus L* (11.13), *Bougainvillea glabra Choisy* (9.16), *Terminalia cattapa L* (9.14), *Muntingia calabura L* (6.85) and *Tamarindus indica L* (6.03) showed high degree of tolerance, these plants can be used as bio indicators and also be grown as bio accumulators.

Key words: Indicators, air pollution tolerance index, pH, relative water content, ascorbic acid, total chlorophyll

INTRODUCTION

Plants play an important role in monitoring and maintaining the ecological balance by actively participating in the cycling of nutrients and gases like carbon dioxide, oxygen and also provide enormous leaf area for impingement, absorption and accumulation of air pollutants to reduce the pollution level in the air environment. The harmful effects of air pollution on vegetation have already been well documented [1], [2], [3], [4] and have recorded the ability of plants to reduce air pollution .The efficiency of plants in absorbing pollutants is such that it can produce pockets of clean air [5]. Plants growing in air polluted environment often responded and showed significant changes in their morphology, physiology and biochemistry [6]. Plants were assessed for their tolerance index to establish the air pollution level. Air pollution effects on plants have long been known. Singh and Rao [7] have suggested a method of determining air pollution tolerance index (APTI) by synthesizing the values of four different biochemical parameters i.e. leaf extract pH, ascorbic acid, total chlorophyll and relative water content. In the present study tolerant species to

air pollution have been identified in respect to the above four biochemical parameters which may help in proper selection of plant species in plantation at Kuvempu University campus of Shankarghatta.

MATERIALS AND METHODS

The Kuvempu University Campus (13°41'N and 75°38'E; altitude: 680-720m) is located 28 km South-East of Shivamogga City and the campus is only 2 km from the magnificent Bhadra Reservoir across the river Bhadra, one of the important life lines of the area. The University campus sprawls over an area of 230 acres of land with varied habitats, from undulating hilly terrain to man-made wetlands. Before the inception and establishment of the University in this landscape, the area was a barren hill-slope without any prominent vegetation. But in the past 10 years after the establishment of the University, there is considerable secondary vegetation of many plant species establishing gradually. However, most of the plant species in the campus are less than 10 year old. The collected leaf samples were immediately brought to lab in a heatproof container. The leaf fresh weight was taken immediately upon getting to the laboratory, and then samples were preserved in refrigerator for further analysis.

APTI Assessment

Relative Leaf water Content (RWC)

RWC was determined and calculated with the formula as described by Singh [8],

RWC= [(FW-DW)/ (TW-DW)] x 100

Where,

FW= Fresh weight
TW= Turgid weight
DW= Dry weight

Fresh weight was obtained by weighing the leaves. The leaf samples were then immersed in water overnight blotted dry and then weighed to get the turgid weight. The leaves were then dried overnight in a hot air oven at 70^0 C and reweighed to obtain the dry weight.

Total Chlorophyll Content (TCh)

This was carried out according to the method described by Arnon [9]. 3 grams of fresh leaves were blended and then extracted with 10ml of 80% acetone and left for 15 min. The liquid portion was decanted into another test tube and centrifuged at 2,500 rpm for 3 minutes. The supernatant was then collected and the absorbance was then taken at 645 nm and 663 nm using a spectrophotometer. Calculations were done by using the formula given below:

Chlorophyll a =12.7Dx 663 – 2.69DX 645 *V/1000W mg/gm
Chlorophyll b = 22.9 Dx 645 – 4.68 DX663*V/1000W mg/gm
TCh = Chlorophyll a + b mg/gm

Where,

Dx = Absorbance of the extract at the wavelength X nm
V = Total volume of the chlorophyll solution (ml)
W = Weight of the tissue extracted (g)

pH of Leaf Extract

5 grams of the fresh leaves was homogenized in 10ml deionised water. This was filtered and the pH of the leaf extract determined after calibrating pH-meter with buffer solution of pH 4 and 9.

Ascorbic acid (AA) content

Ascorbic acid contents were determined by the method of Aberg et al. [10]. 5ml of the working standard solution was pipette out in to a 100 ml conical flask and 10 ml of 4% oxalic acid was added and titrated against the dye (V_1 ml). End

point is the appearance of pink color which persists for a few minutes. The amount of the dye consumed is equivalent to the amount of ascorbic acid. After extracting the sample (0.5-5gm depending on the sample) in 4% oxalic acid, the volume was made up to a known volume and centrifuged. 5ml of this supernatant was taken in the conical flask and 10 ml of 4% oxalic acid was added and titrated against the dye (V_2 ml). Amount of ascorbic acid mg/100 grams sample was calculated by,

Ascorbic acid = [0.5 mg/V_1 ml *V_2/5 ml * 100 ml/wt. of sample] * 100

Calculation of APTI

The air pollution tolerance index (APTI) was computed by the method suggested by Singh and Rao [7] using the equation,

APTI = [A (T + P) + R] / 10

Where,

A = Ascorbic acid content (mg/g)
T = Total Chlorophyll (mg/g)
P = pH of the leaf extract
R = Relative water content of leaf (%)

RESULT AND DISCUSSION

Air Pollution Tolerance Index [APTI] was calculated for 14 different plant species and the data is presented in Table 1, from the Table 1 it has evidence that the plants showed varied degree of tolerance index to air pollution. The result of the present study reveals that different plants respond differently to air pollution. The variation of the APTI can be attributed to the variation in any of the four physiological factors which govern the computation of the index. These plants that are constantly exposed to environmental pollutants absorb, accumulate and integrate these pollutants into their systems. It is observed that depending on their sensitivity level, plants show visible changes which would include alteration in the biochemical processes or accumulation of certain metabolites. In this study, changes in parameters such as ascorbic acid, total chlorophyll, relative water content, and pH of leaf extract were used in evaluating the degree of tolerance to air pollution by the plant species. Similar observations have been recorded by Sirajuddin and Ravichandran [11] while working on air pollution tolerance index (APTI) of selected plant species of Tiruchirappalli city, India. From their result it is concluded that *Azadirachta indica A Juss*is most tolerant species for the air pollution followed by the *Psidium guajava* The present study also revealed that air pollution tolerance index (APTI) of 14 plant species has been evaluated. High values of APTI were recorded in *Azadirachta indica A Juss* (29.46), *Eucalyptus mysoresins* (22.63), *Mangifera indica L* (20.7), *Carica papaya L* (19.96), *Polyalthia longifolia BENTH. & HOOK. F.* (16.65), *Ricinus communis L* (15.84), *Calotropis gigantea L (*14.11), *Nerium indicum* (13.4), *Psidium guajava* (12.69), *Parthenium hysterophorus L* (11.13), *Bougainvillea glabra* (9.16), *Terminalia cattapa* (9.14), *Muntingia calabura L* (6.85) and Tamarindus *indica L* (6.03). The similar observation has been recorded *by* Jissy and Jaya [12] while working on six different plant species, *Polyalthia longifolia BENTH. & HOOK. F.* expressed highest APTI values and proved to be a tolerant variety and the others as sensitive species to air pollutants. The urban areas should grow such plant species which can sustain at the site and can tolerate the air pollution.

CONCLUSION

An attempt has been made to study the air pollution tolerance index of selected plant species of Kuvempu University campus , Shankarghatta has been evaluated and it is concluded that *Azadirachta indica A Juss* is most tolerant species among all the fourteen plant species followed by *Eucalyptus mysoresins* and most sensitive species are *Tamarindus indica L* and *Muntingia calabura.* So this study is useful for the better understanding and management of air quality as well as in selection of suitable plant species (with high APTI) for plantation in industrial area as well as roadside and this may become one of the strategy for the abatement of city's air pollution because it will have a marked effect on many aspects of the quality of the urban environment and the richness of life in a city.

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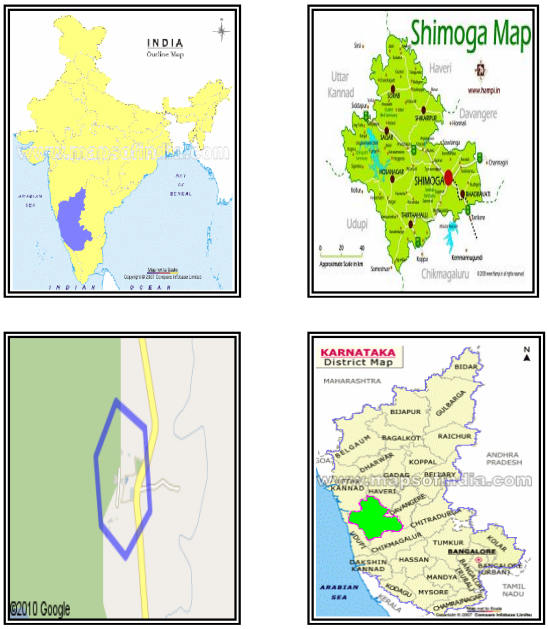

MAP SHOWING THE STUDY AREA
Kuvempu University campu

Table 1: Air pollution tolerance index (APTI) of plant species growing in Kuvempu University campus, Shankarghatta

S. No	Plant species	RWC (%)	TCH(mg/g)	pH	AA(mg/g)	APTI
1	*Carica Papaya*	71.09	0.6	6.2	18.91	19.96
2	*Calotropis gigantea*	57.8	0.18	5.7	14.18	14.11
3	*Eucalyptus mysoresins*	75.54	0.13	5.2	28.3	22.63
4	*Parthenium hysterophorus*	77.92	0.38	6.7	4.72	11.13
5	*Nerium indicum*	78.63	0.06	5.8	9.45	13.4
6	*Polyalthia longifola*	79.59	0.2	5.9	14.18	16.6
7	*Mangifera indica*	53.14	0.15	4.5	33.1	20.7
8	*Ricinus communis*	75.04	0.18	5.7	14.18	15.84
9	*Psidium guajava*	74.32	0.67	4.9	9.45	12.69
10	*Muntingia calibra*	41.88	0.15	5.5	4.72	6.85
11	*Bougainvillea glabra*	65.85	0.06	5.4	4.72	9.16
12	*Terminalia cattapa*	70.29	0.29	4.2	4.72	9.14
13	*Azadirachta indica*	77.29	0.15	5.6	37.8	29.46
14	*Tamarindus indica*	44.94	0.07	3.2	4.72	6.03

RWC = Relative water content, TCh = Total Chlorophyll content, AA = Ascorbic acid and APTI = Air Pollution tolerance index

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RESEARCH ARTICLE

A New Approach for the Design and Development of Poly Herbal Tablets of Nilavembu Kudineer Churanam

Ramya Devi. D [1*], Gopinath. V [2], Vedha Hari. BN [1]

[1] Department of Pharmaceutics, School of Chemical and Bio-Technology (SCBT),

SASTRA University, Thanjavur – 613 401, Tamil Nadu, India

[2] Centre for Advanced Research in Indian System of Medicine (CARISM),

SASTRA University, Thanjavur – 613 401, Tamil Nadu, India

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[1*]Address for correspondence
Ramya Devi. D

Department of Pharmaceutics, School of Chemical and Bio-Technology (SCBT),

SASTRA University, Thanjavur – 613 401, Tamil Nadu, India

E mail ID: ramya@scbt.sastra.edu

ABSTRACT

The present work aims on optimization of the poly herbal tablet formulation of siddha preparation Nilavembu Kudineer churanam, based on experimental method by formulating various trails with different concentration of binders like Poly Vinyl Pyrrolidone (PVP) and starch, and other excipients. The tablets are prepared by wet granulation technique. The powder and granules are studied for pre-formulation parameters and optimized for tablet compression. The formulated tablets are evaluated for the physical parameters such as appearance, weight variation, hardness, friability, and thickness and disintegration time. The microbial load is studied for the tablets to be within the standard limits. The optimized formulation containing 7 % starch mucilage binder in addition with MCC (Micro Crystalline Cellulose) and lactose as diluent, talc and aerosil as glidant and lubricant showed good free flowing property and resulted in efficient tablet compression. The friability of less than 1% and hardness of 2.5 kg/cm^2 indicated that tablets had a good mechanical resistance and the disintegration time less than 1 minute proves the tablets as suitable candidate for improving patient compliance and commercial acceptance.

Key words: Poly herbal tablet, Nilavembu Kudineer churanam, poly vinyl pyrrolidone, starch.

INTRODUCTION

Patient compliance can be improved for any medication by administering them as oral solid unit dosage form like tablets or capsules. The easy administration, unit dose accuracy and less adulteration in the formulation of siddha preparations as tablets make them suitable for commercial acceptance [1]. Nilavembu Kudineer Churanam contains the mixture of *Andrographis paniculata Nees*, *Chrysopogon zizanioides L*, *Vetivera zizanioides L*, *Santalam album L*, *Trichosanthes Cucumerina L*, *Cyperus rotundus L*, *Zingiber officinalis Rocoe*, *Piper nigrum L*, *Mollugo cerviana L* [2]. It is

used as antipyretic indicated for all types of fever like malarial fever, swine flu fever, chikungunya and fevers with shivering. Nilavembu exhibits anti-inflammatory, anti HIV, antibacterial, antioxidant, antiparasitic, antispasmodic, antidiabetic, anticarcinogenic, antipyretic, hepatoprotective, nematocidal and various other activities.[3] The Churanam is prepared as decoction and administered at the dose of 30-60 ml every day.[4, 5] The patient acceptance of the dosage form is poor due to the non accurate dose, dosage form preparation and administration. So, an attempt has been made for the development of poly herbal tablets of the siddha preparation Nilavembu Kudineer Churanam. A solid pharmaceutical dosage formulation of the dry plant powder mixture (Churanam) using various excipients[6] viz., poly vinyl pyrollidone K30, starch, microcrystalline cellulose, Lactose, Talc and Aerosil was formulated by wet granulation tablet compression method. The present communication also deals with the study of pre-formulation parameters and evaluation of the formulated tablets [7] such as weight variation, friability, hardness and disintegration time and microbial load.

