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RESEARCH ARTICLE

Effect of Organic Carbon on Soil Moisture.

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ABSTRACT

Soil hydraulic property governs soil metabolism and greatly affects the soil management; hence an optimum level of Soil Organic Carbon (SOC) is required to hold water and nutrients which decrease the risks of erosion and degradation. SOC also acts as a bio-membrane that filters pollutants and alleviates eutrophication in streams and coastal ecosystems; Further, SOC improves soil texture and aggregation and provides energy to microorganisms that play an important role in the nutrient cycling of the soil system. Soil moisture and water retention capacity of soil may be affected by changes in SOC that occur because of both climate change and land management practices. An increase of soil Carbon in cropland soils may increase crop yield as well as enhance food security. Carbon sequestration has the potential to offset fossil fuel emissions by 0.4 to 1.2 Giga-tons of Carbon per year or 5 to 15% of the global fossil-fuel emissions.

Key words: Organic carbon, Water holding capacity, Moisture content, Soil Organic Carbon (SOC).

INTRODUCTION

Soils are the largest Carbon reservoirs of the terrestrial Carbon cycle. About three times more Carbon is contained in soils than in the world's vegetation. Soils hold double the amount of Carbon that is present in the atmosphere. Worldwide the first 30 cm of soil holds 1500 Pg (1pg=1015 grams). Carbon (Batjes,1996); Soils contain 3.5% of the earth's Carbon reserves, compared with 1.7% in the atmosphere, 8.9% in fossil fuels, 1.0% in biota and 84.9% in the oceans (Lal et al., 1995). The amount of CO2 in the atmosphere steadily increases as a consequence of anthropogenic emissions, but there is a large interannual variability caused by terrestrial biosphere (Erbrecht and Lucht, 2006). Moving to organic-rich systems of agriculture with much-improved soil-water relations greatly reduces the hazard of soil erosion at a given place, because the soil is better protected against raindrop damage and is more porous and absorptive. By controlling gaseous losses of C via mineralization, soil moisture may affect the extent to which C can

accumulate in burrow soil altering the effects on earthworms on C storage and soil structure (Burrow et al., 2005). Agro -forestry practices by sequestering Carbon can increase crop yields by increasing the diversity of products grown and also monitor the climate change (Paul Schroeder, 1993; Dhayani et al., 2009) Modeling of changes of organic Carbon content in soils and related changes in ecosystem productivity attracts significant attention with regard to climate changes and management changes. Existing models lack the feedback effect of organic Carbon content accumulation on water retention and saturated hydraulic conductivity (Rawls et al., 2004, Andre Leu, 2007).
An increase of soil Carbon in cropland soils may increase crop yield as well as enhance food security; Carbon sequestration has the potential to offset fossil fuel emissions by 0.4 to 1.2 Giga-tons of Carbon per year or 5 to 15% of the global fossil-fuel emissions (Lal, 2004a). It is the soil hydraulic property that governs soil metabolism and greatly affects the soil management (Falloon et al., 2007). Therefore, an optimum level of Soil Organic Carbon (SOC) is required to hold water and nutrients which decreases the risks of erosion and degradation (Post and Kwon, 2006). SOC also acts as a bio-membrane that filters pollutants and alleviates eutrophication in streams and coastal ecosystems (Kaiser and Zech, 1997 & 1998). Further, SOC improves soil texture and aggregation and provides energy to microorganisms that play an important role in the nutrient cycling of the soil system (Palaniappan et al., 2009). Soil moisture and water retention capacity of soil may be affected by changes in SOC that occur because of both climate change and changes in land management practices (Laporte et al., 2002)

The vadose zone is an integral component of the hydrological cycle, directly influencing infiltration, storm runoff, evopotranspiration, inter flow, and aquifer recharge. Since a substantial fraction of SOC is confined within the root zone (a part of vadose zone), degradation of soil in terms of quality and quantity, affects SOC dynamics which subsequently have severe negative effects on the food production and food security; mainly in the developing countries, like India (Lal, 2004 a & b; Bhattacharyya et al., 2000).The soil in vadose zone will be unsaturated and under tension with variable moisture content (van Genuchten, and Nielsen, 1985). Thus, interactions between SOC and soil moisture/water retention capacity of soil should be studied and quantified for the sustainable management of land resources. The water retention characteristic of the soil describes the soil's ability to store and release water. The study of soil-water movement under unsaturated conditions is highly nonlinear and has created interest in scientists from diverse disciplines. Water retention and movement in unsaturated soils has been studied and simulation models are presented in literature (Soraganvi, 2005). The study here aims to explore the relation between Soil Organic Carbon (SOC), Soil Moisture Characteristic (SMC) and hydraulic conductivity for saturated and unsaturated soils of different class, resulting into the understanding of impacts of SOC variation on soil-constitutive relationships with respect to the regional soil texture classes and climate. Soil hydraulic property governs soil metabolism and greatly affects the soil management (Falloon et al., 2007). Therefore, an optimum level of Soil Organic Carbon (SOC) is required to hold water and nutrients which decreases the risks of erosion and degradation (Post and Kwon, 2006). SOC also acts as a bio-membrane that filters pollutants and alleviates eutrophication in streams and coastal ecosystems (Kaiser and Zech, 1997 & 1998). Further, SOC improves soil texture and aggregation and provides energy to microorganisms that play an important role in the nutrient cycling of the soil system (Palaniappan et al., 2009). Soil moisture and water retention capacity of soil may be affected by changes in SOC that occur because of both climate change and changes in land management practices (Laporte et al., 2002)

The study area is Raichur, a city and district head quarter in the Indian state of Karnataka located between Krishna and Tungabhadra rivers at 16.2°N 77.37°E . It has an average elevation of 407 metres (1335 ft). It is located at a distance of 409 km from the state capital. It has a traditional agricultural based economy.

MATERIALS AND METHODS

Four classes of soils namely Black Cotton Soil, Marshy Soil, Red Soil and Mountainous Soil were taken from different locations by removing the top 5cm soil with ten samples from each location. Such of the collected samples are analyzed in its natural form for particle size distribution by Sieve analyzing as per IS: 460-1962 and grouped accordingly in soil class, determined field density by the cores taken from the field, also analyzed for SOC and field

moisture content. Soils along with organic carbon are then analyzed for organic carbon (walkley-black, 1934) and moisture content. These soils are then added with Organic carbon for the volume up to 80% from top of the soil column by replacing the equal quantity of soil by SOC and maintaining the same field density in the soil column. Two sets of soil columns for each percentage of added carbon were made for all the class of soils. Water was ponded in the Soil columns from the top through the measuring cylinder and the quantity of water added was recorded, quantity of water drained from soil columns was measured and the corresponding time was recorded. Addition of water was made as and when the surface of soil column was dried and the corresponding time was recorded. The addition of water to the soil columns was continued till the consistent drain volume and the time intervals were obtained. For each consistent drain readings water Holding Capacity and moisture content was determined.

RESULTS AND DISCUSSION

Soil samples were sieve analyzed in laboratory for their fine and coarse contents. Data of the sieve analysis is presented in the table 1 and figure 1.

Table 1: Particle Size Distribution of Soil Samples

SIEVE SIZE	% FINER Mountainous	% FINER Red	% FINER B C	% FINER Marshy
4.75mm	98.948	99.681	94.768	92.682
2.36mm	94.278	97.462	74.208	66.71
1.36mm	70.27	75.912	46.852	21.148
600µ	50.85	57.564	32.851	9.261
425µ	28.43	36.499	17.437	4.12
300µ	24.495	32.71	14.552	3.457
212µ	9.18	16.359	5.885	1.595
150µ	7.365	13.057	4.039	1.269
75µ	2.311	3.232	1.038	0.573
0	0.557	0.32	0.27	0.18

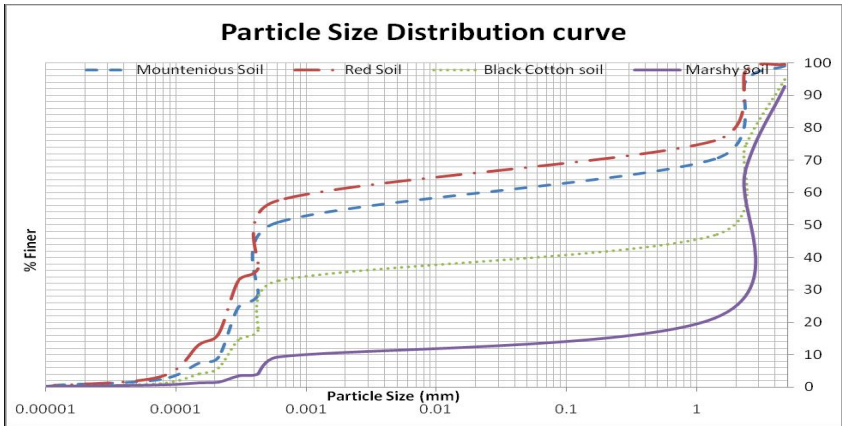

Figure1: Particle Size DistributionCurves

Carbon content in soils and organic matter: Organic matter and carbon content in soils and Organic carbon (walkley-black, 1934) are tabulated in table 2.

Table 2: Carbon and Organic Matter Content

Soil	% C	% Organic matter
Red	0.35	0.8
Marshy	0.4	0.9
Black cotton	0.44	1.01
Mountainous	0.34	0.77
Humus	0.6	1.4

Water holding capacity for various soils

All the collected soil samples were added with varying organic carbon content from zero to eighty percentages and then were analyzed for water holding capacities and moisture contents. Mountainous soils and Red soils had only 29.7% & 24.1% of fines in them hence low organic carbon input didn't exemplified the water holding capacity of these soils, it was the high organic carbon input as high as 50% for Mountainous soils & 40% for Red soils proved beneficial for these soils with respect to water holding capacity. Organic carbon in these soils acted as a fine medium of sorption to hold water as well improved the soil aggregation (Roth, C.H. 1985). The results of which are shown in Table 3 & 4 respectively.

Table 3: Mountainous Soil (1000 ml. columns)

% (Vol.) CARBON ADDED	WATER HELD(ml)	% (Vol.) Water Held	% Moisture Content
0	691	69.1	31.61
10	604	60.4	39.825
20	627.5	62.8	25.755
30	700.5	70.1	31.765
40	516	51.6	25.415
50	762.5	76.3	47.84
60	693.5	69.4	46.965
70	556.25	55.6	55.61
80	560.5	56.1	43.665

Table 4:Red Soil (800ml. columns)

% (Vol.) CARBON ADDED	WATER HELD(ml)	% (Vol.) Water Held	% Moisture Content
0	576.5	72.1	35.945
10	571.5	71.4	26.575
20	601.5	75.2	30.18
30	622.5	77.8	26.615
40	631	78.9	36.985
50	572.25	71.5	43.305
60	590	73.8	38.17
70	484.75	60.6	54.21
80	583.5	72.9	57.7

Table 5:Black Cotton Soil(1000ml. columns)

% (Vol.) CARBON ADDED	AVG. WATER HELD(ml)	% (Vol.) Water Held	% Moisture Content
0	891.75	89.2	32.55
10	865.5	86.6	43.22
20	780.5	78.1	45.03
30	928	92.8	50.49
40	884	88.4	50.11
50	561	56.1	44.495
60	510.5	51.1	44.285
70	490.75	49.1	46.88
80	419.75	42.0	39.71

Table 6:Marshy Soil(1200ml. columns)

% (Vol.) CARBON ADDED	AVG. WATER HELD(ml)	% (Vol.) Water Held	% Moisture Content
0	784	65.3	32.14
10	1058.5	88.2	55.925
20	875	72.9	43.41
30	869.5	72.5	57.795
40	883.5	73.6	57.72
50	838	69.8	52.595
60	895	74.6	45.785
70	717.5	59.8	59.88
80	605	50.4	41.465

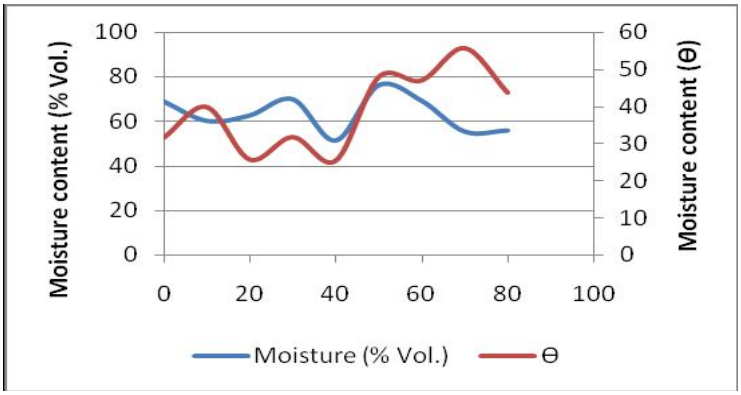

Figure:2 Water held Vs Moisture Content

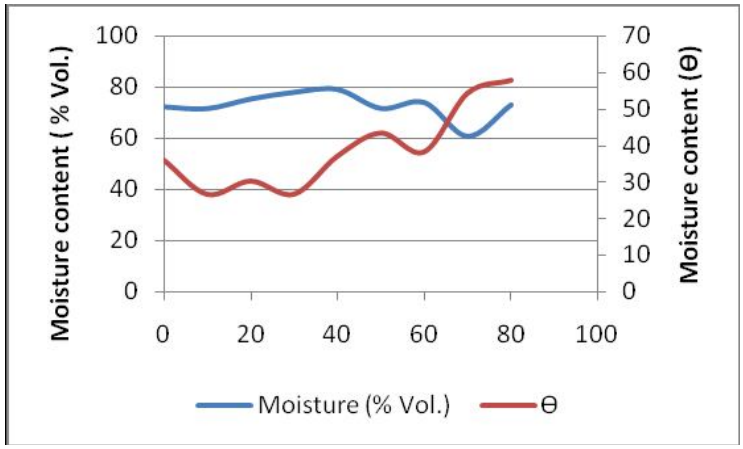

Figure:3 Water held Vs Moisture Content

Marshy soil and black cotton soil had 78.8% & 53.1% fines (dia. Less than 1mm) respectively and were already rich in organic matter and organic carbon as depicted in Table 2, both soils exemplified the good water holding capacity for a very less percentage of organic carbon input viz. 10% for marshy soil and 30% for black cotton soil as shown in Table 5&6. Soil organic matter tends to increase as the clay content increases. This increase depends on two mechanisms. First, bonds between the surface of clay particles and organic matter retard the decomposition process. Second, soils with higher clay content increase the potential for aggregate formation. Macro-aggregates physically protect organic matter molecules from further mineralization caused by microbial attack (Rice, C.W. 2002).