MATERIALS AND METHODS

Materials

Poly herbal powder mixture (Nilavembu Kudineer Churanam) was obtained from Lakshmi Seva Sangam, Gandhigram. Poly vinyl pyrrolidone (PVP K30), Starch, Micro Crystalline Cellulose (MCC), Lactose, Magnesium Stearate, and Iso propyl alcohol (IPA) were purchased from SD fine Chemicals, Mumbai. Aerosil and Talc was purchased from Otto Chemicals and Loba Chemie respectively. All the materials were pure and of analytical grade.

Methods

Preparation of Tablets

The poly herbal tablets were prepared by wet granulation method [8, 9, 10 and 11]. In the present study, wet granulation was trialed with two different binders. Formulation F1 and F2 were prepared using PVP K30 dissolved in Iso propyl alcohol as the granulating agent. Formulations F3 to F12 were formulated with starch mucilage (starch dissolved in warm water to make as paste) as granulating agent. (Table1). The poly herbal powder mixture (Nilavembu Kudineer Churanam) and all other excipients were sieved individually using sieve no: 44. The fibers and other coarse particles retained on the sieve were milled and sieved again to get fine particles of uniform size. All the powder ingredients were weighed accurately and blended well. The granulating fluid was added gradually to the dry powder mixture to form a smooth coherent mass. The wet mass was sieved through sieve no: 16 and the wet granules were dried at 100 ^{o}C for 15-20 minutes until it was dried to the moisture level of 1-2 %. The dried granules were again sieved using sieve no: 25 to get the uniform sized granules. These granules were lubricated with the required concentration of lubricant (aerosil) and glidant (talc) and then compressed into tablets of fixed average weight using the single punch tablet compression machine (Khera, New Delhi) with 6mm punches and dies.

Pre-formulation Studies

The poly herbal mixture powder and the prepared granules were subjected for pre-formulation studies [12]. The percentage of moisture present in the herbal powder mixture was estimated by loss on drying method. The weighed (w_1) quantity of the powder was dried in hot air over at 105 ^{o}C for a period of time until the final weight (w_2) becomes constant and the percentage loss on drying was calculated. Bulk density and tapped density were calculated from the mass of powder and its bulk volume and tapped volume respectively. The flow ability of the granules was assessed by the application of compressibility index calculated from its bulk volume and true volume, and by determining the angle of repose.

Evaluation of physical parameters of tablets

The organoleptic characters such as colour, shape, odour and appearance were observed visually. Tablet thickness and diameter were measured by using Vernier Caliper (Mitutoyo Corporation, Mumbai). Twenty tablets from each formulation were selected randomly, weighed individually and the average weight was calculated. Not more than two of the individual weights deviate from the average weight by more than the percentage and none deviates by more than twice that percentage as given in Indian Pharmacopoeia (I.P) [13]. Hardness of the tablets was determined using Monsanto hardness tester (Dolphin, Mumbai). Percentage friability of formulated tablets was determined using Roche Friabilator (Veego, Mumbai). Disintegration time was evaluated by placing six tablets on the tablet

disintegration test apparatus (Veego, Mumbai) containing water as the medium maintained at 37 ^{o}C. The tubes are allowed to move up and down and the time taken for the disintegration of the tablets was noted.

Microbial Load evaluation

The tablet was subjected to microbial analysis.[14, 15, 16] 1gm of sample of the powdered tablet was taken and added to 9 ml of sterile distilled water for preparing the serial dilution. The samples in the flask were kept in a mechanical shaker for few minutes to obtain uniform suspension of microorganisms. The dilution was 1:10 or 10^{-1}. From that 1ml of the 10^{-1} dilution is transferred to 9ml of sterilized distilled water. This was 1: 100 or 10^{-2}. This procedure was repeated up to 10^{-7} dilution. 0.1 ml of serially diluted samples was inoculated into the sterile plate containing Nutrient agar, Salmonella Shigella Agar (SSA) and Potato Dextrose Agar (PDA) Medium by spread plate method. Nutrient agar, and SSA plates were incubated at 37ºC for 24 hours and PDA plates were incubated at room temperature for 3-5 days. Bacterial and fungal colonies were counted using colony counter. *Salmonella, Shigella* and *E.coli* can be identified using SS Agar medium. The standard of limits was studied as per WHO guidelines [17].

RESULTS AND DISCUSSION

The herbal tablets were prepared by wet granulation technique and the formula and process was optimized by experimental method. The herbal powder mixture absorbed more moisture and the percentage loss on drying was found to be 3.6 %. The pre-formulation studies performed for the powder indicated a poor compressibility index and flow property (Table 2). So the granules were prepared with different concentration of binders and lubricants to optimize the formula for tablet preparation. Formulations F1 and F2 were prepared with 3 % and 5 % concentration of PVP K30 in IPA as binder, respectively. Talc and magnesium stearate was added for lubrication of the granules. The binding capacity was poor and so resulted in more friable tablets (friability > 2%) with less hardness (0.5 Kg/Cm^3). Since the powder absorbed more moisture, formulations F3 to F6 were trailed with starch mucilage at concentration of 5, 10, 15 and 20 % respectively. The binding capacity and hardness was good at the concentration of above 15 %, but the friability was more than 1 % limit. To reduce the high concentration of starch binder and to get tablet of enough hardness and friability, the other excipients such as micro crystalline cellulose and lactose was incorporated. Formulations F7 to F12 were prepared with starch mucilage at concentration of 20, 15, 12, 10, 7 and 5 % respectively in addition with micro crystalline cellulose and lactose as filler. Since the powder contains more fibre content it was elastic in nature, so the addition of MCC and lactose may act as plasticer to provide enough binding and compactability to the herbal plant mixture. Talc and aerosil was added as lubricant and glidant to the formulation F7 to F12 to get uniform flow of granules with less friction during tablet compression. The poly herbal tablets prepared were elegant in appearance (Fig. 1) and patient acceptable. The physical parameters were observed to be fairly good and confirming to requirements. The weight variation of the tablets of all the formulations F1 to F12 is within the I.P. limits ($\pm$ 5 %). The average weight of tablets was found to be within the range 295- 310 mg. The thickness of the tablets was in the range of 2.5 – 2.7 mm. The friability test results showed F7 to F12 formulations were hard enough and less friable (0.01 to 0.1 %). The hardness of formulation F7 to F11 was in the range of 2.5 – 3.0 Kg/Cm^3 due to high concentration of starch in addition to the diluents. The other formulations F1 and F2 containing PVP K30 as binder showed poor hardness (less than 1 Kg/Cm^2) whereas in formulation F3 to F6, the hardness increased gradually with increase in concentration of starch mucilage binder. The disintegration time of all the 12 formulations was < 1 min (Table 3). In the microbiological analysis, the total microbial load was determined. E.coli, total heterotrophic bacteria and total fungi count were within the WHO limits (Table 4 and Fig. 2). The absence of *Salmonella* sp. and *Shigella* sp. in the formulation as per WHO standards prove the poly herbal tablets as suitable for commercial acceptance.

CONCLUSION

The various trial formulations of the tablet preparation show that the composition of starch mucilage binder at 7 % concentration in addition with 7 % micro crystalline and 7 % lactose as diluent, 2 % of talc and aerosil as lubricant and glidant respectively is an optimized formula for this poly herbal tablet formulation. The evaluation tests results

prove the statement as the parameters are within the standard limits. So the solid unit dosage form of tablets was optimized and formulated for easy administration of the medication and improving the patience compliance.

Table 1: Formulation variables of Nilavembu Kudineer churanam Poly-Herbal Tablets

Ingredients	Quantity for each tablet (mg)											
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Poly Herbal Mixture	200	200	200	200	200	200	200	200	200	200	200	200
PVP K30	9	15	-	-	-	-	-	-	-	-	-	-
Starch	85	73	73	58	43	28	-	-	-	-	-	-
Starch (For Mucilage)	-	-	15	30	45	60	60	45	36	30	21	15
Microcrystalline Cellulose	-	-	-	-	-	-	15	21	21	21	21	21
Lactose	-	-	-	-	-	-	13	22	31	37	46	52
Talc	3	6	6	6	6	6	6	6	6	6	6	6
Magnesium Stearate	3	6	6	6	6	6	-	-	-	-	-	-
Aerosil	-	-	-	-	-	-	6	6	6	6	6	6
Isopropyl Alcohol	q.s.	q.s.	-	-	-	-	-	-	-	-	-	-
Distilled water	-	-	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Table 2: Pre-formulation Studies for Herbal Powder and Granules

Preformulation Parameters	Herbal Powder Mixture	Herbal Tablets Granules (F11)
Moisture Content (%)	3.6	1.0
Bulk Density (g / cc) *	0.3125	0.4166
Tapped Density (g / cc) *	0.4761	0.4761
Compressibility Index (%) *	34.37	12.5
Angle of Repose (θ) *	39.95	21.03

* Average of three trials

Table 3: Physical Parameters of Nilavembu Kudineer Churanam Poly Herbal Tablets

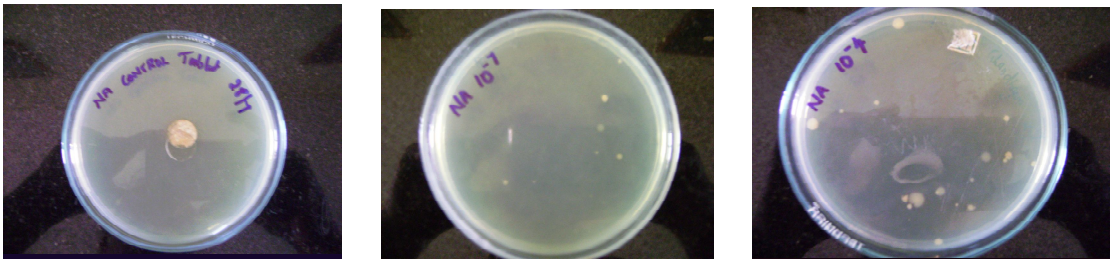
Formulation Code	Hardness (kg/cm^3) *	% Friability	Thickness (mm) *	Average Weight (mg ± 5 %) **	Disintegration Time (seconds)
F1	0.58 ± 0.18	2.65	2.66 ± 0.04	302.27 ± 5.51	10 ± 0.89
F2	0.66 ± 0.23	2.50	2.65 ± 0.07	303.22 ± 6.43	14 ± 1.41
F3	0.75 ± 0.25	1.91	2.56 ± 0.04	304.04 ± 6.28	14 ± 0.37
F4	1.25 ± 0.25	1.52	2.66 ± 0.07	302.72 ± 6.63	17 ± 2.79
F5	2.08 ± 0.18	1.21	2.61 ± 0.06	302.34 ± 5.31	20 ± 3.59
F6	3.16 ± 0.23	1.10	2.63 ± 0.07	303.09 ± 5.74	35 ± 5.77
F7	3.41 ± 0.18	0.02	2.70 ± 0.05	302.88 ± 5.64	60 ± 7.28
F8	3.33 ± 0.23	0.05	2.70 ± 0.08	300.94 ± 7.11	53 ± 8.68
F9	3.16 ± 0.23	0.06	2.63 ± 0.11	303.28 ± 6.75	52 ± 8.37
F10	2.33 ± 0.23	0.09	2.61 ± 0.06	303.37 ± 6.52	50 ± 7.31
F11	2.5 ± 0	0.09	2.66 ± 0.07	304.22 ± 3.11	45 ± 2.88
F12	1.41 ± 0.34	0.15	2.60 ± 0.05	302.27 ± 5.81	28 ± 4.65

***** Average of 3 ****** Average of 2

Table 4: Total Microbial Count of Nilavembu Tablets

S. No.	Bacterial Name	Cells in Sample / g	WHO Limit	Inference
1.	E.coli	Nil	10^2	With in Limits
2.	Salmonella sp.	Nil	Absent	Absent
3.	Shigella sp.	Nil	Absent	Absent
4.	Total Heterotrophic Bacteria	18 x 10^4	10^7	With in Limits
5.	Total Fungal Count	62 x 10^2	10^4	With in Limits

Figure 1: Optical Microscopic Images of Nilavembu tablets



a) Positive Control b) Total Bacterial count c) Total Fungal count

Figure 2: Microbiological study for Nilavembu tablets

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RESEARCH ARTICLE

Cardio protective Effect of Soybean (*Glycine Max (L) Merril*) on Isoproterenol Induced Myocardial Infarction in Wistar Rats

Ramesh kannan. N[1], I. Antonie Lebel[2] and Natarajan. E[1*]

[1]PG & Research Department of Botany, National College, Trichy–620001, Tamil Nadu, India.

[2]Department of Plant Biology and Biotechnology, Loyola College, Chennai – 600 034, Tamil Nadu, India.

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[1*] Address For Correspondence

Dr. Natarajan.. E

PG & Research Department of Botany, National College,

Trichy–620001, Tamil Nadu, India.

Email ID: enr69@yahoo.com

ABSTRACT

Isoproterenol induced myocardial infarction was confirmed by changes in serum and heart tissue marker enzymes as lactate dehydrogenase (LDH), creatine phospho kinase (CPK), aspartate transaminase (AST) and alanine transaminase (ALT), increased level of lipid peroxidation (LPO) and histopathological changes in the heart of isoproterenol administered rats. Pretreatment with *Glycine max* (soybean rich diet as recommended by NCLAS, Hyderabad) for 30 days was found to ameliorate the effect of isoproterenol induced pathological changes, reduced the lipid peroxide formation and retained the myocardial marker enzyme activities at near normal level. The above results indicate the cardioprotective effect of *Glycine max* (Soybean) against isoproterenol induced myocardial infarction in rats.

Key words: Cardiovascular diseases, Myocardial Infarction, *Glycine max*, Soybean, Isoproterenol, Lipid Peroxidation, Heart enzyme markers.

INTRODUCTION

Myocardial infarction, ranks first in the list of life threatening diseases of the mankind. For that, the phytotherapy is emerging as an effective alternative to the chemotherapeutic treatments, which instead of ameliorating the condition, aggravates it [1, 2]. Of the several thousands of medicinal species present in our country, Soybean (*Glycine max* (L) Merril) occupies its prime importance in conferring protection against cardiovascular diseases due to the presence of a typical phytochemical namely isoflavones [3,4]. Because it was already demonstrated to possess antioxidant [5, 6], antithrombotic [7] lipid lowering effect [8] of which the antioxidant property is due to the presence of a typical component known as isoflavones [9-11]. During myocardial infarction, produced by isoproterenol, there is an increase in the levels of lipid peroxides in the myocardium due to the overproduction of free radicals, reactive oxygen species (ROS) etc., causing myocardial membrane damage and dysfunction [12]. Since isoflavones possess several cardio protective attributes, it was hypothesized that the protection of soybean against myocardial damage might be due to the abundant availability of isoflavones in it. Soybean has complex carbohydrates, protein, dietary fiber, oligosaccharides, phytosterol, saponin, lecithin, isoflavone, phytic acid, trypsin inhibitor, and minerals. The

cholesterol lowering effect of soy milk and its role of heart disease was widely recognized in the mid 90s when the results of a meta-analysis of 38 clinical studies were published. The results demonstrated that a diet with significant soy protein reduces Total Cholesterol, LDL cholesterol (the "Bad" cholesterol) and Triglycerides. Complex carbohydrates and dietary fiber contents contribute to low glycemic indexes, which benefit diabetic individuals[13] and reduce the risk of developing diabetes. Also, soybean reduces cholesterol levels[14]. This hypothesis is substantiated with the alteration in the activities of Lactate Dehydrogenase (LDH), Creatine Phospho Kinase (CPK), Aspartate Transaminase (AST) and Alanine Transaminase (ALT), increased level of Lipid Peroxidation and histopathological changes in the heart of isoproterenol administered rats.

MATERIALS AND METHODS

Male albino rats weighing 100 – 150 g were used as experimental animals. These were divided into four groups of six each (n=6). Group I, served as control fed with normal diet (Table 1) recommended by NCLAS, Hyderabad, Group II was given isoproterenol hydrochloride (1 mg / kg body weight, subcutaneously) for three consecutive days at a time interval of 24 hrs fed with normal diet (Table 1). Group III served as experimental group fed with specially designed diet (Table 2), Group IV served as experimental group fed with special diet (Table 2) rich in roasted soybean flour was administered with isoproterenol (1 mg / kg body weight subcutaneously) like Group II. The animals of group I and II were fed with normal diet (Table 1) and Group III & IV were fed with special diet (Table 2) for 30 days. After 30 days of experimental period, isoproterenol was administered to induce myocardial infarction. The isoproterenol and other requisite chemicals for the analysis were procured from Himedia Company. Following the administration of the third dose of isoproterenol HCl, the animals were sacrificed and the blood sample was collected, allowed to clot and serum was separated at 5000 rpm for 10 minutes. The activities of LDH[15], CPK[16], AST[17] and ALT[17] and LPO[18] were determined. Statistical analysis was carried out using student's 't' test[19]. For histopathological studies, the lower portion of heart tissue were excised and fixed in 10% formalin for 24 hour and stained by haematoxylin.

RESULTS

At the end of the experimental period, a significant decreased LDH activities in Group II when compared to Control group. The LDH activity in the Soybean with isoproterenol treated and Soybean treated groups were increased significantly. A significant decrease in the activity CPK was observed in myocardial infarcted rats when compared to control rats. Soybean with isoproterenol treated and Soybean treated rats significantly increased the activity of CPK near to control level. The myocardial infarcted rats showed a significant decrease in the activities of both AST and ALT as compared to infarcted rats. Soybean with isoproterenol treated and Soybean treated rats regained the activities of AST and ALT like control groups (Tables III). The concentration of lipid peroxidase (LPO) was significantly increased in infarcted rats when compared to the control group. Administration of soybean with isoproterenol treated and Soybean treated rats significantly decreased levels of the lipid peroxidation to near normal level (Table III). Microscopic examination of heart tissue of the Group I control rats (Fig. a) showed normal myocardial fibers and muscle bundles with normal architecture. Heart tissue of Group 2 isoproterenol myocardial infarcted rates (Fig. b) showed separation of myocardial fibers with inflammatory mononuclear collections, edema and myocardial necrosis. Soybean alone treated Group 3 rats (Fig. c) showed normal myocardial fibers with no pathological changes. Myocardial section of soybean pretreated Group 4 rats showed slightly separated myocardial fibers with small focus of inflammatory mononuclear collections (Fig. d) with absence of necrotic damage.

DISCUSSION

The present investigation is aimed to evaluate and explore the cardioprotective of soybean on isoproterenol induced myocardial infarction in rats. Myocardium contains an abundant concentration of diagnostic marker enzymes of myocardial infarction like CPK, LDH and transaminases and once metabolically damaged, releases its content into the extra cellular fluid [20]. In isoproterenol myocardial infarcted rats (Group II), the increased activities of the serum marker enzymes accompanied by their concomitant reduction in the heart homogenate confirm to onset of myocardial necrosis. Hence the total concentration of the marker enzymes were found to be decreased in heart tissue

of isoproterenol infarcted rats as compared to control, which may be the reflection of consequences of cellular injury due to lipid peroxides. Isoproterenol is well known to generate free radicals and to stimulate lipid peroxidation, which may be a causative factor for irreversible damage to the myocardium [21]. The increased levels of TBARS indicate excessive formation of free radicals and activation of lipid peroxidation system resulting in irreversible damage to the heart in animals subjected to isoproterenol stress. The significant increase observed in the levels of lipid peroxides in heart of isoproterenol infarcted rats compared to control, was in accordance with the observation of previous reports [22].Upon soybean pretreatment Heart marker enzyme of rats were found to be significantly increased as compared to isoproterenol myocardial infracted rats and this could be due to antioxidant and free radical quenching effect of soybean[23]. Soybean, a principal isoflavones compound has potent free radical scavenging activity and protective effect against altered changes in AST and ALT activities caused by toxicant [24]. Isoflavones have been reported to possess protective action against myocardial ischemia and diminishes the release of AST and LDH enzymes [25]. Similar observations have been recorded with soybean pretreatment in the present study, which could be attributed to cardioprotective action of soybean. Soybean pretreated (Group IV) rats showed a significant decrease in LPO level in heart tissue as compared with isoproterenol myocardial infracted rats. Previous investigations have shown that Isoflavones exhibit a cardioprotective effect against myocardial ischemic injury by inhibiting lipid peroxidation and thus enhancing the recovery of cardiac function [26]. This could be the reason for the reduced formation of LPO in soybean pretreated rats. Histological examination of heart tissue of Group II rats showed myocardial necrosis and separation of myocardial fibers with inflammatory mononuclear infiltrate whereas the examination of heart tissue of Soybean pretreated (Group IV) rats showed maximum protective effect by reduced histological changes as compared to isoproterenol myocardial infracted rats. The therapeutic efficacy of soybean may be due to its antioxidant, antilipidperoxidative, free radical scavenging, immunomodulatory and cardiotonic property that could have prevented isoproterenol induced tissue injury. Thus it could be concluded that soybean protects experimental myocardial infarction as revealed by the amelioration of histological changes and biochemical markers of cardiac tissue damage without any adverse effect which merit further detailed studies to develop it as cardioprotective drug.

Table 1: Diet Composition for control groups I & II (as recommended by NCLAS, Hyderabad**)**

Wheat flour	22.5%
Roasted Bengal gram flour	60%
Skimmed milk powder	5%
Casein	4%
Refined groundnut oil	4%
Salt mixture	4%
Vitamin mixture	0.5%

Table II: Dietary composition for experimental group III & IV

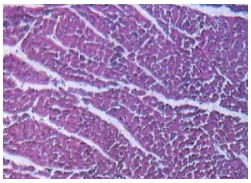
Wheat flour	22.5%
Roasted soybean flour	60%
Skimmed milk powder	5%
Casein	4%
Refined groundnut oil	4%
Salt mixture	4%
Vitamin mixture	0.5%

Table III: Effect of Soybean on LDH, CPK, AST, ALT and LPO in control and experimental group of rats [Values are expressed mean±SD for 6 animals in each group]

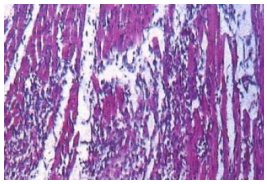
Parameter	Group I	Group II	Group III	Group IV
Heart tissue marker enzymes				
LDH§	129.67±8.70	57.73±9.84*	131.33±2.50	122.00±3.13a
CPK§§	17.09±1.11	7.83±1.80*	18.11±0.49	15.72±0.58a
AST§	51.98±1.69	31.77±1.49*	53.34±2.15	47.18±2.22a
ALT§	24.89±0.59	12.37±0.47*	26.50±1.21	21.96±1.03a
Lipid Peroxidation				
LPO§§§	6.25±0.20	9.12±0.40*	5.95±0.44	6.62±0.35a

§ (nmoles of pyruvate liberated/min/mg/protein)
§§ (µmoles of phosphorous liberated/min/mg/protein)
§§§ (mmoles of TBARS/mg of protein)

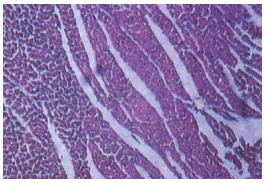
P values: *<0.001 statistically significant when compared with Group 1;
a<0.001 statistically significant when compared with Group 2.



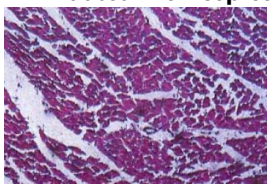
(a) Normal Control



(b) Group II fed with normal diet, induced with Isoproterenol



(c) Group III fed with soy rich diet



(d) Group IV fed with Soy rich diet, induced with Isoproterenol

Figure: Sections of heart from apical region stained with haematoxylin

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RESEARCH ARTICLE

A Preliminary Assessment of Certain Heavy Metals on Plants in and Around the Shivamogga City of Karnataka State, India

Hina Kousar*, Adamsab. M. Patel*, Semema Naik*, Ramaraj. S **, Sirajuddin.M.Horaginamani *** and Ravichandran. M***

*Department of Environmental Science, Kuvempu University, Jnana Sahyadri, Shankarghatta-577 451, Shivamogga, Karnataka, India. **Plant Stress Physiology and Biotechnology Lab, Department of Plant Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli- 620 024, India ***Department of Environmental Management, School of Environmental Sciences, Bharathidasan University, Tiruchirappalli - 620 024, Tamil Nadu. India.

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****Address for correspondence**
Mr. S.Ramaraj
Plant Stress Physiology and Biotechnology Lab, Department of Plant Science,
School of Life Sciences, Bharathidasan University, Tiruchirappalli- 620 024, India
E-mail ID: ramarajbiotech@gmail.com

ABSTRACT

Metals are ubiquitous in the modern industrialized environment. Heavy metals like cadmium, iron, molybdenum, lead, nickel, tin and zinc are present in the environment in trace concentrations .These metals are universally present in soil, water, air and biota. Eight plant species were chosen for the study purpose in and around Shivamogga city (Experimental site). Shankarghatta was chosen as a control site. The selected plant species are *Polyalthia longifolia, Bauhinia purpurea, Anacardium occidentale, Artocarpus heterophyllus, Syzygium cumini, Terminalia catappa, Psidium guajava, and Hibiscus rosa- sinensis*. The heavy metals analyzed were found to be in extremely high concentration. The industrial areas of Machenahalli and Mandli were found to be severely polluted and hence the concentration of heavy metals in these areas was found to be very high. From the present investigation, it is evident that Shivamogga city (experimental site) is severely polluted, as comparing the data with control site (Shankarghatta).

Keywords: Heavy metals, plant leaves, Shivamogga, plant stress, Shankarghatta

INTRODUCTION

The atmosphere is a dynamic system consisting of four principal zones; troposphere, stratosphere, mesosphere and the Ionosphere. The zone closet to the earth is the troposphere and is of greatest concern for the transport of pollutants. Temperature and winds are a major influence on the rate and volume of movement of pollutants in the atmosphere. It could be observed that the plants particularly leaves act as good receptors of the particulate emission produced by automobile exhaust, industries and miscellaneous anthropogenic activities .Many plants can be used as indicators of pollutants in air and the responses of these can be related to known concentrations of specific air pollutants [1], [2], [3]). So plants can indicate both the presence and also monitor concentrations of the air pollutants .Many studies have shown that trace elements, such as Cd, Pb, Zn, Cu, Mn, Fe, Cr and Ni are found deposited on

plant leaves [4]; [5]; [6]. Higher vascular plants and many lower non –vascular plants are also used in the abatement of pollutants [7]. The study site (Shivamogga) has witnessed for the location of many small scale industries and the national high way NH 206, is also passes through this area .Therefore, the most of the industries located along the highway and further development is also restricted on the same line because of convenient accessibility a result, road dust automobile exhaust and industrial emissions are the common scenario around the area. The aim of the present study is to find out the extent of air pollutants deposition on plants growing in and around the Shivamogga city of Karnataka state especially with reference to the trace elements deposition, namely Cu, Mn, Zn and Fe.

MATERIALS AND METHODS

Study area

Shivamogga is one of the most important city of Karnataka State and is situated on the banks of river Tunga and spread over an area of 50 km² (19.31 square miles) with a total population of 4,97.373 as per 2007 census. The geographical location of the city is 13°55′18″ N, 75°34′12″ E. Its height is 584 meter above MSL (Mean Sea Level). It is a blend of history, tradition and a thriving commercial city. It is exposed to Southwest monsoon. Humidity is more during the month of July (78%). The annual rainfall is 200.97 mm. The average wind velocity is 9.7 km/h from the Southwest.