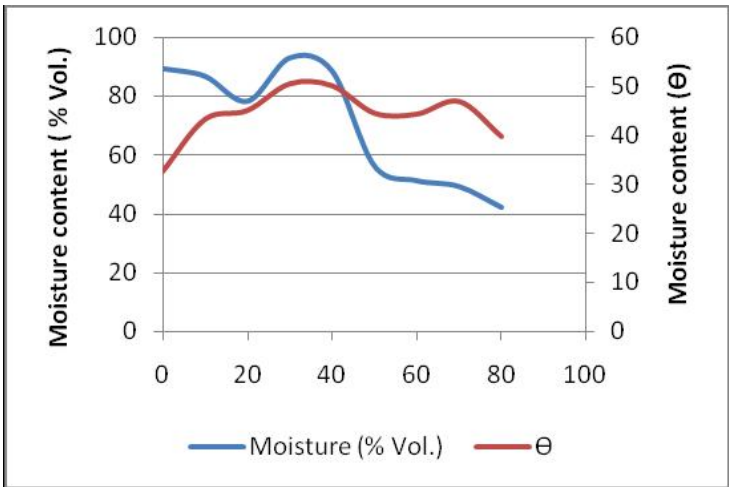

Figure:4 Water held Vs Moisture Content

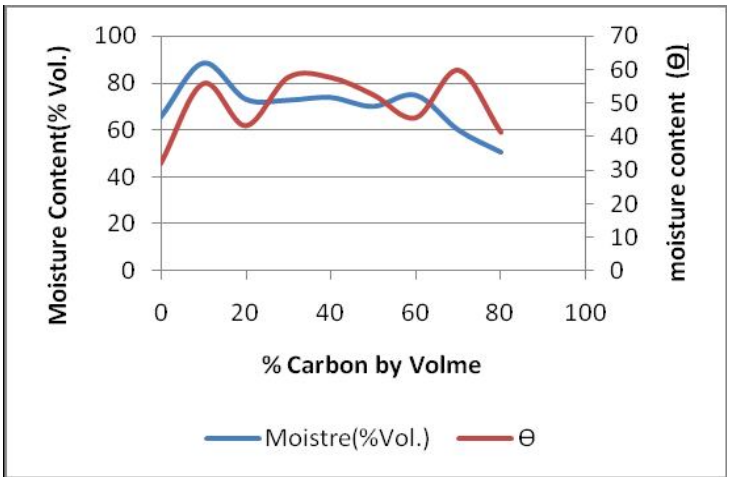

Figure:5 Water held Vs Moisture Content

CONCLUSION

The maximum limit for (SOC) intrusion was found to be 50%, 40%, 30% & 10% for Mountainous soil, Red soil, Black cotton soil and Marshy soil respectively any further addition did not boost water holding capacities.Marshy and Black cotton soils collected were already rich in organic matter, hence hardly any percentage input was sufficient. Red and mountainous were scarce in organic matter that made them to implicate good percentage SOC input.For fine soils when the Carbon input was maximized pores were clogged and maximum resistance to flow was observed.If Organic carbon is imposed as per the optimized levels in the soils, soils can hold water and nutrients which decrease the risks of erosion and degradation. SOC improves soil texture and aggregation and provides energy to microorganisms that play an important role in the nutrient cycling of the soil system.

REFERENCES

1. Andre Leu, 2007 March "Organics and Soil Carbon: Increasing soil Carbon, crop productivity and farm profitability,Managing the Carbon Cycle", Katanning Workshop 21-22 www.amazingCarbon.com.

2. Batjes, N, H., "Total C and N in soils of the world". Eur J Soil Sci 1996 , 47:151-163.

3. Bhattacharya, T., Pal, D,K., Mondal, C., Velayutham M., "Organic Carbon stock in Indian soils and their geographical distribution", Current Science 2000, 79(5):655-660.

4. Burrow soil L., Jose´ A. Amadora, Josef H. Go¨rresa, Mary C. Savinb., 2005. "Role of soil water content in the Carbon and nitrogen dynamics of Lumbricus terrestris", Applied Soil Ecology 28:15–22

5. Dhayani S.k.,.Kareemulla K, Ajit and Handa A.K.., 2009."Agroforestry potential and scope for development across agro-climate zones in India", Indian Journal of forestry, Vol.32(2): 181-190

6. Erbrecht T, Lucht W., "Impact of large scale climatic disturbances on the terrestrial Carbon cycle"., Carbon Balance and Management 2006

7. Falloon, P., Jones, C.D., Cerri, C.E., Al-Adamat, R., Kamoni, P., Bhattacharyya, T., Easter, M., Paustian, K., Killian, K., Coleman, K., Milne, E., 2007. "Climate change and its impact on soil and vegetation Carbon storage in Kenya, Jordan, India and Brazil", Agriculture, Ecosystem & Environment 122, 114–124.

8. Kaiser, K., and Zech, W., 1997, "Competitive sorption of dissolved organic matter fractions to soils and related mineral phases", Soil Science Society of America Journal 61, 64–69.

9. Kaiser, K., and Zech, W., 1998. "Soil dissolved organic matter sorption as influenced by organic and sesquioxide coatings and sorbed sulfate", Soil Science Society of America Journal 62, 129–136.

10. Lal R, Kimble JM, Levines E, Whiteman C., "World soil and greenhouse effect", SSSA Special Publication Number Madison, WI 1995 , 57:51-65.

11. Lal, R., 2004a. "Soil Carbon sequestration impacts on global climate change and food security", Science 304, 1623-1627.

12. Lal, R., 2004b. "Soil Carbon sequestration in India", Climatic Change 65, 277–296

13. Laporte, M.F., Duchesne, L.C., Wetzel, S., 2002., "Effects of rainfall patterns on soil surface CO2 efflux, soil moisture, soil temperature and plant growth in a grassland ecosystem of northern Ontario, Canada: implications for climate change", BMC Ecology 2, 10.

14. Palaniappan, S, P., Chandrasekaran, A., Kang, D,S., Singh, K., Rajput, R.P., Kauraw, D.L., Velayutham, M., Lal, R., 2009. "Sustainable management of natural resources for foosecurity and environmental quality: case studies from India – A Review. In: E. Lichtfouse (ed.), Climate Change, Intercropping, Pest Control and Beneficial Microorganisms", Sustainable Agriculture Reviews, pp. 339-372.

15. Paul Schroeder., 1993."Agro forestry systems: integrated land use to store and conserve Carbon", Climate Research Vol.3: 53-60.

16. Post, W.M., Kwon, K.C., 2006. Soil carbon sequestration and land-use change: processes and potential. Global Change Biology 6, 317-328

17. Rawls W.J., Nemesl A. and Pachepskl Y.A.., 2004. "Effect of Soil Organic Carbon On Soil Hydraulic Properties", Developments In Soil Science Volume 30 ISSN 0166-2481/DOI 1O.1016/S0166-2481(04)30006-1 Elsevier

18. Rice, C.W. 2002. Organic matter and nutrient dynamics. In: Encyclopedia of soil science, pp. 925–928. New York, USA, Marcel Dekker Inc

19. Roth, C.H. 1985. Infiltrabilität von Latosolo-Roxo-Böden in Nordparaná, Brasilien, in Feldversuchen zur Erosionskontrolle mit verschiedenen Bodenbearbeitungs-systemen und Rotationen. Gött. Bod. Ber., 83: 1–104.

20. Soraganvi Veena S., 2005 July, Numerical And Stochastic Modeling of Flow And Transport In The Vadose Zone, Ph.D Thesis Department of Civil Engineering Indian Institute Of Science Bangalore.

21. Van Genuchten, M. T., and Nielsen. D.R., 1985. "On Describing and Predicting the Hydraulic Properties of Unsaturated Soils", Annales Geophysicae, 3(5), 615-628.

22. Walkley, A. and I. A. Black. 1934. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37:29-37.

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RESEARCH ARTICLE

Study of Pathogenesis of Alzheimer's disease-an *Insilico* Approach.

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ABSTRACT

Alzheimer's disease is a progressive neurodegenerative disorder characterized by deposition of amyloid plaques composed of aggregated amyloid beta plaques, and neurofibrillary tangles composed of hyperphosphorylated tau that leads to synaptic defects resulting in neuritic dystrophy and neuronal death. In the present study, huge amounts of data relating to the Pathogenesis of Alzheimer's disease has been extracted from Swiss-Prot and found that there are 132 proteins are responsible. It was evaluated by employing multiple sequence alignment using ClustalW, MEGA5 tool and constructed a Phylogenetic tree using the functional protein sequences. The phylogenetic tree was constructed using Neighbour – Joining Algorithm in Bioinformatics approach. The results of this study suggest that four proteins UBB, UBC, UBA52, RPS27A have a dominant role in the pathogenesis of Alzheimer's disease. Secondary and Teritiary Structure Prediction of those proteins was done using bioinformatics tools. Three acetylcholinesterase inhibitors (AChE-I) such as donepezil (Aricept), rivastigmine (Exelon), and galantamine (Reminyl) are used for the treatment of Alzheimer's disease (AD). 1AAP was taken as target proteins from PDB and Rivastigmine derivatives were obtained from PUBCHEM and were taken as ligands. The bioactivity of the Rivastigmine derivatives was predicted using molinspiration. Docking studies of Rivastigmine derivatives with target protein was done using glide and the best score was evaluated.

Key words: Alzheimer's disease, neurodegenerative disorder, Swiss-Prot, bioinformatics tools.

INTRODUCTION

Alzheimer's is the most common form of dementia. This incurable, degenerative, and terminal disease was first described by German psychiatrist and neuropathologist Alois. Alzheimer in 1906 and was named after him. Most often, it is diagnosed in people over 65 years of age, although the less-prevalent early-onset Alzheimer's can occur much earlier [3].

Alzheimer's disease (AD) has been identified as a protein misfolding disease, caused by accumulation of abnormally folded Amyloid beta and tau proteins in the brain. Research indicates that the disease is associated with plaques and tangles in the brain. The Amyloid plaques are responsible for the pathology of Alzheimer's disease. Plaques are dense, insoluble deposits of amyloid-beta peptide. Tangles are aggregates of the microtubule-associated protein tau. The molecular mechanisms and hypotheses of Alzheimer's disease (AD) can be incredibly complex [4]. The key event leading to AD appears to be the formation of a peptide (protein) known as amyloid beta (beta amyloid, Aß) which clusters into amyloid plaques (senile plaques) on the blood vessels and on the outside surface of neurons of the brain which ultimately leads to the killing of neurons. A first sketch of the amyloid cascade of events in AD would therefore be: Aß formation => amyloid plaques => neuron death => dementia

The first fact to know in creating a second sketch is that the amyloid beta peptide is created by enzyme clipping of the normal neuron membrane protein known as Amyloid Precursor Protein (APP). APP is actually thought to be a natural neuroprotective [2] agent induced by neuronal stress or injury, which reduces Ca^{2+} concentration and protects neurons from glutamate excitotoxicity.Enzymes can clip APP in ways that do not result in amyloid beta formation.Tau is an important protein that maintains the structural integrity of microtubules. But in AD the tau proteins become hyper-phosphorylated and lose the capacity to bind to microtubules. Instead, the phosphorylated tau proteins bind to each other, tying themselves in "knots" (paired helical filaments two threads of tau wound around each other) known as NeuroFibrillary Tangles (NFTs). Neurons full of NFTs rather than functional microtubules soon die[1]. With these facts in mind, the second sketch of the amyloid cascade would be: APP =>$Aß_{42}$ =>fibrillarAß =>amyloid plaques =>inflammation/NFTs => neuron death

The enzymes that cleave APP are known as secretases. The two enzymes that initially compete to cleave APP are alpha-secretase and beta-secretase. If alpha-secretase cleaves APP there is no formation of $Aß_{42}$. If APP is cleaved by beta-secretase it can then be further cleaved by gamma-secretase to form either a 40 amino acid amyloid peptide ($Aß_{40}$) which is soluble and innocuous or a 42 amino acid peptide ($Aß_{42}$) which clumps together to form insoluble amyloid plaques. Alpha-secretase cleavage occurs at the cell surface, whereas beta-secretase acts at the endoplasmic reticulum [5].

MATERIALS AND METHODS

Protein Sequences corresponding to AD was retrieved from UniProtKB

↓

Multiple Sequence Alignment was done using ClustalW and MEGA5

↓

Structure Prediction of the proteins were done

- Secondary structure Prediction using Psipred, Jpred, PredictProtein, Gor
- Tertiary Structure Prediction using Phyre

↓

Target sequence was collected from PDB

↓

Rivastigmine derivatives were used as ligands & collected from PUBCHEM

↓

Bio Activity Prediction of Rivastigmine derivatives was done using Molinspiration

↓

Docking of target (AD) protein with Rivastigmine derivatives as ligands was done using Schrodinger Maestro

RESULTS AND DISCUSSION

The work was carried out using all tools as mentioned in materials and methods. The results have been obtained and are as follows.

Structural Analysis of the proteins responsible for pathogenesis of Alzheimer's disease using ClustalW

Protein sequences responsible for causing AD have been extracted from NCBI. It is found that there are 132 proteins that may cause Alzheimer's disease. The similarities of these proteins were evaluated by employing multiple sequence alignment using ClustalW tool and constructed a Phylogenetic tree. Phylogenetic tree was constructed using Neighbour – Joining Algorithm in Bioinformatics approach.

ClustalW Multiple Sequence Alignment

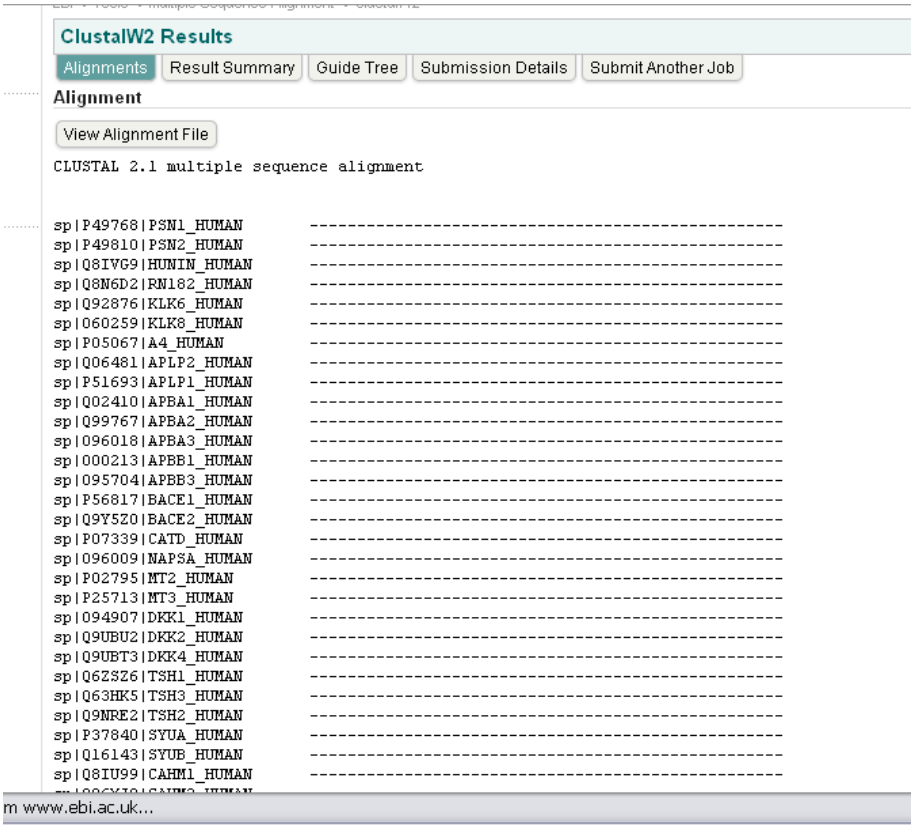

Fig: 1. ClustalW Multiple sequence Alignment of 132 proteins causing Alzheimer's disease.

Phylogram of Alzheimer's disease causing proteins

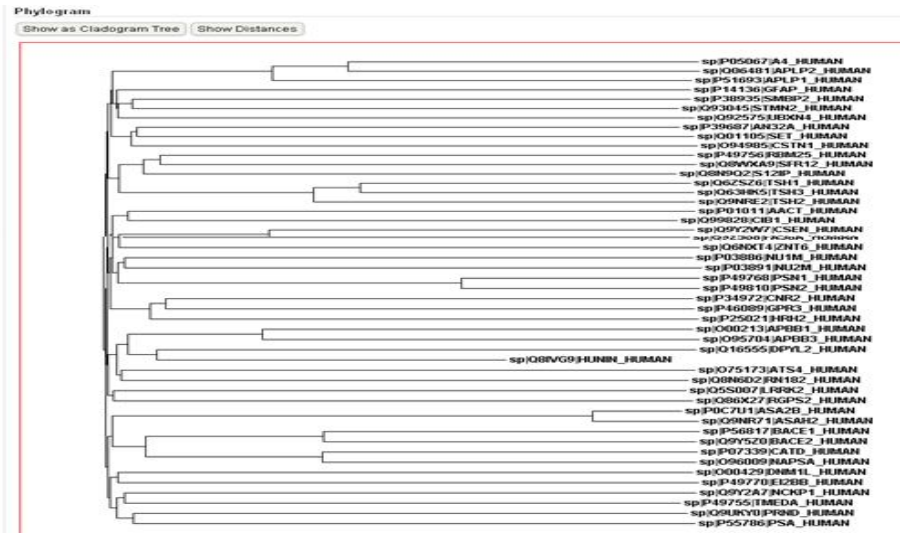

Fig.2: Phylogram showing the evolutionary relationships of 132 proteins causing AD

Cladogram of Alzheimer's disease causing proteins

Fig.3: Cladogram highlighting the proteins RL40, UBB, UBC, RS27A which are closely related.

The above two Figures represents the evolutionary relationships of 132 proteins which are found to be involved in the pathogenesis of AD and highlights the closely related proteins RL40, UBB, UBC, RS27A

Multiple Sequence Analysis using MEGA5

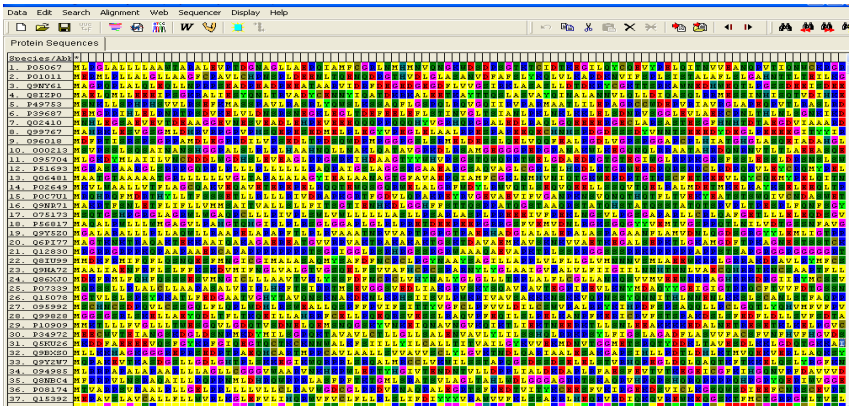

Fig.4: MEGA5 Alignment Explorer showing AD proteins

MEGA5-ClustalW results

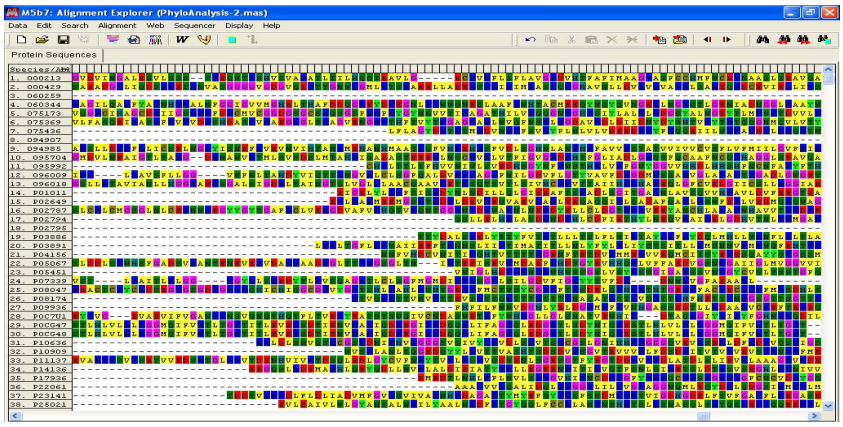

Fig.:5 ClustalW results in MEGA5

Mega-Neighbour Joining Method

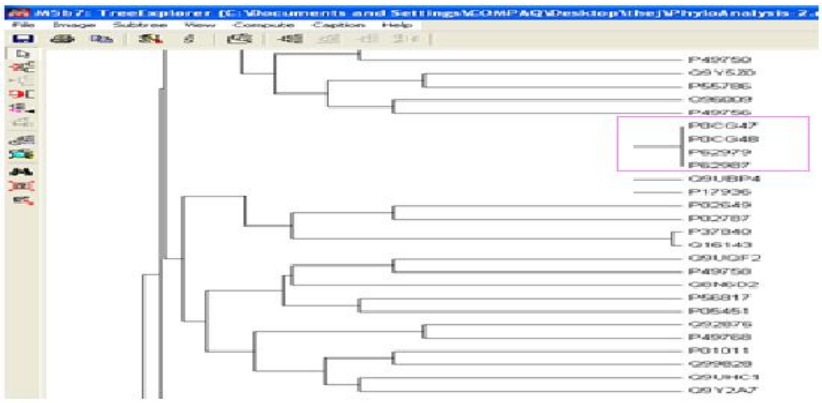

Fig.6: Mega-NJ Method result highlighting closely related proteins

Mega-Maximum Parsimony

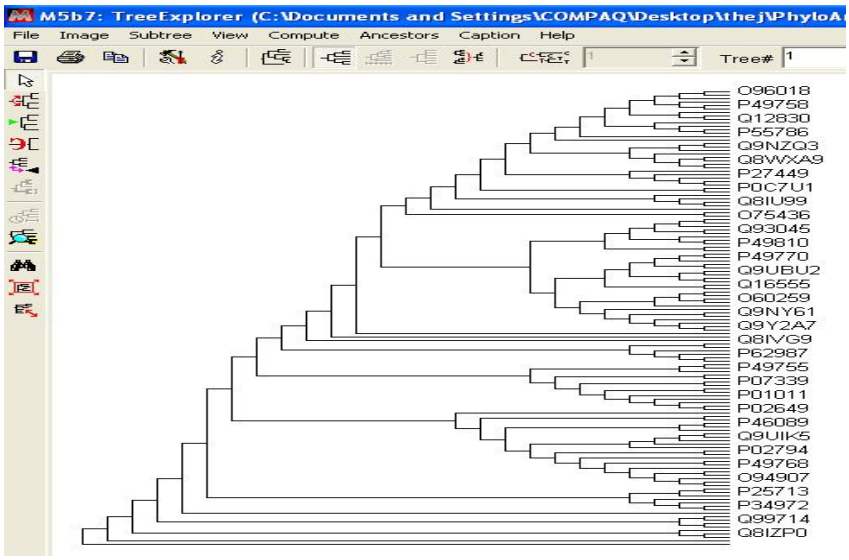

Fig.7: Mega-Maximum Parsimony

Secondary Structure Prediction
Ubiquitin-60S ribosomal protein (P62987- UBA52)
Output for PSIPRED Predictions

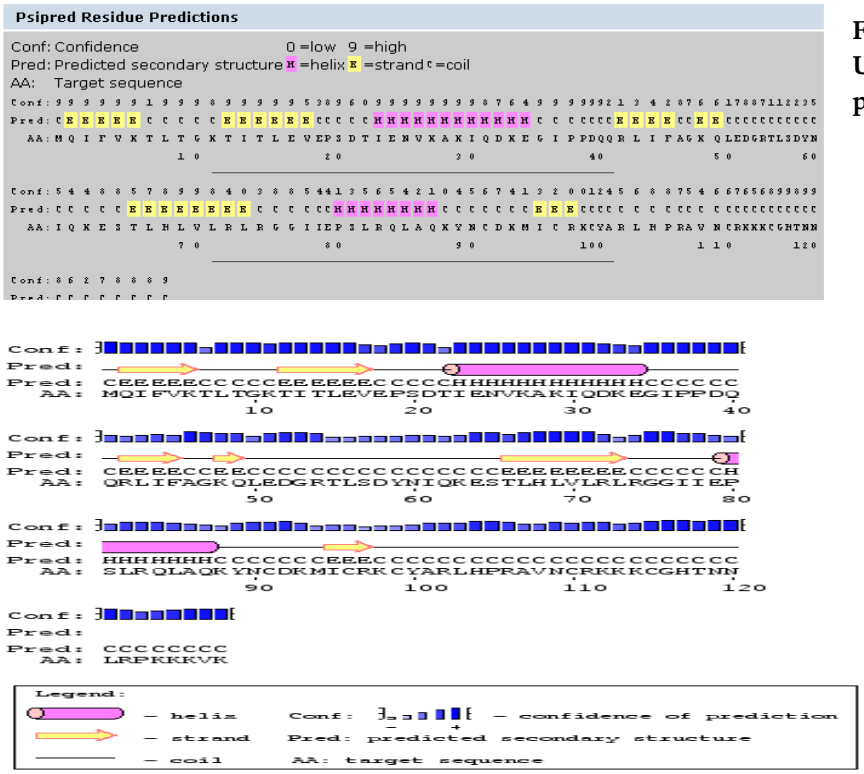

Fig.8: PSIPRED output for Ubiquitin-60S ribosomal protein

Output for Jpred Prediction

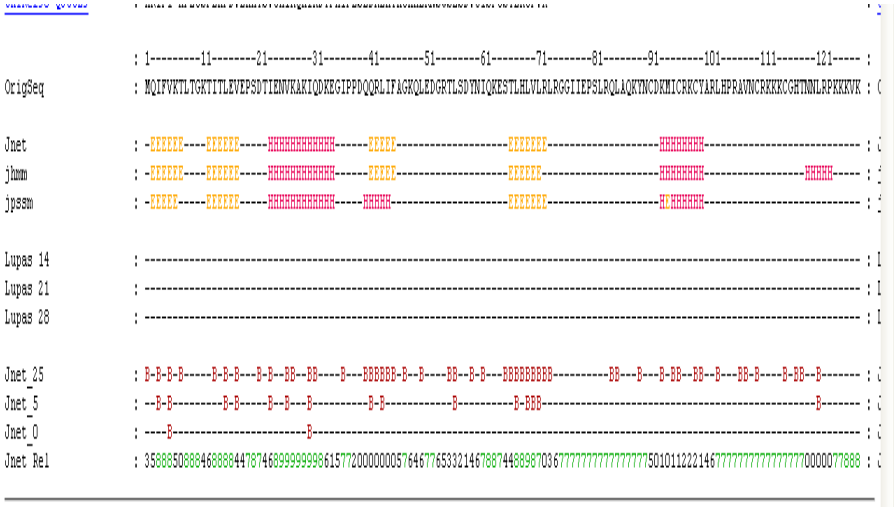

Fig.9: JPRED output for Ubiquitin-60S ribosomal protein

Output for PredictProtein Prediction

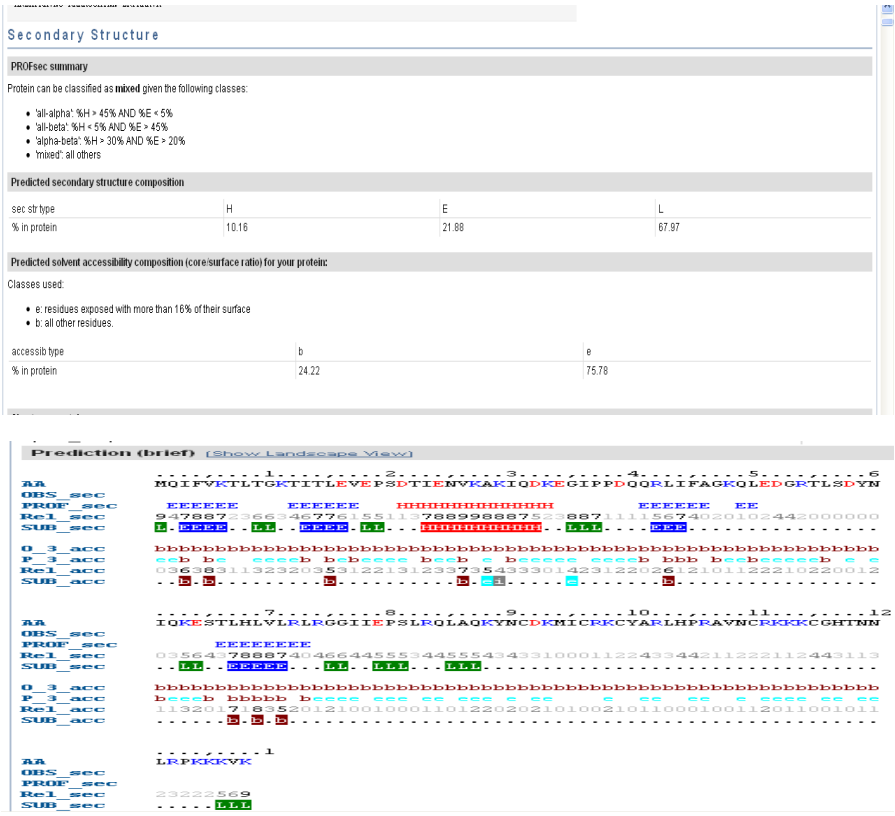

Fig.10: PredictProtein output for Ubiquitin-60S ribosomal protein

Output for GOR Prediction

GOR is running, please wait...