Sampling Sites

The selection of sampling sites for the present study was based on the location, population, regional background and other such factors. Commercial and Industrial areas were chosen for the present study. Bus stand and Gandhi Bazar were the commercial areas; Mandli and Machanahalli were the industrial areas. Eight plant species were chosen for the study purpose in and around Shivamogga city (Experimental site). Shankarghatta was chosen as a control site. The selected plant species are *Polyalthia longifolia, Bauhinia purpurea, Anacardium occidentale, Artocarpus heterophyllus, Syzygium cumini, Terminalia catappa, Psidium guajava, and Hibiscus rosasinensis*. The reason for selecting these plants was due to their common occurrence and easy availability.

Sample collection

The leaves of the selected plants of both experimental as well as control were collected from February to April 2010. The leaves were collected approximately at a height of 5-7 ft from the ground level. About 10-12 leaves were collected from each plant species and were cut with the help of stainless steel scissor to avoid contamination.

Sample preparation

The major veins were carefully removed from the unwashed leaves and were dried in a hot air oven in individually labeled Petri plates. At the time of drying, the temperature was gradually increased from 50 to 80°C. After 48 hrs the Petri plates were taken out from the oven. Grinding of the leaves to a fine powder was done and sieved with the help of 0.2mm nylon sieve plate. The prepared samples were digested using tertiary acid mixture, concentration of Perchloric, Nitric and Sulphuric acids in the ratio of 10:1:4. This method is called Triacidic method. 0.5gm fine powder leaf sample is taken to which 10ml Concentrated HNO3 was added and kept for 24 hours without disturbance. Later 6 to 8 ml Triacidic mixture is added which gives a white precipitate. By adding 50ml distilled water, samples were filtered through Whatman no.44 filter paper and the solutions were used to determine heavy metal concentration using Atomic Absorption Spectrophotometer. Acetylene is the carrier gas flame type used in this atomic absorption spectrophotometer (model GBC 932 AA).

RESULTS AND DISCUSSION

The present investigation was the preliminary survey conducted to determine the air pollution in Shivamogga city. As stated earlier, the major air pollutants, which are usually generated from any vehicles and industry, include heavy metals. Hence, it is very essential to record their concentration before implementing the control measures. Table 1 gives the average concentration of heavy metals in the selected plant leaves of experiment site. The results reveal the heavy metal concentration in *Syzygium cumini* : Cu-14.**7**ppm ,Mn-141.1ppm, Zn-58.3ppm Fe-1690.0ppm and in *Terminalia catappa* it was as follows: Cu -15.3ppm, Mn-178.8ppm, Zn-60.5ppm, Fe-2622.0ppm.Similarly the plant

leaves analyzed for heavy metals in Gandhi bazaar area showed the following results**:** *Hibiscus rosasinensis* - Cu-10.9ppm, Mn-205.6ppm, Zn-45.3ppm, Fe-2847.5ppm , *Psidium guajava* Cu -11.4ppm, Mn-132.3ppm, Zn-29.8ppm, Fe-1042.3ppm. In Mandli area the results obtained were as follows: *Anacardium occidentale* **-** Cu-14.0ppm, Mn-148.4ppm, Zn-60.4ppm, Fe-3225.0ppm and *Artocarpus heterophyllus*- Cu -27.2ppm, Mn-225.1ppm, Zn-96.4ppm, and Fe-5240.0 ppm. Machanalli area is considered as an industrial area of Shivamogga city exhibited the following results: *Polyalthia longifolia***-**Cu-5.5ppm, Mn-155.9ppm, Zn-56.0ppm, Fe-2920.0ppm and *Bauhinia purpurea*- Cu-6.0ppm, Mn-132.4ppm, Zn-40.0ppm, Fe-4647.5ppm. From the table 2 indicates the heavy metal concentration in the plant leaves at control site (Shankarghatta). The concentration of heavy metals analyzed in the plant leaves is as follows: (All values are in ppm). *Syzygium cumini*: Cu-20.2, Mn-17.8, Zn-10.8, Fe-259.2.*Terminalia catappa*: Cu-8.1, Mn-60.1, Zn-29.6, Fe-259.7,*Hibiscus rosa-sinensis*: Cu-0, Mn-68.1, Zn-23.0, Fe-149.8. *Psidium guagaua* : Cu-16.4, Mn-55.4, Zn-14.7, Fe-264.9,*Anacardium occidentale* : Cu-0, Mn-30.2, Zn-26.0, Fe- 247.4,*Artocarpus heterophyllus* : Cu-15.5, Mn-21.9, Zn-21.0, Fe-195.4,*Polyalthia longifolia* : Cu-10.9, Mn-40.2, Zn-20.4, Fe-195.5,*Bauhinia purpurea* : Cu-2.4, Mn-34.4, Zn-19.8, Fe-132.5. From the graphs, it is evident that Cu ranges from 5.5 to 27.2ppm; Mn ranges from 132.3 to 225.1ppm; Zn ranges from 29.0 to 60.5ppm and Fe ranges from 1042 .3 to 5240.0ppm in the experimental site. Peri-urban and urban areas of developing countries continue to receive raising level of heavy metals through atmospheric deposits. [8].Deposition of Fe appeared higher than the other elements in the present study which ranked in the order Fe> Mn> Zn> Cu. A similar observation has been recorded by [9]and [10]. Compared to all the heavy metals the concentration of Fe is high. The graph indicates that the concentration of Fe in experimental site is more compared to control. A similar finding has been observed by [10]and [11] Iron comes from corrosion of pipes, containers and wear and tear of vehicles. The very high deposition of Fe is also attributed to the local soil dust, which is rich in Fe concentration. A very interesting observation has been recorded in the present study. Although all the heavy metals which were analyzed showed higher concentration in experimental site than the control, Cu displayed a peculiar behavior. It was found that the concentration of Cu in the control area was more than that of experimental site. The reason attributed for this is the presence of Chalcopyrite i.e. the copper ore at some places in the control site which has resulted in the higher concentration of Cu. Among the experimental sites the industrial areas of Mandli and Machanahalli recorded the highest concentration of heavy metals. Similar observation has been recorded by [12]while working on air pollution effect on boreal forest vegetation in the Russian-Norwegian Border due to Nickel-Copper smelter. Variation in heavy metal concentration in plants could be probably due to variable capabilities of plants to absorb and accumulate the heavy metals [13]as well as to variable concentration of heavy metals in air atmospheric deposits and in soil [14]; [15], variation in growth period and growth rates [16]. All the plant samples at the experimental sites showed a higher concentration of heavy metals. Shivamogga is a fast growing city with major constructions going on inside the city limits including road widening work. The ever increasing vehicles and emission from several industries located in and around Shivamogga city have lead to an increasing concentration of pollutants.

CONCLUSION

An attempt has been made to study the air pollution status of Shivamogga city pertaining to heavy metals accumulation in plants. The heavy metals analyzed were found to be in extremely high concentration. The industrial areas of Machenahalli and Mandli were found to be severely polluted and hence the concentration of heavy metals in these areas was found to be very high. From the present investigation, it is evident that Shivamogga city is severely polluted and the reason attributed is vehicular emission, industries, demolition of buildings road widening, etc.

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Table 1: Heavy metal concentration in experimental sites Shivamogga

SITES	Cu	Mn	Zn	Fe
1. Bus stand				
Syzygium cumini	14.7	141.1	58.3	1690.0
Terminalia catappa	15.3	178.8	60.3	2622.0
2.Gandhi bazar				
Hibiscusrosa-sinensis	10.9	205.6	45.3	2847.5
Psidium guagaua	11.4	132.3	29.8	1042.3
3. Mandli				
Anacardium occidentale	14.0	148.4	60.4	3225.0
Artocarpus heterophyllus	27.2	225.1	96.4	5240.0
4. Machanahalli				
Polyalthia longifolia	5.5	155.9	56.0	2920.0
Bauhinia purpurea	6.0	132.4	40.0	4647.5

Table 2: Heavymetal concentration at control sites Shankarghatta (ppm)

SITES Shankarghatta	Cu	Mn	Zn	Fe
Site 1				
Syzygium cumini	20.2	17.8	10.8	259.2
Terminalia catappa	8.1	60.1	29.6	259.7
Site 2				
Hibiscus rosa-sinensis	0	68.1	23.0	149.8
Psidium guagaua	16.4	55.4	14.7	264.9
Site 3				
Anacardium occidentale	0	30.2	26.0	247.4
Artocarpus heterophyllus	15.5	21.9	21.0	195.4
Site 4				
Polyalthia longifolia	10.9	40.2	20.4	195.5
Bauhinia purpurea	2.4	34.4	19.8	132.5

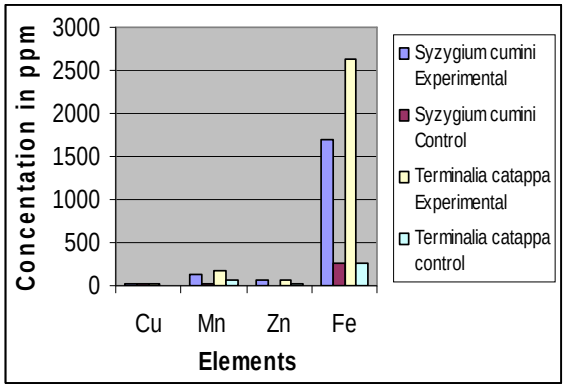

Figure 1. Graph depicting comparison of heavy metals in site 1 and Bus stand area

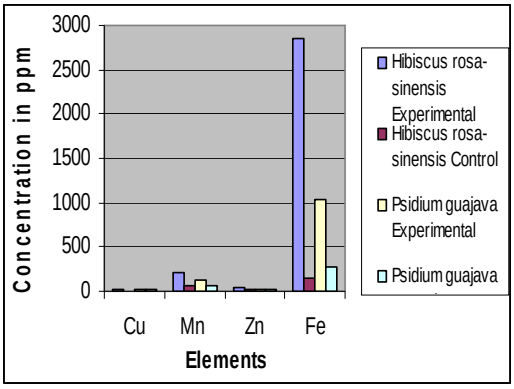

Figure 2. Graph depicting comparison of heavy metals in site 2 and Gandhi bazaar

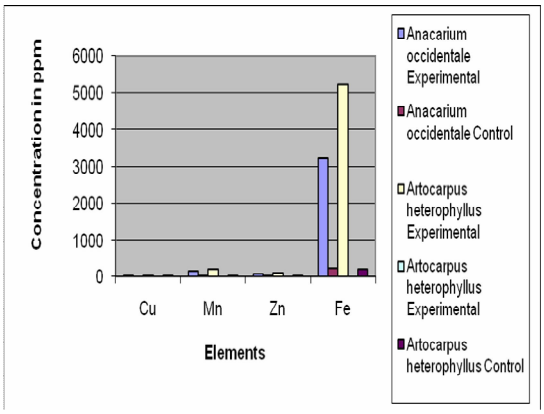

Figure 3. Graph depicting comparison of heavy metals in site 3 and Mandli.

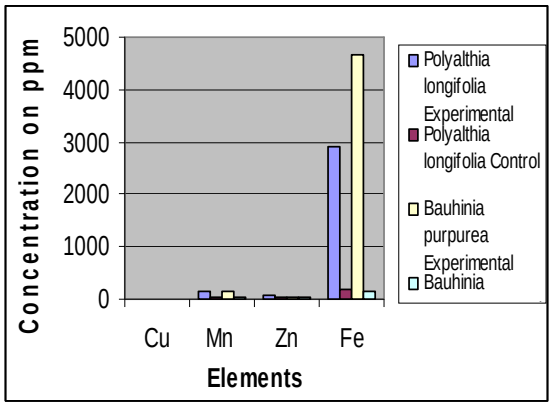

Figure 4. Graph depicting comparison of heavy metals in site4 and Machenahalli.

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RESEARCH ARTICLE

Investigation of Anti-Microbial Activity of *Nymphoides macrospermum Vasud*

Neelakanthan Vinod. T, Yasmin Begum. A, Nagappan. RM, Vedha Hari. BN*, Sudha. C

Department of Pharmaceutical Technology, SCBT, SASTRA University, Thanjavur - 613 401. Tamil Nadu, India.

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***Address for correspondence**
Mr. B.N. Vedha Hari
Department of Pharmaceutical Technology,
SCBT, SASTRA University,
Thanjavur - 613 401, Tamil Nadu
E mail ID: vedhahari@scbt.sastra.edu

ABSTRACT

The present study aimed at evaluating the *in vitro* antimicrobial activity of alcoholic extracts of *Nymphoides macrospermum Vasud* (Menyanthaceae) against bacterial species and fungal species. Despite its traditional use, *Nymphoides macrospermum Vasud*has not been subjected to detailed phytochemical and anti-microbial studies. Thus this plant species was analyzed for the anti-microbial activity by zone of inhibition using agar plate technique shows antimicrobial activity and promising results are observed with bacterial and fungal species. The preliminary phytochemical analysis reveals the presence of steroid, tannins, saponins and sugars.

Key words: Antimicrobial, Antioxidation, Phytochemical.

INTRODUCTION

Medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents. Tagara (*Valeriana jatamansi* Jones) (Valerianaceae) is an important ayurvedic drug employed in several preparations used in the treatment of various diseases. In south India, a formulation is used in the name Granthika tagara (Tamil), botanically identified as *Nymphoides macrospermum Vasud* (Menyanthaceae), is used as Tagara for the same ayurvedic preparation under the same formulations. It is employed in several preparation used in the treatment of various illness such as epilepsy, anemia, jaundice, tuberculosis, mental disorders, fevers, cough and asthmatic conditions and also as a general and brain tonic [1]. The accepted botanical source of Tagara is *Valeriana jatamansi* Jones (Valerianaceae) [2]. The genus *Nymphoides* consists of about 20 species, of which five are found in India; they are aquatic herbs, floating or creeping with white or yellow flowers [3]. *Nymphoides macrospermum Vasud* is recorded as a new taxon from south India [4]. Recently a report shows that the plant has Anti-sedative activity [5]. The present study aimed at evaluating the antimicrobial activity of plant *Nymphoides macrospermum Vasud* extracts against Gram-positive and Gram-negative bacterial strains. The antimicrobial activity was analyzed due to the factor each microbe will be inhibited with certain components in plant materials like steroids, sugar, tannins and saponin.