GOR run is completed.

This is the secondary structure prediction:

```
CCCEEEECCCCEEEEECCCCCHHHHHHHHHCCCCCCCCHHHHHHHHHHCCCCEEEEECCCCHHHHHHHHHCCCCEE
EEEEECHHHHHHCCCCCCCCHHHHHHHCCCCCCCCCCCCCCCCCCCCC

Column information:
1) Sequence index
2) Amino acid type
3) Helix probability
4) Sheet probability
5) Coil probability
6) GOR V prediction

1 M 0.062 0.123 0.816 C
2 Q 0.070 0.195 0.735 C
3 I 0.097 0.412 0.490 C
4 F 0.081 0.796 0.123 E
5 V 0.114 0.770 0.116 E
6 K 0.138 0.734 0.128 E
7 T 0.261 0.449 0.290 E
```

Fig.10: GOR output for Ubiquitin-60S ribosomal protein

Tertiary Structure Prediction USING PHYRE
Ubiquitin-60S ribosomal protein

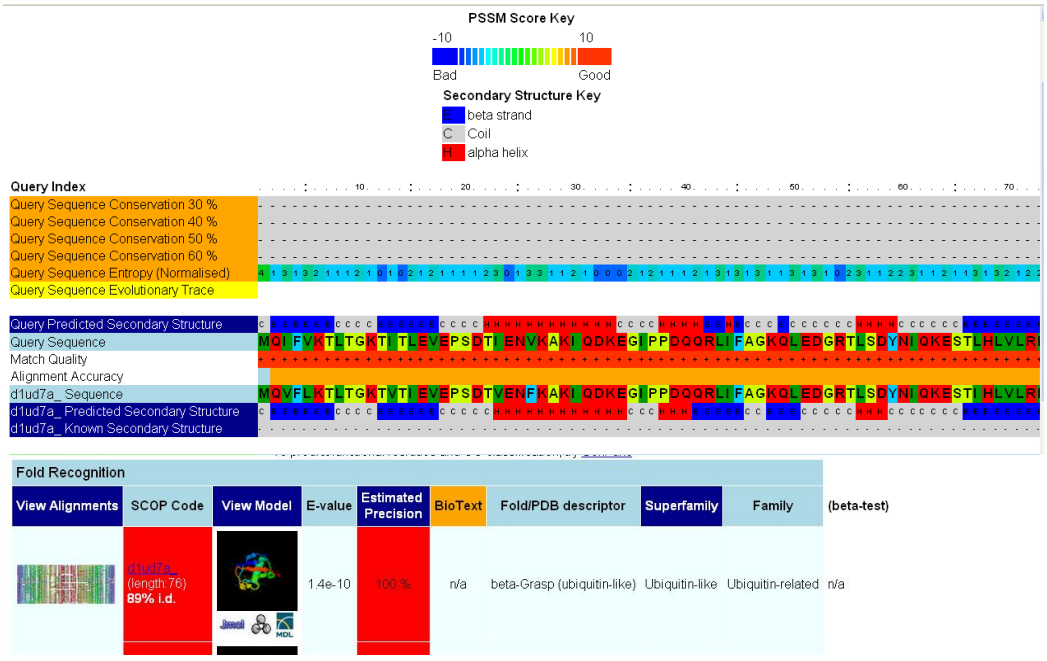

Fig.11: PHYRE output for Ubiquitin-60S ribosomal protein

The results from ClustalW and MEGA5 shows that four proteins UBB, UBC, UBA52, RL40 have major role in the pathogenesis of AD.

Secondary and tertiary structure predictions have been done for these four proteins using PSIPRED, Jpred, PredictProtein, GOR and PHYRE and it was found that all the four proteins belong to the same family ubiquitin.

Drug Molecules Bioactivity Prediction Using Moliinspiration

Table 1: Bioactivity of Rivastigmine Derivatives.

S.No	ID	Drug Likeliness Score	GPCR Ligand	Ion channel modulator	Kinase Inhibitor	Nuclear receptor Inhibitor
1	CID5077	v2008.12	0.07	-0.04	-0.43	-0.55
2	CID77991	v2008.12	0.07	-0.04	-0.43	-0.55
3	CID10586926	v2008.12	0.07	-0.04	-0.43	-0.55
4	CID10729795	v2008.12	0.07	-0.04	-0.43	-0.55
5	CID10777494	v2008.12	0.07	-0.04	-0.43	-0.55
6	CID10848426	v2008.12	0.07	-0.04	-0.43	-0.55
7	CID12133267	v2008.12	0.07	-0.04	-0.43	-0.55
8	CID25207757	v2008.12	0.07	-0.04	-0.43	-0.55
9	CID25230720	v2008.12	0.07	-0.04	-0.43	-0.55
10	CID25230721	v2008.12	0.07	-0.04	-0.43	-0.55
11	CID25230723	v2008.12	0.07	-0.04	-0.43	-0.55
12	CID25230724	v2008.12	0.07	-0.04	-0.43	-0.55
13	CID25241428	v2008.12	0.07	-0.04	-0.43	-0.55
14	CID9823072	v2008.12	-0.00	0.05	-0.46	-0.61
15	CID23645310	v2008.12	-0.17	-0.10	-0.56	-0.63
16	CID46844528	v2008.12	-0.17	-0.10	-0.56	-0.63
17	CID9817108	v2008.12	0.16	0.06	-0.42	-0.47
18	CID9838539	v2008.12	0.16	0.06	-0.42	-0.47
19	CID25230725	v2008.12	-0.14	-0.58	-0.28	-0.17
20	CID11359764	v2008.12	0.09	0.00	-0.33	-0.25
21	CID25175200	v2008.12	0.18	0.04	-0.33	-0.39
22	CID25204947	v2008.12	0.03	0.06	-0.30	-0.22
23	CID4689202	v2008.12	0.03	0.06	-0.30	-0.22
24	CID11066683	v2008.12	0.12	0.12	-0.49	-0.45
25	CID21767524	v2008.12	-0.09	-0.20	-0.55	-0.17
26	CID25271873	v2008.12	-0.09	-0.20	-0.55	-0.17
27	CID29010878	v2008.12	-0.09	-0.20	-0.55	-0.17
28	CID42604975	v2008.12	0.04	-0.04	-0.30	-0.30
29	CID42604976	v2008.12	0.02	-0.00	-0.52	-0.84
30	CID23037471	v2008.12	-0.32	-0.19	-1.25	-1.20
31	CID10989924	v2008.12	0.13	0.16	-0.60	-0.45
32	CID22025986	v2008.12	0.12	0.06	-0.42	-1.04
33	CID23037475	v2008.12	0.15	-0.02	-0.76	-1.23
34	CID23037476	v2008.12	0.24	0.07	-0.39	-0.83
35	CID25095376	v2008.12	0.12	0.06	-0.42	-1.04
36	CID45986	v2008.12	-0.05	-0.13	-1.19	-1.22

37	CID46093	v2008.12	0.12	0.09	-0.64	-1.39
38	CID46111	v2008.12	0.11	-0.14	-0.82	-1.22
39	CID5744837	v2008.12	0.04	0.06	-0.65	-0.41
40	CID10062018	v2008.12	0.09	0.06	-0.47	-0.37
41	CID25098268	v2008.12	0.12	0.09	-0.64	-1.39
42	CID23037472	v2008.12	-0.36	-0.36	-1.65	-1.58
43	CID23037473	v2008.12	-0.38	-0.30	-1.53	-1.37

There are 43 Rivastigmine Analogues which obeys the Rule of Lipnski, extracted from PUBCHEM. The bioactivity of the Rivastigmine analogues was found by using Molinspiration. Ion Modulator channel, Kinase Inhibitor, Nuclear Receptor Inhibitor was found. The compound with ID 23037476 has highest Ion Modulator channel value i.e 0.24. Kinase Inhibitor values ranges from -0.28 to -1.65. Nuclear Receptor Inhibitor values ranges from -0.17 to -1.58.

Docking Results

Table 2: Docking results for Rivastigmine Derivatives.

S.No	Compound-(IUPAC) Name	Distance	Glide Energy	Dope Score
1	[3-[(1S)-1,2,2,2-tetradeuterio-1-(dimethylamino)ethyl]phenyl]N-ethyl-N-methylcarbamate	3.010	-2.895138	-30.512471
2	[3-[(1S)-1-(dimethylamino)ethyl]phenyl]N,N-diethylcarbamate	1.974	-2.793992	-30.329125
3	[3-[(1R)-1-(dimethylamino)ethyl]phenyl]N-ethyl-N-methylcarbamate	2.104	-2.771487	-29.468781
4	[3-[(1S)-1-(diethylamino)ethyl]phenyl]N-ethyl-N-methylcarbamate	2.132	-2.411204	-27.709963
5	[3-[(1S)-1-(dimethylamino)ethyl]phenyl]N-ethyl-N-methylcarbamate	1.852	-2.756695	-26.808259
6	[3-[(1S)-1-(dimethylamino)ethyl]phenyl]N,N-dimethylcarbamate	2.129	-2.628146	-26.672909
7	[2,3,4,6-tetradeuterio-5-[(1S)-1(dimethylamino) ethyl]phenyl]N-ethyl-N-methylcarbamate	2.188	-2.616197	-26.610137
8	[2-deuterio-3-[(1S)-1-[dideuteriomethyl (methyl)amino]ethyl]phenyl]N-ethyl-N-methylcarbamate	2.159	-2.564000	-26.426501
9	[3-[1-(dimethylamino)ethyl]phenyl]N,N-dimethylcarbamate	1.875	-2.856698	-26.413473
10	[3-[(1S)-1-(dimethylamino)ethyl]-2-tritiophenyl] N-ethyl-N-methylcarbamate	2.169	-2.398154	-25.779974
11	[3-[1-(dimethylamino)ethyl]phenyl]N-methylcarbamate	2.253	-2.631287	-23.156961
12	[3-[1-(dimethylamino)propyl]phenyl]N-methylcarbamate	2.000	-1.774592	-23.122989

The Table 2 represents the distance, glide energy, dope score of Rivastigmine derivatives docked with the target protein 1AAP. [3-[(1S)-1,2,2,2-tetradeuterio-1-(dimethylamino)ethyl]phenyl]N- ethyl-N-methylcarbamate was found to be best ligand which has a highest dope score value -30. 512471.

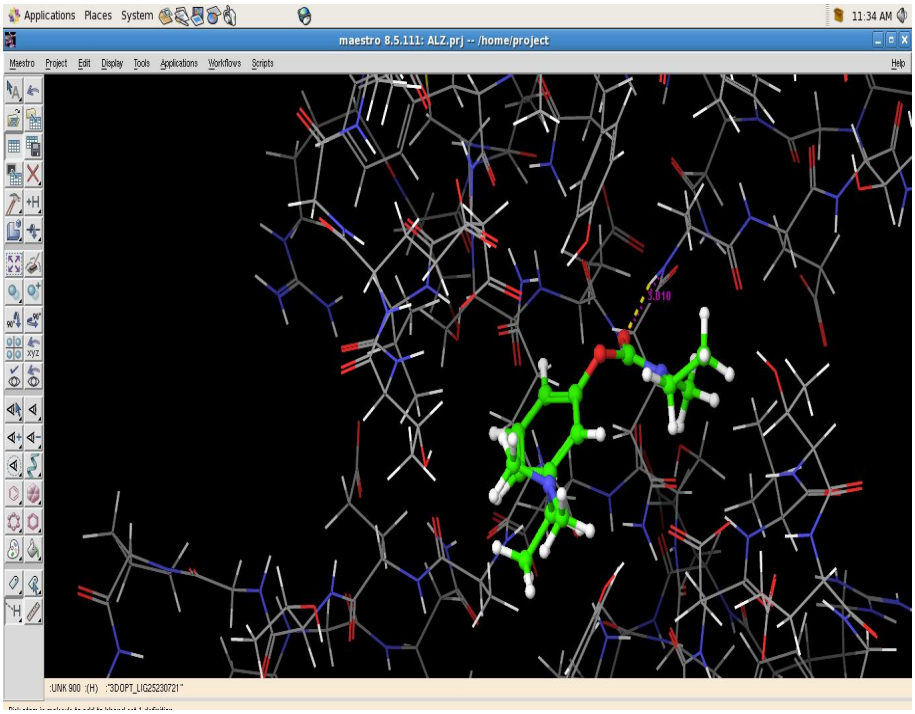

Fig.12: Best Ligand Screenshot

Figure represents the screenshot of [3-[(1S)-1,2,2,2-tetradeuterio-(dimethylamino)ethyl]phenyl]N-ethyl-N-methylcarbamate, This was found to be best ligand.

CONCLUSION

Alzheimer's disease is a progressive neurodegenerative disorder characterized by deposition of amyloid plaques composed of aggregated amyloid beta plaques, and neurofibrillary tangles composed of hyperphosphorylated tau that leads to synaptic defects resulting in neuritic dystrophy and neuronal death. In the present study, huge amount of data has been extracted from Swiss-Prot and found that there are132 proteins that may cause Alzheimer's disease. These proteins were evaluated using ClustalW and MEGA to find the proteins having dominant role. The results shows that UBB, UBC, UBA52, RPS27 have a dominant role in the pathogenesis of Alzheimer's disease. The present study raises the possibility that genetic components are more important in Alzheimer's disease compared to environmental, metabolic, and age related factors. The bioactivity of the Rivastigmine derivatives was predicted. The target protein (1AAP) was docked with Rivastigmine derivatives and best ligand was found. In future, the best ligand i.e [3-[(1S)-1,2,2,2-tetradeuterio-1-(dimethylamino)ethyl]phenyl]N-ethyl-N-methylcarbamate could be used as lead molecule in drug development.

REFERENCES

1. Ballatore, C., Lee, V.M.Y. and Trojanowski, J.Q. (2007). "Tau-mediated neurodegeneration in Alzheimer's disease and related disorders". *Nature Reviews Neuroscience*, 8: 663-672.
2. Becker, R.E. (2008). "Alzheimer's disease Drug Development in 2008 and beyond: Problems and Opportunities", *Curr Alzheimer Res*.5(4):346-357.
3. Chopra, K. (2011). "Current perspectives on pharmacotherapy of Alzheimer's disease". Expert Opinion on *Pharmacotherapy*. 12(3): 335-350.
4. Hampel, H. (2010). "Biological markers of amyloid β-related mechanisms in Alzheimer's disease". *J Experimental Neurology*. 223(2): 334-346.
5. Timo, G. and Hans, F. (2009). "Beta Amyloid in Alzheimer's disease: Increased Deposition in Brain Is Reflected in Reduced Concentration in Cerebrospinal Fluid", *J Biological Psychiatry*. 65(11): 927-934.

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RESEARCH ARTICLE

The Studies on the Environmental Stress in Traffic Police Population in Tiruchirappalli, TamilNadu, India.

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ABSTRACT

Several man-made chemicals find their way into the environment and pose health risk to human population. The comet assay is one such state-of-the-art technique for quantitating DNA damage and repair *in vivo* and *in vitro* in any eukaryotic cell. Result shows that in the lowest age group of <20 years the lymphocytes of the study group showed very good integrity as nearly 90% of the cells had a diameter ranging from 20 to 40 μm, which means that their DNA were intact without any SSBs and no significant tail formation was seen. The remaining 10% showed very little damage as only tail lengths of about 40 to 60 μm were seen. This might be due to the environmental factors inducing the SSBs.

Key words: Traffice Police, Comet assay chromosomal damage and Pollution.

INTRODUCTION

Air Pollution is a chemical, physical (e.g. particulate matter), or biological agent that modifies the natural characteristics of the atmosphere. The atmosphere is a complex, dynamic natural gaseous system that is essential to support life on planet Earth. Stratospheric ozone depletion due to air pollution has long been recognized as a threat to human health as well as to the Earth's ecosystems. Worldwide air pollution is responsible for large numbers of deaths and cases of respiratory disease. 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]. Both the developed and developing countries are faced with the problems related to environmental pollution, 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. Existing cities are expanding, new cities are being created, and adjacent cities are merging. As transportation systems are increasing everywhere. Therefore, air pollution has become a growing problem in cities through out 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. Despite the improved performance of technology is presently insufficient to counteract the growth of vehicles [2] and associated pollution problems. Thus, it is necessary to evaluate the status of urban air pollution continuously and to assess its impact on human health and plants.The traffic policemen's peripheral blood lymphocytes were obtained and the cells were first tested for their integrity, because it is well established that the exposure to chemicals (air pollutants) by blood cells, especially lymphocytes have chromosomal aberrations and micronuclei.

MATERIALS AND METHODS

The present investigation was undertaken to study the effects of various environmental factors and their level of chromosomal damage in peripheral lymphocytes obtained from traffic police men blood samples. Accordingly the comet assay was employed to study the rate of DNA single strand breaks (SSBs) induced in the lymphocytes after their exposures (as whole blood samples) to different environmental factors such as Na_2, So_2, Co_2, Co etc. The method of Singh *et al*., (1988)[10]about 5 ml peripheral blood was collected aseptically in heparinised vials from traffic police population. The samples were divided into five groups based on age. Lymphocytes from all fractions were separated immediately, washed and suspended in PBS Solution. The slides was prepared and observed under Microphotography was done using the Nikon camera attached to the Nikon (Optiphot-2, Japan) microscope. For microphotography in artificial light, 400 ASA film was used.

RESULTS AND DISCUSSION

The various age groups and the relevant comet formation were tabulated and indicated using histograms. The tests yield large amount data in form of comet tail lengths with regard to increasing age. The overall view present by the experiment was that as age increase the damage, namely, single stranded breaks, increase in a directly proportional way and reached a peak value of damage at the age group of > 50 (Fig.1). Table 1 shows that in the lowest age group of <20 years the lymphocytes of the study group showed very good integrity as nearly 90% of the cells had a diameter ranging from 20 to 40 μm, which means that their DNA were intact without any SSBs and no significant tail formation was seen. The remaining 10% showed very little damage as only tail lengths of about 40 to 60 μm were seen. This might be due to the environmental factors inducing the SSBs. Beyond that point the cells exhibited different tail lengths indicating the various degree of damages (Fig.2).

In normal people and traffic policemen there is age related increase in the comet tail lengths that corresponds to the damages accumulated at the individual strand levels. This phenomenon is seen in several such age related studies and that gives rise to another supporting proof to the varicaies of time that leads to the degradation of the genetic material in all living species including the highest intellectual form of beings, namely Humans. The environmental effects are seen in earlier exposures of even < 20 years age group also compared to the controls, thereby indicating that the sequence of damage are linear in nature and do not require any threshold levels to lead to SSBs, this is important because, even a small exposure can lead to incremental damages culminating in the highest levels of damages in the highest exposed group of > 50 years (Fig. 2)Finally the interesting thing in the SSBs assay is that of the correlation between the two age groups. In this respect the two age groups show good correlation in the first the categories as <20, 21-30, and 31-40, were the values of above 0.9** are seen among the two groups. The result highlights the nature of pollutants in the area of Trichy having more RSPM and SPM level because a rapid increase in the number of vehicles, urbanization of industries [6].The study area results help to visualize the impact of the air

pollution on the population of the city in general and traffic policemen in particular.In normal people and traffic policemen there is age related increase in the comet tail lengths that corresponds to the damages accumulated at the individual strand levels. This phenomenon is seen in several such age related studies and that gives rise to another supporting proof to the varicaies of time that leads to the degradation of the genetic material in all living species including the highest intellectual form of beings, namely Humans. Similar study supported to age correlated with an increasing percentage of sperm with highly damaged DNA (range: 0–83%) and tended to inversely correlate with percentage of apoptotic sperm (range: 0.3%–23%)[8].The environmental effects are seen in earlier exposures of even < 20 years age group also compared to the controls, thereby indicating that the sequence of damage are linear in nature and do not require any threshold levels to lead to SSBs, this is important because, even a small exposure can lead to incremental damages culminating in the highest levels of damages in the highest exposed group of > 51 years. Finally the interesting thing in the SSBs assay is that of the correlation between the two age groups. In this respect the two age groups show good correlation in the first the categories as <20, 21-30, and 31-40, were the values of above 0.9** are seen among the two groups.

Similarly, the frequencies of DNA damage in peripheral blood lymphocytes many workers occupationally implicated in photocopying industry were studied. DNA frequency of the workers increased with length of the chemical exposure period [7][3] reported age and gender factors have been found to be strongly associated with the frequency of DNA and it's reported the relationship between aging and structural genetic damage.Similarly, the low dose ionizing radiation also increased frequency of genetic damage with increase in duration of work suggesting a cumulative genotoxic effect by various workers [9,1,4,11,12]. The urban pollution including ozone exposure may cause DNA damage in children, reported in Mexico City by Garciduenas *et al*.,1996[5] using "comet" assay which measured strand breaks of DAN in respiratory epithelial cells. The similar result showed that significantly high level of damage are present in epithelial cells of exposed individuals compared to newcomers who had never been exposed to the urban pollution of Tirucirappalli city.

Table 1. Relative Comet Lengths Obtained in General Public (GP) and Traffic Policemen (TP) of Different Age Groups.

DNA MIGRATION *[Comet tail length in μm]* **& Grading**	**Age in Years**									
	<20		**21-30**		**31-40**		**41-50**		**>50**	
	GP	**TP**	**GP**	**TP**	**GP**	**TP**	**GP**	**TP**	**GP**	**TP**
Excellent (20-40 μm)	90%	75%	85%	65%	75%	55%	70%	50%	60%	45%
Good (41-60 μm)	10%	15%	15%	20%	15%	25%	20%	10%	20%	10%
O.K. (61-80 μm)	-	10%	-	10%	5%	10%	10%	10%	15%	20%
Damaged (81-100 μm)	-	-	-	5%	-	10%	-	25%	5%	25%
Highly Damaged (101-120 μm)	-	-	-	-	-	-	-	5%	-	5%
Correlation	0.9902		0.9880		0.9529		0.8402		0.5613	

Sample Size = 10 / groups, Number of Cells Scored per Sample = 1000, Total Number of Cell Scored per Age Group = 10000

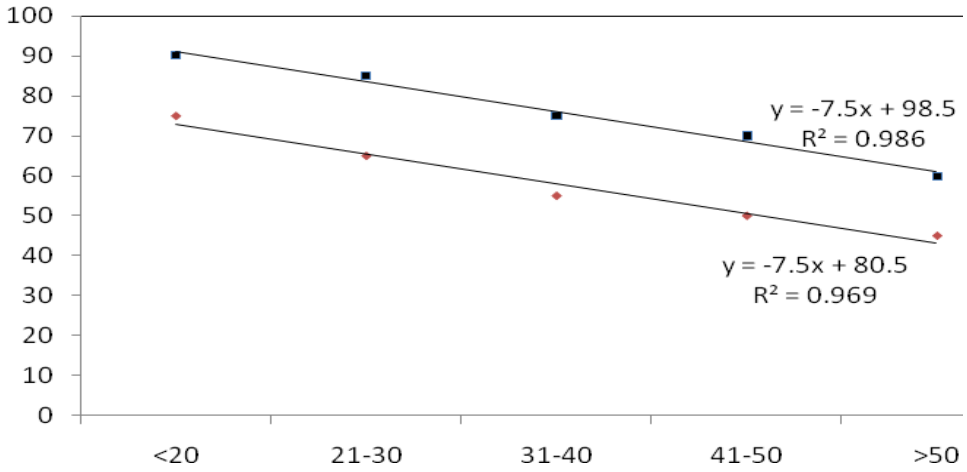

Fig.1. Linear trendline of intact (excellent) cells as seen by comet assay in normal people (np) and traffic policemen (tp)

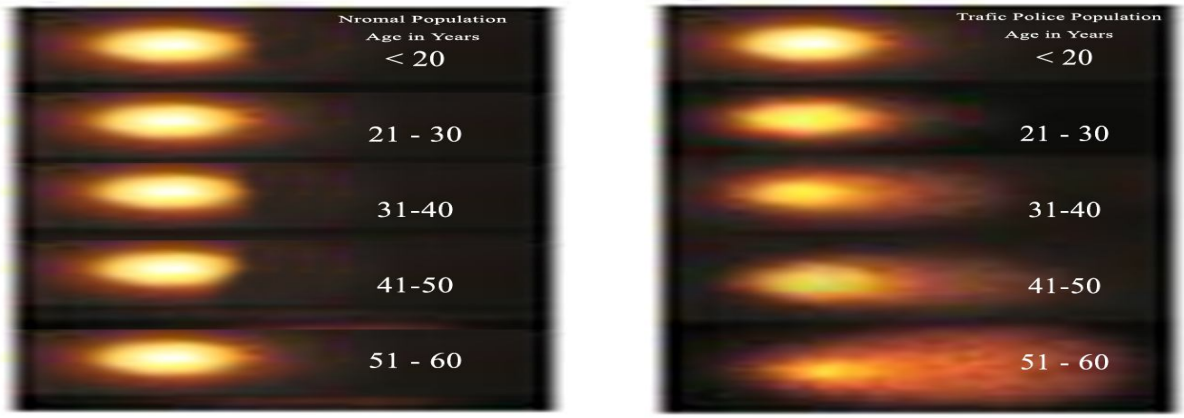

Fig.2. A photographics showing the DNA migration pattern from Normal Population and Traffic Police Population.