MATERIALS AND METHODS

Material

Plant

The root and rhizome constituting the drug of commerce of *Nymphoides macrospermum Vasud* was procured from the crude drug market of Coimbatore, Tamil Nadu, India, during March 2010. The characters found in the market sample were pharmacobotanically analyzed, identified as *Nymphoides macrospermum Vasud* [6] was authenticated by Dr. N. Ravichandran, SASTRA University, Thanjavur.

Chemicals

Absolute ethanol 99.9% (Qualigens, Mumbai), Petroleum ether 40:60 (Qualigens, Mumbai), Ciproflaxin (Chatan & Chatan, Chennai), Chloramphenicol (Microlabs, Hosur), Nutrient agar (Himedia, Mumbai, India).

Extraction

Shade dried roots and rhizomes (75gms) were powdered by milling and sieved using 40 mesh sizes for uniform size distribution. Then the powdered plant material was treated with petroleum ether (40:60) to remove the fats and unnecessary compounds for 48hours using soxhlet extraction procedure. Then the wet content was allowed to dry to remove the petroleum ether for overnight. The dried powder was treated with ethanol 99.99% (absolute ethanol) to extract the active components from the plant materials in a Soxhlet apparatus, the process was carried out for 48 hours. The extract was collected and preserved in desiccator to prevent any contamination and moisture absorption and used to carry out antimicrobial activity and phytochemical studies.

Phytochemical studies

Ethanol extract was tested to determine the various phytochemical constituents using standard methods [7] [8]. The anti-microbial properties of the plants may be attributed to the secondary metabolites present in it, phytoconstituents like phenolics [9] [10], tannins [11] and alkaloids [12] are found to be effective anti-microbial substance against a wide range of micro organism [13].

Anti-microbial activity

Anti-microbial activity was studied by agar well diffusion method [14]. On the solidification of agar, wells of 6mm diameter were punched with sterile bores and sealed with molten agar to prevent the escape of extract through bottom. Stock cultures of *Salmonella typhi, Shigella sonni, E. coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus* were prepared by using nutrient broth taking one loop full of organisms from old cultures and incubated for 24hours. After overnight incubation, these were firmly swept over the agar (nutrient agar medium) using sterile cotton swab to make uniform culture lawns in laminar air flow to prevent further contamination. The plant extract of 1200µg/ml along with the drug were poured in each well and incubated for 18-24 hours after that clear zone around the well are observed. The different standard drugs were used for bacterial strains such as Ciprofloxacin 100mcg/ml and Chloramphenicol 50mcg/ml

RESULT

The anti-microbial activity results revealed that the ethanolic extract of plant were effective against various tested organisms of bacterial strains were compared to standard drugs; plant extract shows good inhibition against microorganisms. (Table 1)

CONCLUSION

From the above performed antimicrobial activity it is clear that the plant extract of *Nymphoides macrospermum Vasud* have antimicrobial activity and it inhibit the four different microbial species. These plant extracts are most effective on bacterial strains. Thus from these results we conclude that the plant *Nymphoides macrospermum Vasud* have antibacterial activity

Table 1: Antimicrobial Activity of *Nymphoides macrospermum Vasud* extracts against various organisms by agar well diffusion method

Organism name	Zone of Inhibition(mm)			
	1	**2**	**3**	**4**
Salmonella typhi	2	16	15	NI
Shigella sonnei	4	19	17	NI
E. Coli	2	12	15	NI
Pseudomonas aeruginosa	6	18	20	NI
Staphylococcus aureus	NI	17	16	NI

1. Ethanolic extract (1200 μg/ml),
2. Ciprofloxacin (100μg/ml),
3. Chloramphenicol (50μg /ml),
4. Control, NI- No Inhibition

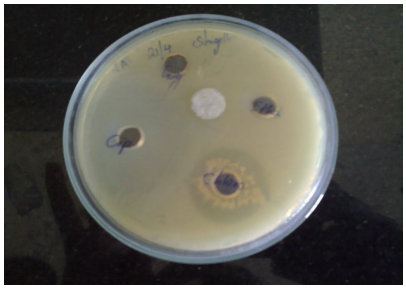

Figure 1: Antimicrobial activity of *Nymphoides macrospermum Vasud* extracts against *Pseudomonas aeruginosa* by agar well diffusion method

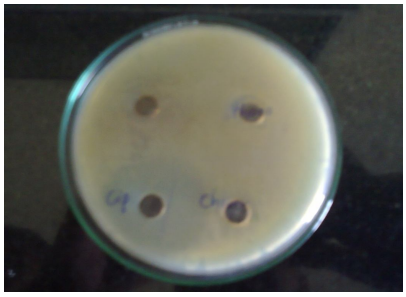

Figure 2: Antimicrobial activity of *Nymphoides macrospermum Vasud* extracts against *Salmonella typhi* by agar well diffusion method

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REVIEW ARTICLE

THALASSEMIA: AN OVERVIEW

Ganesh. S* and Jannet. JV

Department of Bioinformatics, Karunya University, Coimbatore – 641114. Tamil Nadu, India.

Received: 26 July 2010 Revised: 1 Aug 2010 Accepted: 10 Aug 2010

***Address for Correspondence**
S.Ganesh,

Ph D Scholar,

Department of Bioinformatics, Karunya University,

Coimbatore – 641114, TamilNadu

Email ID: ganesh_bioinfo77@yahoo.com

ABSTRACT

Thalassemia is an inherited autosomal recessive blood disease. In thalassemia, the genetic defect results in reduced rate of synthesis of one of the globin chains that make up hemoglobin, which causes the anemia. In this article, the types of Thalassemia and the inheritance pattern are described in such away to make awareness. The signs and symptoms of thalassemia are due to lack of oxygen in the bloodstream. This occurs because the body doesn't make enough healthy red blood cells and hemoglobin. The severity of symptoms depends on the severity of the disorder. Diagnosis of thalassemia is done using blood tests, including a complete blood count (CBC) and special hemoglobin tests. This paper clearly focuses the occurrence, types, symptoms, screening, diagnosis, prevention and management of thalassemia.

Key words: Inherited blood disorders, α thalassemia, β thalassemia, Cooley's anemia, Thalassemia intermedia.

INTRODUCTION

Thalassemia is an inherited blood disorders. Thalassemia causes the body to make fewer healthy red blood cells and less hemoglobin than normal. Normal hemoglobin, also called hemoglobin A, has four protein chains—two α globin and two β globin. The two major types of thalassemia, α and β, are named after defects in these protein chains. The disorders are treated with blood transfusions, medicines, and other procedures [1]. Thalassemia usually results in underproduction of normal globin proteins, often through mutations in regulatory genes. Since thalassemia is not a single disorder but a group of related disorders that affect the human body in similar ways, it is important to understand the differences between the various types of thalassemia. The alterations of hemoglobin patterns in patients with thalassemia led to the discovery of HbH (β4) [2] and Hb Barts (γ4) [3]. Experiments revealed a quantitative or qualitative deficiency of specific messenger RNA in many thalassemia syndromes as well as defects in the translations of the messenger RNA to protein [4]. This latter stage requires ribosomal units that can initiate (promote or enhance), elongate, and terminate the globin chain.

OCCURRENCE

Thalassemia is a lot more common than most people think, especially in parts of South East Asia including Thailand. Up to 40% of Thais will be a carrier of a thalassemia trait or of HbE. Carrier rates are also high in people from some other ethnic groups, for example the Mediterranean, while the carrier rate is much lower in other ethnic groups.

Thalassemia gene is found in only 1 in 1000 people from Northern Europe. Thalassemia may or may not have a family history of the disease. When two carriers of β thalassemia have a child, there is a 1 in 4 chance (25%) their child will have thalassemia, a 1 in 2 chance (50%) that their child will be a carrier like them, and a 1 in 4 chance the child will have normal genes. Thalassemia may be curable by stem cell or bone marrow transplantation, but it is preventable by screening [5]. Screening can help to more accurately assess the risk, because the thalassemia gene is inherited in blood relatives and have a high chance of carrying the gene error. In the case where both parents test positive as thalassemia carriers, there are several options. Couples can have a baby naturally and take the risk of having an affected child, couples can have prenatal testing at around 12-14 weeks of pregnancy and choose to terminate the pregnancy if fetus is affected, or couples can have PGD, Preimplantation Genetic Diagnosis, and transfer an embryo not affected by thalassemia. It is now possible to undergo PGD for Human Leukocyte Antigen (HLA) matching to you for the purpose of having a HLA-matched Hematopoietic Progenitor Cell (HPC) donor for your thalassemia affected child. The goal is to select those embryos that are a match and transfer them. In this process, we can significantly increase the chance to have a child that is HLA-matched to your affected child [6].

CAUSES

Hemoglobin contains two chains, α and β globin. Genetic abnormalities, which cause an imbalance in the production of either chain, may be inherited. β thalassemia is caused by a mutation in the β globin chain. Blood transfusions may modify some of the signs of the disease, but iron overload from the transfusions may cause damage to the heart, liver, and endocrine systems. The mild form of β thalassemia produces small red blood cells, with no symptoms. Risk factors include a family history of thalassemia and an ethnic background that has shown susceptibility to the disease. β thalassemia occur in people of Mediterranean origin, and to a lesser extent, Chinese, other Asians, and blacks. α thalassemia occur most commonly in people from Southeast Asia and China, and are caused by deletion of a gene or genes from the alpha globin chain [6] . The most severe form of alpha thalassemia causes stillbirth (death of a fetus before delivery).

TYPES OF THALASSEMIA

The thalassemias are classified according to which chain of the hemoglobin molecule is affected. In α thalassemia, production of the α-globin chain is affected, while in β thalassemia production of the β globin chain is affected. Thalassemia produces a deficiency of α or β globin, unlike sickle-cell disease which produces a specific mutant form of β globin. β globin chains are encoded by a single gene on chromosome 11α-globin chains are encoded by two closely linked genes on chromosome 16. Thus in a normal person with two copies of each chromosome, there are two loci encoding the β chain, and four loci encoding the α-chain.

α Thalassemia: The α-thalassemia involves the genes HBA1 (Online 'Mendelian Inheritance in Man' (OMIM) 141800) and HBA2 (Online 'Mendelian Inheritance in Man' (OMIM) 141850), inherited in a Mendelian recessive fashion. It is also connected to the deletion of the 16p chromosome. α thalassemia result in decreased α-globin production, therefore fewer α-globin chains are produced, resulting in an excess of β chains in adults and excess γ chains in newborns. The excess β chains form unstable tetramers (called Hemoglobin H or HbH of 4 β chains) which have abnormal oxygen dissociation curves [6]. There are four genetic loci for α globin, two of which are maternal in origin and two of which are paternal in origin. The severity of the α thalassemia is correlated with the number of affected α globin loci (the greater the number of affected loci, the more severe will be the manifestations of the disease). The Inheritance of α Thalassemia is illustrated in Figure 1. The figure shows the α globin genes are located on chromosome 16. A child inherits four α globin genes—two from each parent. In the above figure, the father is missing two α globin genes and the mother is missing one α globin gene. Therefore, each child has a 25 percent chance of inheriting two missing genes and two normal genes (thalassemia trait), three missing genes and one normal gene (hemoglobin H disease), four normal genes (no anemia), or one missing gene and three normal genes (silent carrier). α-Thalassemia mutations affecting 3 α-globin genes cause Hb H disease [7]. Deletion of all 4 α-globin

genes causes Hb Barts hydrops fetalis. Affected fetuses die in uterus during the second or third trimester of pregnancy or shortly after birth, frequently accompanied by maternal complications. The correct diagnosis is often missed [8]. In some populations, there are 2 to 3 times as many fetuses afflicted with the Hb Barts hydrops fetalis than with β-thalassemia major The thalassemia minor patients should not avoid iron-rich foods by default. A serum ferritin test can determine what their iron levels are and guide them to further treatment if necessary. Thalassemia minor, although not life threatening on its own, can affect quality of life due to the effects of a mild to moderate anemia. Studies have shown that thalassemia minor often coexists with other diseases such as asthma and mood disorders [9].

β Thalassemia: β thalassemia are due to mutations in the HBB gene on chromosome 11 (Online 'Mendelian Inheritance in Man' (OMIM) 141900), also inherited in an autosomal-recessive fashion. The severity of the disease depends on the nature of the mutation. Mutations are characterized as (β^0) if they prevent any formation of β chains, they are characterized as (β^+) if they allow some β chain formation to occur [7]. In either case there is a relative excess of α chains, but they do not form tetramers rather, they bind to the red blood cell membranes, producing membrane damage, and at high concentrations they form toxic aggregates. The Inheritance Pattern for β Thalassemia is illustrated in figure 2. In the above figure, the beta globin gene is located on chromosome 11. A child inherits two beta globin genes—one from each parent. In this example, each parent has one altered beta globin gene. Therefore, each child has a 25 percent chance of inheriting two normal genes (no anemia), a 50 percent chance of inheriting one altered gene and one normal gene (β thalassemia trait), or a 25 percent chance of inheriting two altered genes (β thalassemia major).