REFERENCES

1. Ajoy, K,R,, Geeta, T., Archana, S (1990). Effects of aluminium sulphate on human leukocyte chromosomes in vitro. Mutat. Res. 244: 179–183.
2. Anon, 1980. Motor Vehicle Facts and Figures, American Automobile
3. Balachandar, V., Lakshman Kumar, B., Sasikala, K., Manikantan, P., Sangeetha, R., Mohana Devi, S. (2007). Identification of a high frequency of chromosomal rearrangements in the centromeric regions of prostate cancer patients. J. Zhejiang. Univ. Sci. B. 8(9):638-646.
4. Diaz, S., Fonseca, G., Ferna´ndez, I. (1990). Analysis of lymphocyte and oral mucosa cell micronuclei in Cuban paint industry workers. Hereditas. 113: 77–80.
5. Garciduenas. C., L,Osnaya-Brizuela N, Ramirez-Martinez L, (1996) DNA strand breaks in human nasal respiratory epithelium are induced upon exposure to urban pollution. Environ Health Persp 104 : 160-168.
6. Horaginamani and Ravichandran 2010, Ambiant air quality in an urban area and its effects on plant and human beings: A case study of Tiruchirappalli, India, Kathmandu University, J. Sci., Engg. and Tech Vol. 6. II, pp.13-19.
7. Manikantan.P, Balachandar.V and Sasikala.K (2010) DNA Damage in Workers Occupationally Exposed to Lead, Using Comet Assay International Journal of Biology Vol - 2 No 1.
8. Narendra P. Singh, M.S.,a Charles H. Muller,b and Richard E. Berger.b(2003). Effects of age on DNA double-strand breaks and apoptosis in human sperm. *Fertility and Sterility VOL. 80, NO. 6.*
9. IARC (1989) Monographs on evaluation of carcinogenic risk to humans: Diesel and Gasoline Exhaust and Some Nitroarens, . Lyon:*International Agency for Reasearch on Cancer*.Vol 46.
10. Singh,N.P., M. T. McCoy, R. R. Tice, E. L. Schneider (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp. Cell Res.* 175: 184-191.
11. Yadav, J.S., Seth, N. (1998). Effect of polycyclic aromatic hydrocarbons on somatic chromosomes of coal tar workers. Cytobios. 93: 165–173.
12. Yadav, J.S., Thakur, S. (2000). Cytogenetic damage in bidi smokers. Nicotine. Tobacco. Res. 2: 97–103.

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RESEARCH ARTICLE

Effect of Organophosphate Insecticide and DNA Damage Analysis in *Poecilia sphenops* and *Carassius auratus.*

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ABSTRACT

In ornamental fishes the enzymes play an important role in the growth, respiration and spawning and other process *Poecilia sphenops* and *Carassius auratus* is an easily available fishes which are widely cultivated in fish tanks of Tamil Nadu are selected.

Keywords: Organophosphate, Lethal concentration, Enzymes, *Poecilia sphenops* and *Carassius auratus.*

INTRODUCTION

One type of fish aqua culturists could rear ornamental fishes. Aquarium keeping is amongst the most popular of hobbies with millions of enthusiasts worldwide. Together all countries of the European Union are the largest market for ornamental fish; however, the United States (US) is the single largest importer of ornamental fish in the world. The growing interest in aquarium fishes has resulted in study increase in aquarium fish trade globally. The trade with a turnover of US $5 Billion and an annual growth rate of 8 percent offers for development. In order to sustain the growth it is absolutely necessary to shift the focus from capture to culture base development moreover most of the fish species grown for their ornamental importance.Ornamental fish is often used as a genetic term to describe aquatic animals kept in the aquarium including fishes, invertebrates such as corals, crustaceans, mollusks and also live rock. Aquarium fishes are mainly grouped into two categories. viz; oviparous (egg-layers) and viviparous (live-bearers) further, the fresh water ornamental fish varieties can be broadly grouped into tropical and cold water species.

The Molly is a tropical fish that prefers a little salt in their water. This is a very attractive tropical fish that comes in many different colors such as orange, green and black. Some of the popular varieties include the sail fin, balloon and

Dalmatian.The Goldfish is a favourite fish for many of us didn't keep one at one time or another. They are usually very hardy fish and can live in temperatures ranging from 40-90°F (4-32°C). It is importance to note that Goldfish have an extremely long lifespan if cared for properly, so getting one can become a long term commitment. Many varieties are available with many different markings, fancy varieties and colors including gold, orange, white and black.

In this study analysis the effect of organophosphate insecticide in Ornamental fishes such as *Poecilia sphenops* and *Carassius auratus* and to observe the DNA damage of *Poecilia sphenops* and *Carassius auratus* treated by malathion. A new group of pesticides often organochlorine insecticides was of organophosphorous insecticides. They replaced the organochlorine due to their less persistent life and easy detoxification in animal tissues [4]. Although other groups of insecticides with a shorten life and comparatively very low toxicity organophosphorus (OP) compounds are still used frequently in agricultural practices. Their extensive application may affect fish population as they enter the water through irrigation or rain [3].

Being neurotoxicant, op compounds interfere with many of the vital physiological functions [5] and consequently alter the levels of various body constituents in fishes [1]. Investigations have been shown that changes in carbohydrate and nitrogenous metabolism in fish induced by the stress occurred by pesticide-induces hypoxia. These changes include depletion of proteins, glycogen and pyruvate storage from fish tissues such as liver and muscle [2].Sambasiva Rao (1999)[7] reported an evaluation in free amino acids and protease activity due to pesticide induced hypoxia. The mechanism by which the organophosphorous insecticides exert their action on the arthropods or fishes depends largely on the biochemical processes of animal and the physico-chemical properties of phosphorous compounds. However, the toxic symptoms produced in animals by these organophosphorous compounds are manifested of the inhibition of certain enzyme systems. Organophosphorous insecticides are inhibitors of variety of esterases generally they are associated with inhibition of cholinesterase [6].

MATERIALS AND METHODS

For the present investigation studies were carried out in the field of Angel aqua farm at Panaiyur near ECR, Chennai. The uniform sized ornamental fishes were selected for the present study (wt 10 ± 0.1 gm, length 5 ± 0.3 cm, wt 8 ± 0.1 gm, length such as *Carassius auratus* and *Poecilia sphenops*. Random collection of healthy ornamental fishes of medium size groups were taken and brought to the laboratory in a live aerated polythene bags containing the water. The fishes were exposed to a series of ascending concentration of this insecticide. The enzyme studies were carried out under sub lethal concentration of 1ppm- 3ppm, from the blood sample acid phosphatase, alkaline phosphatase, lactate dehydrogenase, serum glutamate oxaloacetate transminase and serum glutamate pyruvate transaminase were estimated by using standard procedure.

RESULTS AND DISCUSSION

Comet Assay

The comet assay is a versatile and sensitive method for measuring single and double-strand breaks in DNA. The mechanism of formation of comets (under neutral or alkaline conditions) is best understood by analogy with nucleoids, in which relaxation of DNA super coiling in a structural loop to extend into a halo or in the case of the comet assay to be pulled towards the anode under the electrophoretic field.

The comet assay (single-cell gel electrophoresis) is a simple method for measuring deoxyribonucleic acid (DNA) strand breaks in eukaryotic cells. Cells embedded in agarose on a microscope slide are lysed with detergent and high salt to form nucleoids containing super coiled loops of DNA linked to the nuclear matrix. Electrophoresis at high pH results in structures resembling comets, observed by fluorescence microscopy; the intensity of the comet tail relative

to the head reflects the number of DNA breaks the likely basis for this is that loops containing a break lose their super coiling and become free to extend toward the anode. The assay has applications in testing novel chemicals for genotoxicity, monitoring environmental contamination with genotoxins, human biomonitoring and molecular epidemiology and fundamental research in DNA damage and repair. The sensitivity and specificity of the assay are greatly enhanced if the nucleotides are incubated with bacterial repair and nucleases that recognize specific kinds of damage in the DNA and convert lesions to DNA breaks, increasing the amount of DNA in the comet tail. DNA repair can be monitored by incubating cells after treatment with damaging agent and measuring the damage remaining at intervals.

DNA Isolation

A diphenylamine (DPA) indicator will confirm the presence of DNA. This procedure involves chemical hydrolysis of DNA, when heated (e.g ≥ 95^{o}C) in acid, the reaction requires a deoxyribose is converted to w-hydroxylevulinyl aldehyde, which reacts with the compound, diphenylamine, to produce a blue colored compound.DNA concentration can be determined measuring the intensity of absorbance of the solution at the 600nm with a spectrophotometer and comparing to a standard curve of known DNA concentrations. Measuring the intensity of absorbance of the DNA solution at wavelengths 260nm and 280nm is used as a measure of DNA purity.DNA absorbs UV light at 260 and 280 nanometers and aromatic proteins absorb UV light at 280nm; a pure sample of DNA has the 260/280 ratio at 1.8 and is relatively free from protein contamination. A DNA preparation that is contaminated with protein will have a 260/280 ratio lower than 1.8.

The concept underlying the SCGE (single cell gel electrophoresis) assay is that undamaged DNA retains a highly organized association with matrix proteins in the nucleus. When damaged, this organization is disrupted. The individual strands of DNA lose their compact structure and relax, expanding out of the cavity into the agarose. When the electric field is applied the DNA, which has an overall negative charge is drawn towards the cathode. Undamaged DNA strands are too large and do not leave the cavity, whereas the smaller fragments, rather they are free to move in a given period of time. Therefore, the amount of DNA that reveals the cavity is a measure of the amount of DNA damage in the cell.

The comet assay is an extremely sensitive DNA damage assay. This sensitivity needs to be handled carefully it is also vulnerable to physical changes which can affect the reproducibility of results. Essentially, anything that can cause DNA damage or denaturation except the factor(s) being research is to be avoided. The most common form of the assay is the alkaline version although there is as yet no definitive alkaline assay protocol. Due to its simple and inexpensive setup, it can be used in conditions where more complex assays are not available.

The biotic enzyme such as acid phosphatase (ACP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) in the blood of two types of ornamental fishes such as *Poecilia sphenops* and *Carassius auratus* on exposure of different concentration of Malathion from 1ppm-3ppm. On the above said enzymes were studied and the results were recorded from the table (1 and 2). It is clear from the recorded results the enzymes activity was decreased from lower concentration to higher concentration of Malathion treatement. The control fish was recorded highest activity of the entire enzyme analysis.

Result of Enzymatic Assay

Table.1.Shows the amount of tested enzymes - acid phosphatase, alkaline phosphatase, lactate dehydrogenase, serum glutamate oxaloacetate transaminase and serum glutamate pyruvate transaminase present in *Poecilia sphenops* treated by Malathion.

Enzyme	Control (IU/L)	1PPM (IU/L)	2PPM (IU/L)	3PPM (IU/L)
Acid phosphatease	4.2 ± 0.1	3.8 ± 0.3	1.2 ± 0.1	0.9 ± 0.2
Alkaline phosphatase (ALP	112 ± 1	102 ± 2	97 ± 1	93 ± 3
Lactate dehydrogenase (LDH)	47.1 ± 1	38.7 ± 1	33.5 ± 3	29.2 ± 1
Serum glutamate oxaloacetate transminase (SGOT)	73.2 ± 1	68.9 ± 2	65.7 ± 3	59.6 ± 1
Serum glutamate pyruvate transminase (SGPT)	28.1 ± 2	27.5 ± 1	23.2 ± 4	20.8 ± 2

Table.2.Shows the amount of rested enzymes - acid phosphatase, alkaline phosphatase, lactate dehydrogenase, serum glutamate oxaloacetate transaminase and serum glutamate pyruvate transaminase present in *Carassius auratus* treated by Malathion.

Enzyme	Control (IU/L)	1PPM (IU/L)	2PPM (IU/L)	3PPM (IU/L)
Acid phosphatease (ACP)	6.6 ± 0.5	5.7 ± 0.8	3.5 ± 0.6	1.6 ± 0.4
Alkaline phosphatase (ALP	106 ± 0.1	99.7 ± 6	91.8 ± 7	89.5 ± 0.7
Lactate dehydrogenase (LDH)	43.2 ± 0.2	37.7 ± 2	30.8 ± 1	25.8 ± 0.3
Serum glutamate oxaloacetate transminase (SGOT)	67.2 ± 0.1	61.5 ± 0.2	57.7 ± 0.5	51.4 ± 0.1
Serum glutamate pyruvate transminase (SGPT)	27.6 ± 0.1	25.7 ± 0.7	22.4 ± 0.3	19.8 ± 0.8

Malathion is an organophosphorous (OP) insecticide used for the control of insects and other pests on food and non-food crops. It can build up in the bodies of animals that live in Malathion contaminated water. They cause various physico-logical dysfunctions. It is highly toxic to fishes has adverse effect on aquatic system. The present work is designed to understand the level of ill effect caused by Malathion on important enzymes like ACP, ALP, LDH, SGOT, SGPT if the blood serum of ornamental fishes (*Poecilia sphenops* and *carassius auratus*). Healthy medium sized ornamental fishes was selected and exposed to the ascending concentrations. Malathion LC_{50} was recorded 3ppm in 18hrs the enzyme studies more varied out under 1ppm-3ppm at 18hrs quantitative estimation of above said enzymes were carried out in the blood samples using standard procedure. The Malathion treatment under sublethal concentrations (1ppm -3ppm) should decrease the activity.

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REFERENCES

1. Arasta, T., V.B. Bais and P.Thakur. 1996. Effect of Nuvan on some biochemical parameters of Indian catfish *Mystus vittatus*. *Journal of Environmental Research* 80: 80-83.

2. Laui, R.T., P.V.Pradhan and A.M., Bhagwat, 1974. Effect of muscular exercise on glycogen content in *Tilapia mossambica* (in captivity). *Journal of Biological Chemistry,*17:72-77.

3. Li, M., 1975. Pollution in nation's estuaries estuaries origination from the agricultural use of pesticides. In:Estuarine pollution control and assessment. Proceeding of a conference. Washington, D.C., U.S., F-PA, Office of water planning and standards. 451-466 pp.

4. Quaraishi, M.S., 1977. Biological insect control, John Wiely and Sons Publication, New York.

5. Rao, K.S.P. and R.K.V. Rao. 1983. Regulation of phosphorylases and aldolases in tissues of the teleost (*Tilapia mossambica*) under methyl parathion impact. Bulletin of Environmental Contamination and Toxicology 31:427-478.

6. Ravi, G., and Selvarajan V.R. (1990): Influence of phosalone on acetylcholinesterase of Labio rohita (Ham) and *Cyprinus carpio* communis (Lin). *J. Environ. Biol,*11, 211-215.

7. Sambasiva Rao, K.R.S., 1999. Pesticide impact on fish metabolism. 129-149pp.

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RESEARCH ARTICLE

The study of Partial Structure Characterization of Biofouling Antiagonistic Compounds from the Selected Macroalgae (*Ulva lactuca*).

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ABSTRACT

The marine environment the competition for living space in intense, therefore all surface living or innate are susceptible to fouling. This process generally begins with formation of a biochemical conditioning film or molecular fouling. The present study aims to find out the antofouling potential of *Ulva lactuca* and *Hypnea musciformis* by performing antifouling assay against biofilm bacteria. The present result shows the methonolic extract of *Ulva lactuca* exhibited the maximum growth inhibitory activity against all the test organisms while chloroform extract showed inhibitory effect against only 3 strains. Acetone extract of *Hypnea musciformis* did not reflect any zone of inhibition against the biofilm bacterial strains. In the antifouling paint decrease in the bacterial load.

Key words: Antifouling, biofilm, biofouling and Chloroform Extract.

INTRODUCTION

Marine bio-fouling is one of the most important problems currently facing marine transport. In the marine environment any solid surface submerged in sea water will become covered by a complex layer consisting of an organic conditioning film, micro and macro fouling organisms such as marine bacteria, Algae, Protozoa, Barnacle, Mussels and tubeworms[1,2]. All surface in the marine environment influenced by a verity of biological, physical and chemical factors are induced to the formation of a complex layers of attached microorganisms including sessile plants and animals referred to as biofouling[3].Biofouling is a major economic problem concerning man made structures such as, ships, boys, pontoons etc. Biofouling can be described in a 4 main stages process[1] the biochemical conditioning, the microfouling (involving bacteria), the unicellular eukaryote colonisation (yeasts, protozoa and diatoms) and finally the multicellular eukaryote adhesion and growth (larvae and algae spores) [4].These fouling

organisms cause serious technical problems by setting on ship hulls, power plant cooling system, aquaculture systems, fishing net, pipelines and other marine infrastructure. Ship suffer increased drug and other surface corrosion leading to lower speeds, thereby causing higher fuel consumption, additional CO_2 , emission maintenance costs[5]. The living component of biofouling includes microfouling (Eg; Bacteria and Diatoms) and macrofouling (Eg; Algae and Invertibrates). Tremendous effort has been diverted to control macro and microfouling in the world's ocean in order to clean up and maintain agriculture faultiest, ships and sea water pipelines.

Seaweeds produce a wide diversity of secondary metabolites [6]. Some secondary metabolites certainly function as antifouling, antimicrobial or allelopathic agents. The seaweeds under study *Ulva lactuca* belongs to the family Ulvaceae. *Ulva lactuca* is a thin flat green a discold holdfont. The margin is somewhat ruffled and often torn. It may reach 18 cm of more in length, through generally much less, and up to 30cm across. The membrane is two cells thick, soft and translucent, and grows attached, with out a stipe, to rock by a small disc- shaped hold font[7].

The present study aims to find out the antofouling potential of *Ulva lactuca* and *Hypnea musciformis* by performing antifouling assay against biofilm bacteria.

MATERIALS AND METHODS

Collection of Sample

The wood (Ayini) and fiber glass reinforced plastic (FRP) are widely used as substrates in ship hulls, boats, and other marine structures were selected to study. The biofilm formation in offshore of Chinnamuttom, Kanyakumari District, Tamilnadu. 30*30cm thicknesses of 2mm of the panels were chosen. The selected panels were deployed in triplicate at a depth of 1.5cm surface in the offshore and monitored for a period of 72 hours. The biofilm bacterial samples were collected for every 24 hours.

Isolation and identification of biofilm bacteria

The biofilm bacterial strains were isolated by pour plate technique [8].The sampling was carried out in both panels for three consecutive days. Biofilms samples were collected by swabbing with sterile cotton swabs during 24, 48, and 72 hours respectively.The swabs were then transferred into the tubes containing sterile sea water. The biofilm samples were serially diluted and plated in the zobell marine agar and incubated at room temperature for 48 hours. After 48 hours incubation numbers of colonies in each plate were counted and the colony morphology and pigmentation also noted.The isolated biofilm forming bacteria were identified using biochemical tests such as Gram staining, Motility test using hanging drop method, (Indole test, Methyl Red test, and Voges Proskaur test(MR-VP), Citrate test, Urease test, Triple sugar iron test and oxidone test) for the identification of biofilm bacteria and confirmed by reference to Bergey's Manual.

Preparation of seaweed extract

Samples of *Ulva lactuca* and *Hypnea musciformis* were collected by hand, during low tide at kanyakumari sea, kanyakumari District, Tamilnadu. The collected seaweeds were packed tightly in polythene bags individually and brought to the laboratory. Then the seaweeds were washed off thoroughly in running water to remove the soil and dust particles and shade dried completely. Dried seaweeds were cut into small pieces to increase extraction effectively.Ten grams of *Ulva lactuca* and *Hypnea musciformis* was extracted with 100ml of acetone, methanol and chlroform using. The antifouling activity of the *Ulva lactuca* and *Hypnea musciformis* extract against the biofilm bacteria was evaluated by the dics preparation in different concentrations (10µl, 20µl, 40µl and µl). The antibacterial activity of commercially available discs (ampicillin, vanomycin, cefactor, penicillin G, Gentamicin) was tested against

biofilm bacterial isolates using Kirby - Bauer method. The tube dilution method is used to determine the MIC test of the consent isolates.

Preparation of Antifouling plates

For pieces of wood (Ayini) and fiber glass reinforced plastic (FRP) were taken. 4*4cm of 2mm size of the materials was chosen for the present study to assess the biofilm formation. One set of panels were soaked to the 5ml of the seaweeds extracts used a 250ml glass beaker and covered the beaker with paper. The three sets of beaker were kept on the room temperature. After two days, the extract was fully coated with the panels, that pieces were collected and put into the sterile containers. Eight panels were deployed in triplicate at a depth of 1.5cm surface in the offshore of above sampling stations.

RESULTS AND DISCUSSION

The results indicated a gradual increased patterrn of bacterial load in both the wood and FRF panels with respect to increase in time interval from 24 to 72 hours (Table 4&5). 15 biofilm bacterial strains isolated and identified using various biochemical tests are shown in table 1. Antimicrobial activities of commercially available discs were tested against the isolates. Among the strains of 12 bacteria, *Arcanobacterium haemolyticum, Deniobacter grandis,Sporsarcina uveae* and *Shigella* sps showed best results(Table 2). The growth inhibitory effects of the methanol, chloroform and acetone extract of *Ulva lactuca* against the different test organisms are shown in table 3. The methonolic extract of *Ulva lactuca* exhibited the maximum growth inhibitory activity against all the test organisms while chloroform extract showed inhibitory effect against only 3 strains. Acetone extract of *Hypnea musciformis* did not reflect any zone of inhibition against the biofilm bacterial strains.

In the antifouling paint decrease in the bacterial load. The methonolic extract of seaweed showed a better result when compared to the chloroform and acetone extract. The methanol, chloroform and acetone extract of seaweed *Ulva lactuca* gave a better result while *Hypnea musciformis* gave a poor result (Fig1and 2).Antifouling products play a major in shipping industry[9]. Similarly, reported environment consequences of biocide release from antifouling paints are now major obstacles for the development of more efficient antifouling coating [10,11]. Many marine secondary metabolites have been shown to inhibit settlement of fouling organisms.

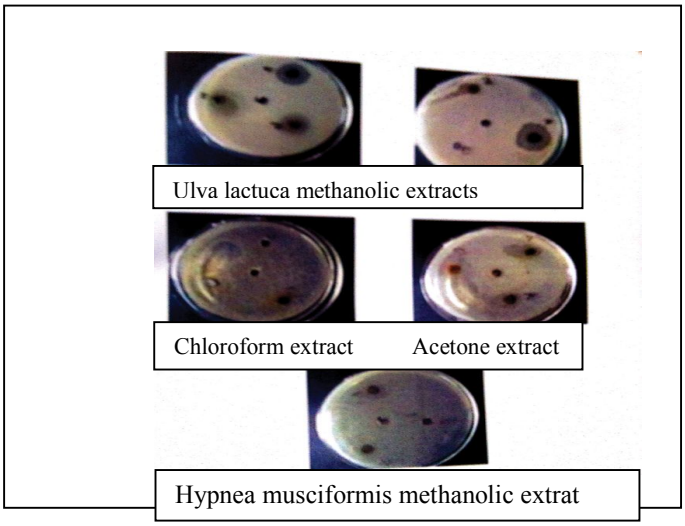

Fig. 1. Showing *Antibacterial* activity in Methanolic, Chloroform and Acetone extracts.

Table 1. Biochemical Test (Gram negative bacteria)

S.No	Organisms	*E.aerogenes*	*P.aeruginosa*	*S.sps.*
1	Indole	-	-	-
2	MR	-	-	+
3	VP	+	-	-
4	Citrate	+	+	-
5	TSI	A/A	K/K	
6	Urase	-	-	-
7	Motility	+	+	-
8	Gram staining	-	-	-

Table 2. Antibacterial activity of commercially available disc on biofilm bacterial isolate.

S.No	Name of the Organisms	Ampicillin (AS10/10)	Vancomycin (VA30)	Cefaclor (CJ10)	PenicillinG (P10)	Gentamicin (G30)
1	*D.grandis*	24	10	17	15	19
2	*D.radiodurans*	-	-	-	-	14
3	*M.halophilus*	24	12	-	12	18
4	*S.roseus*	30	-	20	18	22
5	*S.urea*	39	12	16	39	20
6	*A.erythreum*	23	-	-	-	16
7	*A.haemolyticum*	28	14	23	18	18
8	*B.subtilis*	10	14	13	-	18
9	*M.luteus*	18	18	-	12	-
10	*E.aerogenes*	12	15	18	-	10
11	*P.aeruginosa*	18	-	16	20	18
12	*S.sps.*	16	20	12	20	18

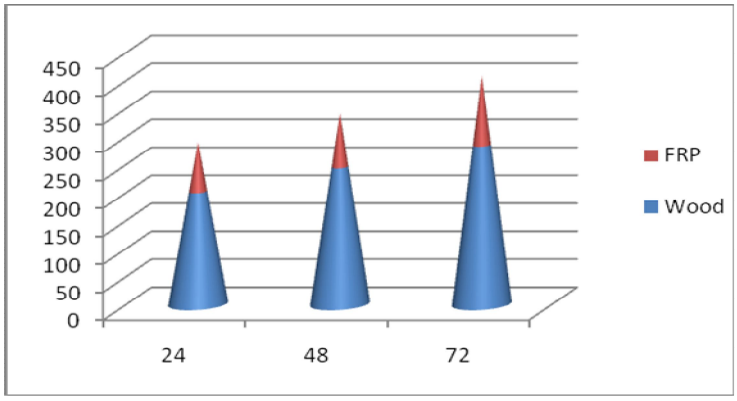

Fig.2 Showing Antimicrobial activity of *Ulva lactuca* in Methanolic Extraction (Antifouling paint preparation)

Table 3.Antimicrofouling activity (Zone of inhibition in mm) of *Ulva lactuca* and *Hypnea musciformis*.

S.No	Name of the Organisms	*Ulva lactuca* Zone of inhibition (mm) on biofilm bacterial isolats			*Hypnea musciformis* Zone of inhibition (mm) against biofilm bacterial isolats		
		Methanol	Chloroform	Acetone	Methanol	Chloroform	Acetone
1	*D.grandis*	24	10	20	12	-	-
2	*D.radiodurans*	16	-	-	-	-	-
3	*M.halophilus*	25	-	-	-	-	-
4	*S.roseus*	18	15	18	16	-	-
5	*S.urea*	10	-	10	-	-	-
6	*A.erythreum*	14	10	-	12	-	-
7	*A.haemolyticum*	20	18	-	-	-	-
8	*B.subtilis*	24	14	18	10	-	-
9	*M.luteus*	20	16	10	-	-	-
10	*E.aerogenes*	14	-	-	12	16	-
11	*P.aeruginosa*	15	15	-	16	14	-
12	*S.sps.*	24	16	25	-	14	-

Table 4. Bacterial density on biofilm of wooden and FRP panel (*Ulva lactuca* extract)

S.No	Exposure duration (hrs)	Before Extract		After Extract	
		Wood	FRP	Wood	FRP
1	24	201	88	151	123
2	48	245	95		
3	72	284	123		

Table 5. Bacterial density on biofilm of wooden and FRP panel (*Hypnea muiformis* extract)

S.No	Exposure duration (hrs)	Before Extract		After Extract	
		Wood	FRP	Wood	FRP
1	24	201	88	284	123
2	48	245	95		
3	72	284	123		

REFERENCES

1. Whal, M., (1989). Marine epibiosis.Fouling and antifouling,Some basic aspects. Mar.*Ecol.Prog*.Ser.58.pp.175-789.
2. Abarzua,S and Jakubowski, S. (1995). Biotechnological investigation for the prevention of biofouling. Biological and biochemical principles for the prevention biofouling . Mar.Ecol.Pro.Sec. 123:pp.301-312.

3. Suhelen Egan, (2001). Production and regulation of fouling inhibitory compounds by marine bacterium *Pseudoalotermonous tunicate*, Ph.D Thesis, the University of new South Wales, Sydney, Australia.
4. Maréchal JP and Hellio C (2009) Green antifouling and potential development for new antifouling technologies. Int. J. Mol. Sci. 10: in press
5. Gitlitz,M.H.,(1981). Recent developments in marine antifuling coationgs. J.Coatings. Technol.53.pp.416-52.
6. Scmitt,T.M., Hay, M.E and Lindquist (1995). Constrains on chemically mediated co-evolution. Multiple functions for seaweed secondary metabolites. *Ecology*, 76:pp107-123.
7. Butman ,C.A.,(1987). Larval settlement of soft sediment invertebrates, the sptil scales of pattern explained by active habitat selection and the emerging role of hydrodynamical processes. Oceanoger.Mar.biol.A Revi.25pp.113-1665.
8. Wahl, M., Kroger, K and Lenz, M(1994). Non toxic protection against epibiosis. *Biofouling* 12(1-3):pp. 205-226.
9. Liu, D.,Magurire.,Lau.,Okamura and Aoyama, I (1997). Wat. Res.,31:pp.2363.
10. Voulvoulis, N., Scrimshaw, M.D and Lester (1999). Chemosphere., 38:pp.3503.
11. Yang,R.,Li.C., Lin,Y Peng, G.,She, Z and Zou (2006). Lactones from a brown alga endophytic fungus (No. ZZF36) from the South China Sea and their antimicrobial activity. *Bioorganic & Medicinal Chemistry Letters, 16:pp.4205-4208*.