SYMPTOMS

Signs and symptoms of thalassemia are due to lack of oxygen in the bloodstream. This occurs because the body doesn't make enough healthy red blood cells and hemoglobin. The severity of symptoms depends on the severity of the disorder. Mild thalassemia usually does not cause any symptoms. People who have more severe forms of the condition may develop symptoms of anemia, which may include:

- Weakness.
- Fatigue.
- Lightheadedness.
- Skin that looks paler than normal.
- Jaundice (skin and whites of the eyes appear yellow).
- Dark urine.
- Decreased appetite and weight loss (poor growth in a child).
- A rapid heartbeat.
- Shortness of breath during exercise.

Less common symptoms of severe thalassemia include:

- Headache.
- Belly pain.
- Ringing in the ears.
- Chest pain.
- A slight fever.

- A sore, smooth tongue.

Children with a more severe form of thalassemia (β thalassemia major, or Cooley's anemia) usually develop symptoms of anemia within the first few months of life. Paler skin is often the first sign of the disease. Infants may grow slowly (failure to thrive) [10]. Other symptoms may include feeding problems, frequent fevers, and diarrhea. Without early treatment, a child may die or develop severe problems, such as:

- A deformed face caused by the bone marrow expanding in the bones. This may cause a bulging forehead (frontal bossing).
- An enlarged liver and spleen.
- Brittle, weak bones (most often the long bones in legs and arms and the bones of the spine).

STANDARD TREATMENTS

Blood Transfusions: Transfusions of red blood cells are the main treatment for people who have moderate or severe thalassemia. A blood transfusion, given through a needle in a vein, gives you healthy red blood cells with normal hemoglobin. Red blood cells live for only about 120 days repeated transfusion is needed to maintain a supply of healthy red blood cells [11]. Hemoglobin H disease or beta thalassemia intermedia, need blood transfusions on occasion. Beta thalassemias major, or Cooley's anemia, need regular blood transfusions (often every 2 to 4 weeks). These will help to maintain normal hemoglobin levels and red blood cell numbers [10]. Blood transfusions are lifesaving, but they're expensive and carry a risk of transmitting infections and viruses (for example, hepatitis). However, this risk is very low in the United States because of careful blood screening.

Iron Chelation Therapy: Because the hemoglobin in red blood cells is an iron-rich protein, regular blood transfusions can lead to a buildup of iron in the blood. This condition is called iron overload. It damages the liver, heart, and other parts of the body. To prevent this damage, iron chelation therapy is needed to remove excess iron from the body. Two medicines are used for iron chelation therapy.

- Deferoxamine is a liquid medicine that's given slowly under the skin, usually with a small portable pump used overnight. This therapy takes time and can be mildly painful. Side effects include loss of vision and hearing [12].
- Deferasirox is a pill taken once a day. Side effects include headache, nausea (feeling sick to the stomach), vomiting, diarrhea, joint pain, and fatigue (tiredness).

Folic Acid Supplements: Folic acid is a B vitamin that helps to build healthy red blood cells. Folic acid supplements are needed in addition to blood transfusions and/or iron chelation therapy.

Blood and Marrow Stem Cell Transplant: A blood and marrow stem cell transplant replaces abnormal or faulty stem cells with healthy ones from another person (a donor). Stem cells are the cells inside bone marrow that make red blood cells and other types of blood cells [13]. A stem cell transplant is the only treatment that can cure thalassemia.

PREVENTION

Thalassemia cannot be prevented because they are inherited (passed on from parents to children). However, these bleeding disorders can be found before birth through prenatal tests. Family genetic studies may help to find out whether people have missing or altered hemoglobin genes that cause thalassemia [14].

DISCUSSION

The genetic defect in thalassemia results in reduced rate of synthesis of one of the globin chains that make up hemoglobin, which causes the anemia. It often occurs through mutations in regulatory genes. The thalassemia minor patients should not avoid iron-rich foods by default. A serum ferritin test can determine what their iron levels are and guide them to further treatment if necessary. Thalassemia minor, although not life threatening on its own, can affect quality of life due to the effects of a mild to moderate anemia. Studies have shown that thalassemia minor often coexists with other diseases such as asthma and mood disorders. Thalassemias are due to lack of oxygen in the bloodstream, because the hemoglobin in red blood cells is an iron-rich protein, regular blood transfusions can lead to a buildup of iron in the blood. A stem cell transplant is the only treatment that can cure thalassemia. Research is looking at a number of ways to treat different types of thalassemia. *In utero blood transfusion* - Doctors give a baby a blood transfusion before birth to treat alpha thalassemia major. *Gene therapy* - Researchers are trying to figure out how to correct or modify flawed genes to decrease or cure different types of thalassemia. *Hemoglobin F therapy* - researchers have tried increasing the level of this type of hemoglobin but it has not had much effect on thalassemia. *Protein therapy* - A protein (AHSP) has been discovered that helps regulate the amount of alpha protein in red blood cells. Researchers are now looking at using AHSP as a thalassemia treatment.

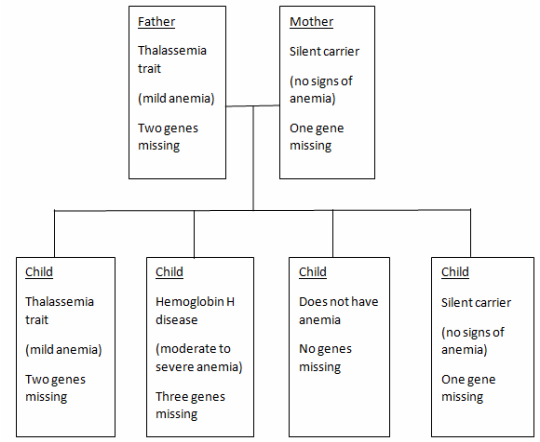

Figure.1: Inheritance of α Thalassemia (National heart heart lung and blood institute diseases and conditions Home: Blood Diseases).

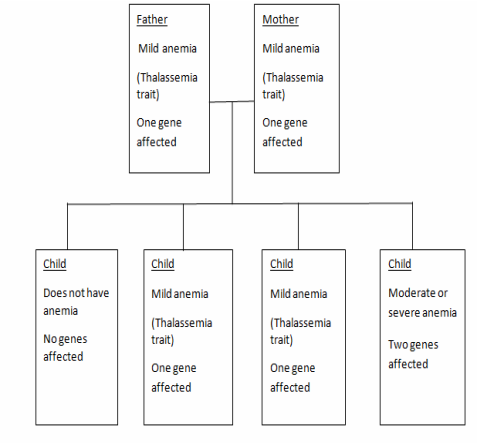

Figure.2: Inheritance of β Thalassemia (National lung and blood institute diseases and index, DCI conditions index, DCI Home: Blood Diseases)

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RESEARCH ARTICLE

The Air Pollution Related Human Health Problems in Tiruchirappalli City

Sirajuddin. M. Horaginamani[1*], Ravichandran. M*, Adamsab.M.Patel** and Hina Kousar**

*Department of Environmental Management, School of Environmental Sciences, Bharathidasan University, Tiruchirappalli-620 024, Tamil Nadu, India.

**Department of Environmental Science, Kuvempu University, Shankaraghatta-577451, Shivamogga District, Karnataka, India.

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[1*]**Address For correspondence**

Mr. Sirajuddin. M. Horaginamani

Department of Environmental Management,

School of Environmental Sciences,

Bharathidasan University,

Tiruchirappalli-620 024, Tamil Nadu, India.

E mail ID: ecosirajuddin@yahoo.com

ABSTRACT

The environment is an integral part of human life, the quality of which plays a critical role in human health. Every component of the biosphere has its own role in different types of epidemics. Though water and land pollution is very dangerous, air pollution has its own peculiarities, due to its transboundary dispersion of pollutants over the entire world. Therefore the study on air pollution and related impacts on human health have a special consideration today. Western countries have conducted several studies in this area, but there are only a few studies in developing countries like India. In this backdrop, a study on air pollution and related problems in Tiruchirappalli city has been undertaken. Ambient air quality was monitored along with micrometeorological data and the results are discussed. The status of air pollution in the area has been evaluated and a questionnaire survey was conducted to estimate the allergic symptoms and exposure to assess the respiratory disorders. The data are analyzed to evaluate the critical situation arising out of the emission of air pollutants and the impact on human health due to respiratory diseases. This may be because of the accumulation of air pollutants, which needs an exclusive study to find out the root cause of the problem. Thus, it is necessary to evaluate the status of urban air pollution and to assess its impact on human health so that proper mitigative measures can be implemented. To evaluate such impacts a fact-finding survey is essential. In this study, attempts have been made to evaluate the status of urban air pollution in Tiruchirappalli city and assessment of its impacts on human health.

Keywords: Air pollution, human health, respiratory diseases, automobiles, asthma

INTRODUCTION

Human health is very closely linked to environmental quality, as the Etiology of most of the human diseases being related to the status of the living environment of man. According to statistics, 25% of all preventable illnesses are caused by detrimental environmental factors [UNEP, United Nations Children's Fund, WHO 2002]. In Africa, the environmental influence on disease incidence is even higher, being about 35%**.** Both the developed and developing countries are faced with the problems related to environmental pollution, sourced in air, water or land, and caused by anthropogenic activities of man, disturbing the habitat around. Smoky indoor air, polluted ambient air, poor sanitation and contaminated water play a crucial role in causing ill health. Air pollution has become a growing problem in cities throughout the globe, and transportation is recognized as the major source of air pollution in many cities. In developing countries the air quality crisis in cities often attributes in large measures (40–80%) to vehicular emission. Because of the source emissions of CO, O3, toxicants and particulates [1] the public health implications [2] [3] are substantial. An improved understanding of the association of the particulates with morbidity suggests the importance of sub-micron particles (PM10) to which motor vehicles are major contributors [4]. Existing cities are expanding, new cities are being created, and adjacent cities are merging. Transportation systems are increasing everywhere. The improved performance of technology is presently insufficient to counteract the growth of vehicles [5]. In this research paper attempts have been made to evaluate the status of urban air pollution in Tiruchirappalli city and its possible impacts on human health.

Study Area

Tiruchirappalli (10.5°N, 78.43°E, 78.8 Mean Sea Level) is a city in the Indian state of Tamil Nadu and the administrative headquarters of Tiruchirappalli District and it is considered as the second capital of Tamil Nadu. It is the fourth largest Municipal Corporation in Tamil Nadu and also the fourth largest urban agglomeration in the state. Tiruchirappalli is the one of the best sanitized city in India. Tiruchirappalli City Municipal Corporation has been ranked sixth in the National rating of cities under the National Urban Sanitation Policy. Situated at a distance of 319 kilometers south of Chennai and 402 kilometers north of Kanyakumari on the national highway NH 45, it is located almost at the geographic centre of the state. Hence it is referred to as the Heart of Tamil Nadu. It is situated on the banks of holy river Cauvery and spread over an area of 146.90 sq. Km with total population of above 7, 50,000, is very rapidly growing in terms of its population and number of vehicles. The 4 major highways NH 45, NH 67, NH 210 and NH 277 pass through the city. The heavy traffic on these highways has been significantly contributed to air pollution in the city.

MATERIALS AND METHODS

To know the ambient air quality monitoring is carried out for major air pollutants viz Suspended Particulate Matter (SPM), Sulphur dioxide (SO_2) and Oxides of Nitrogen (NOX). High volume sampler is used for sampling.SO_2 and NOX were absorbed in Sodium tetrachloromercurate and Sodium hydroxide. This solution was analyzed by West and Gaeke method and Griess – Saltzman method respectively. SPM was collected on pre weighed glass fiber filter (What man). Filter paper was again weighed after sampling and the difference in weight were used to calculate concentrations of SPM in respective areas and expressed as $\mu g/m3$ of air. The monitoring was done for 24 hours. This research work was carried out six months that is from October 2009 to March 2010. Eight sampling stations were selected to represent 8 different traffic volumes and activities. They are Central bus stand, Chattram bus stand, Puthur, Palakarai, Srirangam, Main guard gate, TVS toll gate and Old Paalpanne Circle. At each of these places monitoring was done 3 different days to get the average concentration of pollutants. A questionnaire was prepared and survey was conducted particularly in case of suspected allergic population by inquiring the recurrence of the type of allergic symptoms. The occasions of this onset was recorded with each individual to assess the allergic status.

RESULT AND DISCUSSION

Average concentrations of SPM, SO_2 and NOX at each sampling station were showed in Table-1. The highest concentration of SPM, SO_2 is recorded at Palakarai, while the highest NOX concentrations are recorded at Chattram

bus stand. SPM concentrations ranged from 426.88 to 1301.92μg/m3. SO_2 concentrations ranged from 10.07 to 31.00μg/ m3. NOX concentrations ranged from 149.81 to 182.00μg/ m3. Both SPM and NOX concentrations exceeded ambient air quality standards of Central pollution control board (CPCB) at Central bus stand. High traffic volume in this region is major reason for these high values. But SO_2 is well within the standards of CPCB. At Chattram bus stand, both SPM and NOX concentrations are exceeded the standards of CPCB. High vehicular density in this sampling station is the major reason for these high values of both SPM and NOX. Whereas concentrations of SO_2 within the prescribed limits. While at Puthur, the concentrations of all three major pollutants viz SPM, SO_2 and NOX are well within the prescribed ambient air quality standards of CPCB. The main reason for such values might be the wide roads and fast movements of vehicles in this area. At Palakarai, SPM and NOX concentration exceeded the standards, whereas SO_2 were within the limits prescribed by CPCB. The high levels of SPM were due to slow movement of large number of vehicles. As vehicles move slowly, they emit more smoke. The existing poor road conditions might have been increased the emissions from automobiles. Concentrations of SPM and SO_2 are will within the prescribed limits of CPCB at Srirangam. But NOX values are exceeded the standard at Main guard gate. The data generated from the survey were analyzed to assess the percentage of allergic population and the suspected allergy causing agents. The results are shown in Table-2. The assessment of respiratory disorders (RDs) was obtained from the questionnaire survey from the doctors. On the basis of the survey of the SPM-related RDs each disease was recorded for indexing the imprint class I to IV. The highest imprint score depicts the maximum severity of RDs. Exposure to SPM is also an equally serious risk to health. Inhaleable SPM, particularly less than 10 μ in size, can pass through the natural protective mechanism of human respiratory system. The smallest particulate (2 μ or less), which are coming primarily from diesel pose a much greater risk because of their greater ability to pass through the human respiratory system and cling to inner tissue of the lung. It has been reported that more than 10,000 premature deaths occurred in Kolkata in 1995 due to SPM [6]. SPM includes all air-borne particles in the size range of 0.5 μ to 100 μ. The actual health damage caused by dust particles depends upon its nature and composition [7]. The effects attributed to mild eye irritation mortality [8]. According to the Health Care Institute of India, there is an alarming rise in number of patients in Delhi hospitals with respiratory problems [9]. Automobile exhausts and certain industrial pollutants contain NOX, which by photochemical reaction produces O_3 and effects allergic asthmatics by augmenting allergic responses [10]. Similarly SO_2, NO, particulate matter and acid aerosols effect pulmonary function and cause inflammation of bronchial mucous [11] [12]. It has been observed from several studies that air pollution plays an important role in the genesis and augmentation of allergic disorder and it is described as a disease of civilized society [13] [14].

CONCLUSION

From the results obtained it is clear that Suspended Particulate Matter is the main pollutant within the Tiruchirappalli city. Except Puthur in all four sampling stations, the concentration of SPM exceeded the ambient air quality standard by Central Pollution Control Board. The reason is being the growing number of automobiles and poorly and congested road with heavy traffic. Assessment of the impact of air pollution on human health arising out of RDs in the area showed considerable level of imprint score. This problem can be overcome by adapting advance ecofriendly transport systems and widening of roads. Personal protective devices are also helpful to tackle the air pollution related health problems. A strategic air quality management plan is need of the hour and the adequate mitigation measures should be taken to control the urban air pollution and its related health problems in Tiruchirappalli city.

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Table 1: Average concentrations of SPM, SO_2 and NOX (μg/m3) at different sampling stations from September to November 2008

S. No	Sampling Station	SPM	SO_2	NOX
1	Central Bus Stand	1131.35	21.80	168.67
2	Chattram Bus Stand	1030.43	19.00	182.00
3	Puthur	426.88	12.00	167.93
4	Palakarai	1301.92	31.00	171.21
5	Srirangam	834.65	10.07	149.81

Table 2: Estimation of allergic symptoms

Complaint	Total no. cases	Condition	No. of person	Percent of incidence
Neck block	20	Allergic	19	95
		Non- Allergic	1	5
Sneezing	35	Allergic	28	80
		Non- Allergic	7	20
Cough	60	Allergic	33	55
		Non- Allergic	23	45
Hyperacidity	30	Allergic	21	70
		Non- Allergic	09	30

Table 3: Imprint classification of respiratory diseases

Imprint class	Imprint score	Symptoms
I	0.0	No RD: healthy, free from any respiratory disease
II	2.5	Mild RD: suffering from only upper track respiratory infections (UTRI)
III	5.0	Moderate RD: suffering from UTRI as well as lowest track respiratory infections
IV	10.0	Severe RD: Suffering from bronchitis, asthma, allergic thintis, fibrosis, asbestosis, pneumoconiosis and non-malignant RDs

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REVIEW ARTICLE

Understanding the Role of Biological Clock in Plants – A Review

Ajay. JY[1*], Pradeep Kumar Gajula*, Ramya Devi.D and Vedha Hari. BN

Department of Pharmaceutical Technology, SCBT, SASTRA University, Tanjavur-613401 Tamil Nadu, India.

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[1*]**Address for correspondence**

Ajay. JY

Second year, M.Tech – Pharmaceutical Technology, SCBT,

SASTRA University, Thanjavur-613401, Tamil Nadu, India.

E-mail ID : ajay_mtech87@yahoo.com

ABSTRACT

The environmental cycles like day and night, tides, moon phases and seasons, are very common. An organism has to have evolved biochemical, physiological and behavioral adaptations to all disparate environments, as well as switches that turn these adaptations on or off at appropriate times, often very quickly. If the organism or living cell is removed from the cyclical environment, the switches keep going on and off, and the physiological state of the organism keep oscillating on its own and becoming a timer for a biological clock. The mechanism of such oscillations, as well as changes in various functions of those organisms put their clocks to study by "Chronobiology". The chronobiological characteristics of life which express itself at all levels of organization from unicellular system to man is called "rhythmicity". The Rhythmic phenomena is the physiology, development and behavior of all living systems show period lengths ranging from fractions of a second to hourly, daily and even annual cycles. Here in this article concentrating on the chronobiological characteristics of the plants. The study of biological rhythms in plants has a hoary scientific tradition and first writing was started in fourth century BC itself. Even the Indian Scientist JC Bose wrote significant papers on his findings on diurnal movements of leaves of plants and discovered the entrainment of movement of light, darkness cycles and observed free running periods in continuous light and continuous darkness in early 1919 itself. This chronobiological study of plants is very concern with the growth factor of plants, reproductive capacity of plants, and the response to the environmental changes by the plants in accordance with the time factor.

Key Words: Chronobiology, Circadian, Biological Clock and Rhythm

INTRODUCTION

During ancient times dream of mankind was to prolong life which may be realized through chronobiology. He used to change his lifestyle as time progress. He also regulated the light exposure to the body. He used plant parts as drugs and cured the aliments. Scientifically chronobiology can be defined as a field of science that examines periodic (cyclic) phenomena in living organisms and their adaptation to solar and lunar related rhythms. Thus it is the scientific study of the effect of time on living systems and of biological rhythms. A rabbit in a meadow experiences a very different environment during the day, during the night, and during the moonlit night. The same meadow is very different in winter from what it was last spring, summer or fall. An organism has to have evolved biochemical,

physiological and behavioral adaptations to all disparate environments, as well as switches that turn these adaptations on or off at appropriate times, often very quickly. Because these switches have to act so fast, many of them have evolved to act independently of environmental triggers. The environmental cycles like day and night, tides, moon phases and seasons, are very predictable, thus a switch can get started in advance of the environmental changes, thus rendering the organism "ready" for the new environment just in time for its appearance. Even if the organism is removed from the cyclical environment, the switches keep going on and off, and the physiological state of the organism keep oscillating on its own, becoming a timer: a biological clock. The mechanism of such oscillations as well as various uses that organisms put their clocks to study by chronobiology [1]. Rhythmicity is one of the characteristics of life which express itself at all levels of organization from unicellular system to man. Rhythmic phenonmena is the physiology, development and behavior of all living systems show period lengths ranging from fractions of a second to hourly, daily and even annual cycles [2][3][4].

HISTORY

The first writings, at least in the western canon, to recognize diurnal rhythms come from the fourth century BC [6]. The study of biological rhythms has a hoary scientific tradition. It drew into its fold many brilliant scientists in the 18th, 19th and 20th centuries [7]. The first recorded circadian rhythm was for the sleep movements of the leaves of the tamarind tree by the Greek philosopher Androsthenes. *Tamarindus indica L* that were observed on the island of Tylos (now Bahrein) in the Persian Gulf during the marches of Alexander the Great. It took more than two millennia for this to be experimentally tested. The scientific literature on circadian rhythms began in 1729 by when the French astronomer de Mairan, a blemish less experiment in the modern scientific mode and established that the 'sleep' movement of the 'Touch-me-Not' plant (*Mimosa pudica*) were endogenous. He also removes the plants into the perpetual darkness of deep caves and demonstrated that these rhythms were related to the sleep rhythms of bedridden humans [7]. It took 30 years before de Mairan's observations were independently repeated. Till 1903 these studies excludes temperature variation as a possible zeitgeber driving the leaf movement rhythms [6]. JC Bose wrote significant papers on his findings on diurnal movements of leaves of plants. He discovered the entrainment of movement of light, darkness cycles and observed free running periods in continuous light and continuous darkness as early as 1919. This work was carried by Bunning (1958) in his first monograph on the subject of biological rhythms [8]. Semon (1905, 1908) argued in favour of the genetic inheritance of circadian rhythms [9, 10]. The Dutch botanist working with the leaf movements of the large jack been *Canavalia ensiformis* reported entrainment light. In 1930 Bunning and Stern stressed that the periods of rhythms under constant conditions deviated from 24 hour thus justifying the use of the term circadian [7].

GENERAL CIRCADIAN CLOCK IN PLANTS

Plant clocks control a plethora of biological processes [11]. (a)The expression of several genes shows circadian rhythms. Two examples are the genes encoding the light-harvesting chlorophyll-a/b-binding proteins (Lhcb or CAB) and nitrate reductase (NIA2). Many of these genes are associated with photosynthesis and its related biochemical and physiological activities. It is possible that the timing of expression of such genes (for instance, the predawn rise in Lhcb), indicates a role for the clock in the coordination of metabolism to maximize photosynthetic yields. The use of fluorescent differential display[12] and high density DNA arrays (depicted in the figure) to monitor global expression profiles should give us an indication of the range of genes showing circadian control. (b) Cytosolic concentrations of free calcium have been shown to oscillate with a circadian rhythm in Arabidopsis. Considering the importance of calcium as both a secondary messenger and a cofactor for many enzymes, this might be a means by which the clock regulates a variety of cellular processes. (c) The clock regulates the phosphorylation of some proteins. The best-studied example is in Kalanchoe fedtschenkoi, which exhibits circadian activity of a kinase that phosphorylates phosphoenol pyruvate carboxylase[13]. At a higher level of organization, chloroplast movement [14] (d), stomatal opening (e), hypocotyl elongation [15] (f) and cotyledon and leaf movements [16] (g) in Arabidopsis all show circadian rhythms. In Kalanchoe,

petal opening shows a circadian rhythm (h). The clock is also vital for synchronizing developmental processes such as flowering time (i). Indeed, mutations in all the putative clock-associated genes cause altered photoperiodic control of flowering. The clock's role in the control of flowering has been extensively reviewed [17], [5]. This simple model includes an input pathway (from light and/or temperature) to a circadian oscillator. The oscillator generates signals that are transduced via output pathways to produce overt circadian rhythms (output). The output is depicted as two idealized rhythms (red and green lines) with different phases. Yellow and grey boxes represent light and dark (diurnal) intervals, respectively. Under diurnal conditions, the period of the oscillator (the time between comparable points in the repeating cycle) matches the period of the entraining cycles. Under constant conditions, the clock oscillates with an endogenous period close to 24 hour. Amplitude is half the distance between the peak and trough [5]

TYPES OF RHYTHMS

The biological rhythms are related to the sunlight [1]. The earth rotates on its axis every 24 hour with the result that any position on the earth's surface alternatively faces towards or away from the sun- day and night. That the metabolism, physiology and behavior of most organisms changes profoundly between day and night is obvious to even the most casual observer. These biological oscillations are apparent as diurnal rhythms. It is less obvious that the most organisms have the innate ability to measure the time. Indeed most organisms do not simply respond to surprise but, rather anticipate the dawn and adjust their biology accordingly. When deprived of endogenous time cues, many of these diurnal rhythms persist, indicating their generation by an endogenous biological clock [6]. Thus based on the biological clock, rhythms are broadly classified into three types. They are as follows.

Ultradian: The ultradian rhythms are rhythms that have a period shorter than 24th hours [1].

Circadian: A circadian rhythm is an approximate daily periodicity, a roughly-24-hour cycle in the biochemical, physiological or behavioral processes of living beings, including plants, animals, fungi and cyanobacteria. The term "circadian", coined by Franz Halderg, comes from the latin circa, "around ", and diem or dies, "day", meaning literally "approximately one day". These rhythms allow organisms to anticipate and prepare for precise and regular environmental changes. There are clear patterns of hormonal production, cell regeneration and other biological activities linked to this daily cycle. Circadian rhythm is regulated by endogenous pacemakers, whose activity is modulated by environmental cues, primarily the daily light – dark cycle. All Living organisms, act as 'Biological clock'. Plants may produce leaves or flowers only at certain seasons and flower may open and close at particular times of day. Plants fit to their different ecological niches not only by occupying different parts of habitat, but also by occupying it at different times, dividing up the resources they compete for temporally as well as spacially. To do so, they all have evolved in the form of 'clocks' and hence, they timetable themselves and their activities round the year, across the day, or through the rise and fall of the tides. Ayurvedic experts knew since long time, that many components in flowers and leaves are present only at the certain times of the day; if collected at wrong time, they cannot produce effective medicine. For example, rose petals plucked before sunrise will have more scent than those plucked at 2 p.m. Sunshine and winds are responsible for setting in seasons [1].

Infradian: Seasonal changes are quite spectacular and evident especially at higher latitudes. They are the result of the 23° tilt of the rotating planet earth on its orbit around the sun. Climate, the environment and organisms are profoundly affected by it [18]. In their natural environment, plants develop under daily cycles of light–dark and high–low temperatures. The change of seasons is associated with characteristic fluctuations in day length and in the phase relationship between the photo- and thermo period. In spring and winter, light and temperature change in parallel in a so-called radiation climate. In summer and early fall, the coldest point in the day is around sunrise. At sunset, however, the light "goes off" while the temperature slowly decreases until sunrise. Changes in temperature and light intensity are therefore not synchronous at sunset. Plants have adapted their development to such environmental conditions by the evolution of photo- and thermo periodic responses, e.g. thermo periodic control of phototropic responsiveness [19]. The basis of such responses is endogenous changes in sensitivity to

environmental light- and temperature signals [2, 20]. The temperature and day length both correlate with the seasons but the latter is a more reliable indicator for us to establish the time of year. Based on a method to distinguish between days getting longer (spring) or shorter (fall), day length would be a precise calendar, provided the timing device is independent of the environmental temperature. An alternative calendar for an organism would be an internal annual clock. This is indeed realized in quite a number of organisms, also in plants (e.g. seed germination [21]; water uptake [22]; stomatal movement of bean seeds [23]. However, annual clocks must be synchronized, usually by the photoperiod. Without synchronization, after a few years an annual clock would no longer match the physical year because its period is not exactly 12 months[18].

FACTORS INFLUENCING THE GROWTH OF PLANTS

Plants are very sensitive to changes in their environment responding to such changes with altered growth and development. Most plants have daily rhythm set by environmental conditions such as light. The many external factors exert great influence on the plant growth and development of plants. The most two important factors are (i) Intensity and duration of the light (ii) The temperature of the air and soil around the plant

Intensity and Duration of the Light

The light influences the growth of individual organs or of the entire plant in less direct ways, expecting the effect through photosynthesis. The most striking effect can be seen between a plant grown in normal light and the same kind of plant grown in total darkness. The plant grown in the dark will have a tall and spindling stem and leaves fail to expand, and both leaves and stem, lacking chlorophyll, are pale yellow. Such a plant is said to be *etiolated* [18]. Plants grown in shade instead of darkness show a different response [18]. Moderate shading tends to reduce transpiration more than it does photosynthesis. Hence, shaded plants may be taller and have larger leaves because the water supply within the growing tissues is better. With heavier shading, photosynthesis is reduced to an even greater degree and it results in small and weak plants. The intensity and duration (length) of light both shows different and characteristic effects upon plant growth and development. The length of the daylight period may have a striking effect upon vegetative growth and reproductive activities of plants. The reaction of plants in relation to the length of the day is called *photoperiodism*[18]. Examples of photoperiodic reactions in plants the first is for a unicellular alga. The second example is the succulence of leaves in Crassulaceae. The third is tuber formation in potatoes. Finally, flower induction in short- and long-day plants is described. In the examples chosen, the level of complexity increases from a photoperiodic event in a single cell, to that in an organ such as the leaf, in underground stolons with remote perception of photoperiodic light in the leaves, and in the apex of stems, again with light perception in the leaves[18]. According to photoperiodic effect the plants of temperate region are divided into three categories has upon the vegetative growth and reproduction of the plants. Short – Day plants (SDP) These plants grow vegetatively during the long days of summer and do not produce flowers until the days become shorter in late summer and early fall. Examples for short-day plants are poinsettias, most aster and goldenrods, the rag weeds, chrysanthemums, sorghum and many others. Many short – day plants are extremely sensitive to light exposure during the night. In some types, even vehicle headlights or the light from a flashlight can prevent flowering. *Pharbitis nil* (Chois) cv *violet* and *Chenopodium rubrum* (L.) are examples for short-day plants [18]. Long – Day plants (LDP) These plants will give flowering only under extended periods of illumination and produce only vegetative growth when the photoperiod is long. Examples for long – day plants are hollyhock, radish, garden beet, spinach, iris and clover. *Lolium perenne* for a long-day plant [18]. Day – Neutral plants (DNP): These plants are not particularly sensitive to the length of day. Included in this group are the bean, tomato, vetch, cyclamen, nasturtium, roses, snapdragon, carnation, and many common weeds [24].

Light Quality: Light quality refers to the color or wavelength reaching the plant's surface. A prism (or raindrops) can divide sunlight into respective colors of red, orange, yellow, green, blue, indigo and violet. Red and blue have the greatest impact on plant growth [25]. Green light is least effective (the reflection of green light gives the green color to plants). Blue light is primarily responsible for vegetative leaf growth. Red light, when combined with blue light, encourages flowering. Light quality is a major consideration for indoor growing. For flowering plants that need more red light, use broad spectrum fluorescent bulbs. Incandescent lights are high in red and red-orange, but generally produce too much heat for use in supplementing plant growth [25].

Light Intensity: The more sunlight a plant receives, to a degree, the higher the photosynthetic rate will be. However, leaves of plants growing in low light readily sun scorch when moved to a bright location. Over time, as the wax content on a leaf increases, it will become more sun tolerant. In hot climates, temperature is often a limiting factor related to shade [25]. Landscape plants vary in their adaptation to light intensity. Many gardening texts divide plants into sun, partial sun and shade. However the experienced gardener understands the differences between these seven degrees of sun/shade:

Full sun: Direct sun for at least 8 hours a day, including from 9 a.m. to 4 p.m.

Full sun with reflected heat: Where plants receive reflected heat from a building or other structure, temperatures can be extremely hot. This situation significantly limits the choice of plants for the site.

Morning shade with afternoon sun: This southwest and west reflected heat can be extremely hot and limiting to plant growth [25]

Morning sun with afternoon shade: This is an ideal site for many plants. The afternoon shade protects plants from extreme heat.

Filtered shade: Dappled shade filtered through trees can be bright shade to dark shade depending on the tree's canopy. The constantly moving shade pattern protects under-story plants from heat. In darker dappled shade, only the more shade tolerant plants will thrive.

Open shade: Plants may be in the situation where they have open sky above, but direct sunlight is blocked during the day by buildings, fences and other structures. Here only more shade tolerant plants will thrive.

Closed shade: The situation where plants are under a canopy blocking sunlight is most limiting. Only the most shade tolerant plants will survive this situation, like under a deck or covered patio [25].

Light Duration: Light duration refers to the amount of time that a plant is exposed to sunlight. Travelers to Alaska often marvel at the giant vegetables and flowers that grow under the long days of the arctic sun even with cool temperatures [25].

Temperature

In general, growth is promoted when temperature rises and inhibited if the temperature falls. (e.g. Seedlings) However, the growth rate does not continue to increase indefinitely with temperature rise. High – temperature injury due to *desiccation* (drying) and a runaway metabolic rate eventually occurs. Temperature affects growth though its effect on metabolic activities. Also, high temperatures increase transpiration and thus reduce turgor and growth,

especially during the day. Each species has a minimum temperature, below which it fails to grow; an optimum at which the growth rate is highest; and a maximum, above which, growth comes to an end. The optimum temperature may vary with each stage of development and with the length of time the temperature prevails. Temperature affects not only the rate but also the type of growth. The photoperiod seemed the most important. It is now known that the light response for many plants is modified by temperature. The suitable photoperiod alone may be insufficient to bring about flowering unless it is accompanied by suitable temperatures. Temperature also plays a major role in the cycle of activity and inactivity known as *dormancy* in plants of temperate climates. Dormancy is especially prominent in woody plants; the leaves drop in autumn, the tree is inactive during the winter and with the coming of spring, activity and growth are renewed. The length of the dormant period varies and for many species a period of low temperatures is required to break the dormancy and permit growth to resume. Most deciduous fruit trees, such as the apple, peach and cherry, require extended winter rest periods and therefore can be grown only in temperate climates. While some plants require freezing temperatures to break the rest period, others need only low temperatures above freezing. Most bulbs, tubers and other underground stems require at least a short rest period [24].

CONCLUSION

The diversity of circadian responses in plants demonstrates the potential importance of perceiving many wavelengths in order to run the daily timekeepers. It will be interesting to discover whether additional circadian responses and are also involved in circadian entrainment. There are certainly some good candidates for circadian clock components and their functions in circadian timing, light-signalling and in the control of flowering may soon be clarified with the isolation and characterisation of several other circadian clocks in plants. Newly identified rhythmic processes suggest the importance of time management in plant development; it remains to be determined whether a plant cell's circadian schedule includes resetting the timekeepers of neighbouring cells.

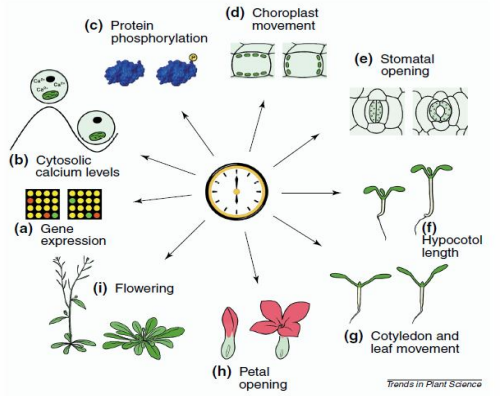


Figure: 1 Plant clocks control a plethora of biological processes [5]

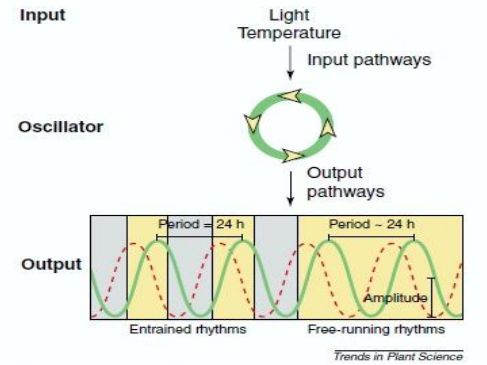
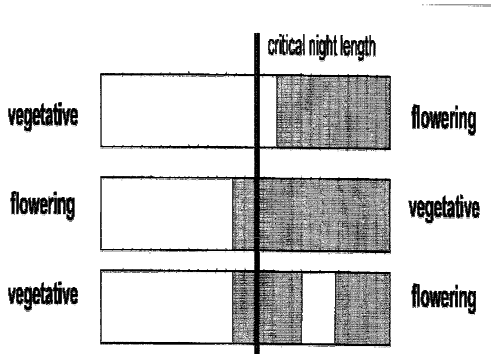
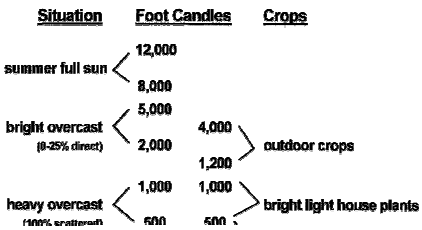


Figure: 2 Components of a circadian system [7]. Schematic Representation of input, oscillator and output rhythms

Short Day Plants Long Day Plants





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SHORT COMMUNICATION

Euphorbia heterophylla L and cancer – a Glance

Parimala Devi. B*, Surendra kumar. M

Department of Phytomedicine and Phytopharmacy, SASTRA University, Thanjavur.

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***Address for correspondence**
Parimala Devi. B
Professor and Head
Department of Pharmacy, SCBT,
SASTRA University, Thanjavur.
E-mail ID: parimaladevi@ scbt.sastra.edu

ABSTRACT

Cancer is projected to become the leading cause of death worldwide in the year 2010, according to a new edition of the *World Cancer Report* from the International Agency for Research on Cancer. Today there are at least 120 distinct chemical substances derived from plants that are considered as important drugs currently in use in one or more countries in the world. The contributing factors that can cause to the development of cancer is the exposure of oneself to one or more of these situations like environment, lifestyle, physical and emotional stresses. Cancer is the dreadful disease among Indians. Medicinal plants can strengthen the immune system, restrain changes, reduce inflammation and lessen the side effects of conventional treatment through chemotherapy or radiation therapy. Developing countries like India heavily depend on the utilization of medicinal plants because of the population and for the people in the line of poverty drastically in need of some therapy which is to be lesser in price or with ease of availability with safety. Based on the natural resources available in India, many of the medicinal herbs being used effectively for their therapeutic potentials by the tribal and most of the rural and some of urban societies too. Recently the diseases like cancer and Diabetes found to be much higher ratio among Indian population , based on known and unknown factors too. Among the medicinal plants used in the management of cancer, the plant *Euphorbia heterophylla* L is also one reputed plant used in the cancer studies. This review highlight the details about the plant and research works carried out already and future prospects.

Euphorbia heterophylla L
Common name: Mexican Fire plant, Wild poinsettia.

Euphorbia heterophylla L, vernacular name is Ya- yang or Bai tong yogg [1]. Traditionally it was used as scabicide. This plant has spread to South and Southeast Asia, having become a weed in India and Thailand, where it has invaded cotton fields and other agricultural terrain. Introduced for ornamental purposes, it quickly spreads, becoming a common sight by the side of the roads and rural pathways. It belongs to the family *Euphorbiaceae.* Euphorbia plants are widespread in nature ranging from herbs and shrubs to trees in tropical and temperate regions all over the world. *Euphorbia heterophylla* L leaf is used in traditional medical practices as laxative, anti gonorrheal, migraine and wart cures. The plant lattices have been used as fish poison, insecticide and ordeal poisons [2] [3]. The skin irritant, tumor-promoting and anti- tumor/anticancer and recently anti-HIV activities of Euphorbia species have also been reported in *E. heterophylla* leaf L [4]. The whole plant and leaves were used as a lactagouge in India [5].

The leaves of *E. heterophylla* have been reported to contain quercetin [6]. Diterpenoids have also been reported in the root of *E. heterophylla* L [7]. In other countries it was used as a laxative [8] [9]. Most of the species of Euphorbiaceae family are used in cancer prevention. *Euphorbia heterophylla* L showed the presence of Ingenane, Tigliane, kansuiphorinA and Kansuiphorin B, 3, 3', 5' – tri – o- methylpiceatannol. The genus Euphorbi is an important source of cytotoxic compounds such as Ingenol-3- hexadeconate [10] Kansuiphorin A and kansuiphorin B [11] 3, 3', 5'- tri-o-methylpiceatanol and 12- deoxyphorbol- 13- (9, 10 methylene) – undecenoate. Some triterpenes such as α – amyrin, β- amyrin, euphyl acetate and moretenone have been found in the leaves 12]. *Euphorbia heterophylla L*found to contain saponins, flavonoids and tannins. The compounds isolated from *Euphorbia heterophylla L* stigmasterol,_-stigmasterol glucoside, benzoic acid and 4 – hydroxyl benzoic acid exhibited good activity against the *Xanthine oxidase* enzymes while the 4-hydroxybenzoic acid showed a marked activity.

The various parts of the plant can be screened for its therapeutic potential especially in the area of cancer. The immunomodulatory property of this plant can be explored and to be utilized in future in the herbal drug research sectors with the outcome of formulation too. So there are ample scopes for the researchers who are doing research in this direction can proceed further to explore the novelty.

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