RESEARCH ARTICLE

Extraction of Bioactive Metabolites from *Aristolochia indica* L.and Docking Studies using Bioinformatics Tool.

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ABSTRACT

The *Aristolochia indica* L. (Aristolochiaceae) valuable medicinal plant in the genus of *Aristolochia*, it is an important medicinal source of plants and physiologically active compounds that belong to different chemical classes is the subject of research in pharmacological and chemical studies. Several referel studies show that the extract of *Aristolachia indica* L. had moderately significant antibacterial and significant antifungal activity. In this research variety of secondary metabolites were extracted and characterized from *Aristolochia indica* L. using UV visible, FTIR and GCMS techniques. The GCMS study shows that the number of phytochemicals, which is present in the plant based on the peak area and percentage. We short listed the phyto compounds were subjected to docking process using bioinformatics tool for future pharmacological application.After docking the compounds were interacted with target receptors and inhibit the activity.

Keywords: *Aristolochia indica* L., pharmacological, phytochemicals, docking, bioinformatics,

INTRODUCTION

Aristolochia indica L is one of the 300 species belonging to the family Aristolochiaceae and it is used in indigenous system of medicine. Leaves of this plant are used to treat cholera, fever and bowel troubles. Roots are bitter, acrid digestive and also used to treat ulcers, leprosy and all types of poisonous bites [1]. The genus *Aristolochia* an important source of physiologically active compounds that belong to different chemical classes is the subject of research in pharmacological and chemical studies. This genus contains a large number of terpenoid compounds; particularly diterpenes [2].India has a rich flora that is widely distributed throughout the country. Herbal medicines

have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced such as Ayurveda, Unani and Siddha. Several plant species are used by many ethnic groups for the treatment of various ailments ranging from minor infections to dysentery, skin diseases, asthma, malaria and a horde of other indications. The past three decades have seen a dramatic increase in microbial resistance to antimicrobial agents that leads to repeated use of antibiotics and insufficient control of the disease [3].

Natural products from medicinal plants either as pure compounds or as standardized extracts provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products, interest particularly in edible plants has grown throughout the world. Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds [4]. Metabolomics is a comprehensive metabolite analysis and it is used to find increasing application as a tool to measure and enable the manipulation of the phytochemical content of foods and to identify the measures of dietary intake and to understand human and animal responses to phytochemicals in the diet [5].

Natural plant compounds have an unexceptional influence in pharmacy as they provide an uncountable number of invaluable lead molecules. Assessing the clinical and biological potential and determining the pharmacokinetics of herbal constituents is also an important area which is related to phytochemical constituents [6].FTIR-Spectrophotometer has become a standard in chemical industry for monitoring the different concentrations of reagents and by-products. However, representing chemical samples by FTIR spectra, which spectra are characterized by hundreds if not thousands of variables conveys their own set of particular challenges because they necessitate to be analyzed in a high-dimensional feature space and many of these features are likely to be highly correlated and surely affected by noise[7].

To evaluate the antimicrobial activities of ethanolic extract of *Aristolachia indica* L. which was a creeper used as traditional folk medicine for the treatment of different infectious diseases and disorders. The antimicrobial activities of the extract against 12 strains belong to bacterial and fungi species were tested by using agar diffusion method. They have observed that ethanolic extract of *Aristolachia indica* L. had moderately significant antibacterial and significant antifungal activity. [8] Essential oil from dry matured stem of *Aristolochia indica* L. Aristolochiaceae was investigated by GC/MS. A total of 15 compounds were identified and representing 91.2% of the total oil. The major constituents of oil were *trans*-pinocarveol and its composition 24.2% and the contribution of Pinene is 16.4% and Pinocarvone is 14.2%[9]. Phytochemical researches nowadays focus on bio-assay guided revealing of the therapeutic profile and synergism of medicinal herbs and their constituents. [10] *Aristolochia indica* has been widely used in the traditional medicine for the treatment of a variety of diseases. The Research study shows the different extracts of roots of *A. indica* were evaluated for their antiinflammatory, antipruritic and mast cell stabilizing activity. [11]

MATERIALS AND METHODS

The Plant *Aristolochia indica* L. was collected from sacred grove forest of Aanaivari village, Rayavaram Panchayat, Arimalam block, Pudukkottai District, Tamil Nadu, India during the month of February 2012. The collected plants and their leaf parts were cleaned with tap water and dried under shade, then ground well to fine powder. About 100 g of dry leaf powder of was *Aristolochia indica* L .extracted with ethanol (Fig.1) at 60^{o}c to 70^{o}c by continuous hot percolation using soxhlet apparatus. The extraction was filtered and kept in oven at 50^{o}c for 24 hours to evaporate the extracts from them.A greenish black waxy residue was obtained. Phytochemical constituents like alkaloids, flavonoids. Carbohydrates, glycosides, phytosterols, fixed oil and fats, proteins, phenolic compounds, tannis and saponins of ethanol solvent extract of *Aristolochia indica* L. were analyzed qualitatively. The secondary metabolites of the plant were extracted and characterized from *Aristolochia indica* L. using UV visible, FTIR and GCMS techniques. Finally the protein structure retrived from PDB (Protein Data Bank) and docking the identified secondary phytocompounds using through HEX tool.The identified structure were correlated with pharmacological application.

RESULTS AND DISCUSSION

Phytochemical constituents like alkaloids, flavoniods, carbohydrates, glycosides, phytosterols, fixed oil and fats, proteins, phenolic compounds, and saponins of *Aristolochia indica* L ., were analyzed by qualitatively and reported in Table 1**.** On the basis of UV-Visible and FT-IR spectral analysis on *Aristolochia indica* L .,we have found the following data.UV-Visible spectra was yielded 4 elevations (355.96nm, 1.2091 and 670.70nm, 0.17306) and the values were interpreted with table values and confirm the presence of phenolics in the given sample. A FT-IR spectrum was yielded Maximum peak level 3930.36 cm^{-1} and Minimum peak level 646.80 cm^{-1}**.** FT-IR studies confirm the presence of functional groups in the compound compared with refereal values, the identified compound was may be phenolics family.GCMS Study was finalized the compound as a phenolic.

The plant Species *Aristolochia indica* L was subjected to docking studies to GCMS study for Phytochemical Analysis. The Gas Chromatography and Mass Spectrometry shows that the number of phytochemicals which is present in the *Aristolochia indica* L. It includes 2-methoxy 4-vinyl phenol, 1-octanol 2,7-dimethyl ricinoleic acid and 13 alpha benzoxy lupanin. It also shows that the structure and functions of phytochemical compounds.

Based on the peak area and percentage we short listed the phytochemical compound 2-methoxy 4-vinyl phenol and this compound was subjected to docking process. Fig 3 shows that the structure visualization of the target protein. The Structure of the protein was retrieved from Protein Data Bank and submitted in to Hex tool (Fig.2). The Structure of the phytochemical compound was retrieved from Pubchem Compound database. Both the structures were docked by using Hex tool. After docking the phytochemical compound was interacted with target receptor and inhibits the activity (Fig 4).

CONCLUSION

The medical plant *Aristolochia indica* L. was selected and subjected to GCMS study for the identification of phytochemical compounds. FTIR Spectrophotometer analysis was also performed to identify the wave length and frequencies. From these results we suggested that *Aristolochia indica* L. might be a source of large amount of proto alkaloids and it has an antibiotic and antioxidant properties. Aristolochia indica L. extracts possess compounds with antioxidant and antimicrobial properties which can be used to treat cancer.

REFERENCES

1. P.B. Kanjilal and Rums Kotoky. (2009) Chemical Composition of the Stem Oil of *Aristolochia indica L*. J. Essent. Oil Res., 21(1): 32-34
2. Alison Geraldo Pacheco, Patrícia Machado de Oliveira, Dorila Piló-Veloso and Antônio Flávio de Carwash Alcântara.(2009)13C-NMR Data of Diterpenes Isolated from *Aristolochia* Species, Molecules 14:1245-1262
3. Prashanth Kumar .V, Neelam S. Chechen, Garish Padh and M. Rajani. (2006). Search for antibacterial and antifungal agents from selected Indian medicinal Plants. Journal of Ethnopharmacology 107:182–188
4. Sasidharan S, Chen Y, Saravanan D, Sundram K.M, Otari K.V, Gaikwad P.S, Shete R.V and Upasani C.D. (2012).Protective effect of aqueous extract of *Spinacia oleracea* leaves in experimental paradigms of inflammatory bowel disease. Inflammopharmacology. 11: 23-28
5. McGhie T.K and Rowan D.D. (2012).Metabolomics for measuring phytochemicals, and assessing human and animal responses to phytochemicals in food science. Mol Nutr Food Res. 56(1):147-58
6. Wang D, Wang S, Chen X, Xu X, Zhu J, Nie L and Long X. (2012) Antitussive, expectorant and anti-inflammatory activities of four alkaloids isolated from Bulbus of Fritillaria wabuensis. J Ethnopharmacol. 139 (1):189-93.

7. Vergara A and Llobet E. (2011) Feature selection versus feature compression in the building of calibration models from FTIR-spectrophotometry datasets. Talanta. 15; 88:95-103.
8. Surendra kumar M, Rajeswari S and Astalakshmi N. (2011). Evaluation of antimicrobial activities of *Aristolochia indica (linn).* International Journal of Pharmacy and Pharmaceutical Sciences 3 (4): 1490-1491
9. Balasubramanian G, Sarathi M, Rajesh Kumar S and Sahul Hameed A.S. (2007)Screening the antiviral activity of Indian medicinal plants against white spot syndrome virus in shrimp Aquaculture 263:15–19
10. Bibi Y, Nisa S, Zia M, Waheed A, Ahmed S and Chaudhary M.F. In vitro cytotoxic activity of *Aesculus indica* against breast adenocarcinoma cell line (MCF-7) and phytochemical analysis. Pak J Pharm Sci. 25 (1):183-7.
11. Jessy Elizabeth Mathew, Srinivasan, Keloth Kaitheri, Seekarajapuram Dinakaran Vachala and Magi Jose. (2011). Anti-inflammatory, Antipruritic and Mast Cell Stabilizing Activity of *Aristolochia Indica L.* Iranian Journal of Basic Medical Sciences. 14 (5) : 422-427

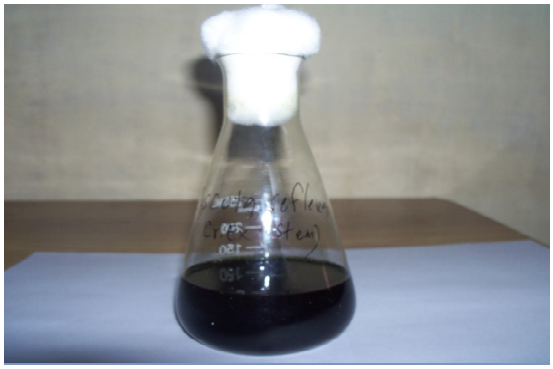

Fig.1.Leaf Extract of *Aristolochia indica* L.

Table 1: Qualitative Analysis of Phytochemicals in *Aristolochia indica* L.

S.No.	Metabolite	Results
1	Alkaloids	+++
2	Falavonoids	++
3	Terpinoids	++
4	Fixed oils	++
5	Phytosterols	++
6	Saponins	-
7	Phenolics	++
8	Fats	++
9	Carbohydrates	++
10	Proteins	+++
11	Glycosides	+
12	Tanins	+

+++ Present in high concentration ++ Present in medium concentration
\+ Present in low concentration - Not present in the sample

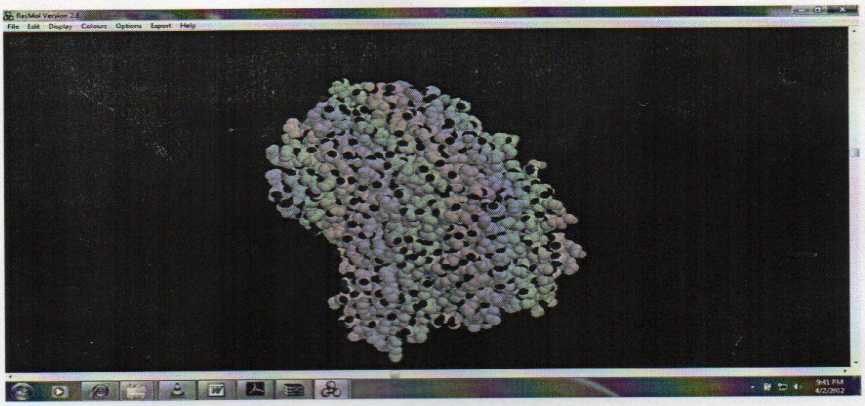

Fig 2: Structure Visualization for 2-methoxy 4-vinyl phenol

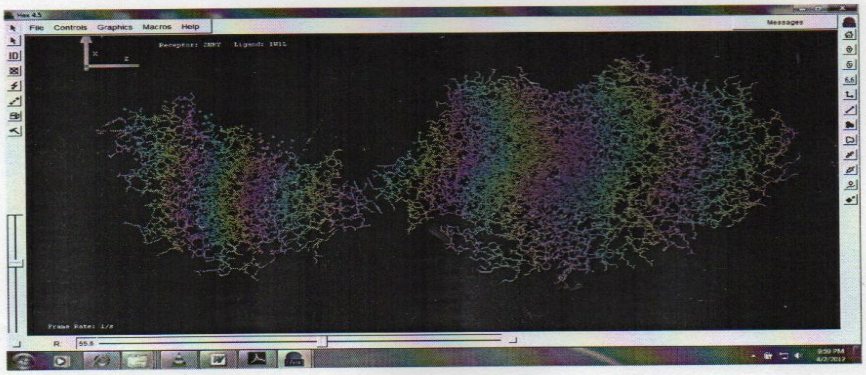

Fig 3: Before docking the phytochemical compound and Target Protein

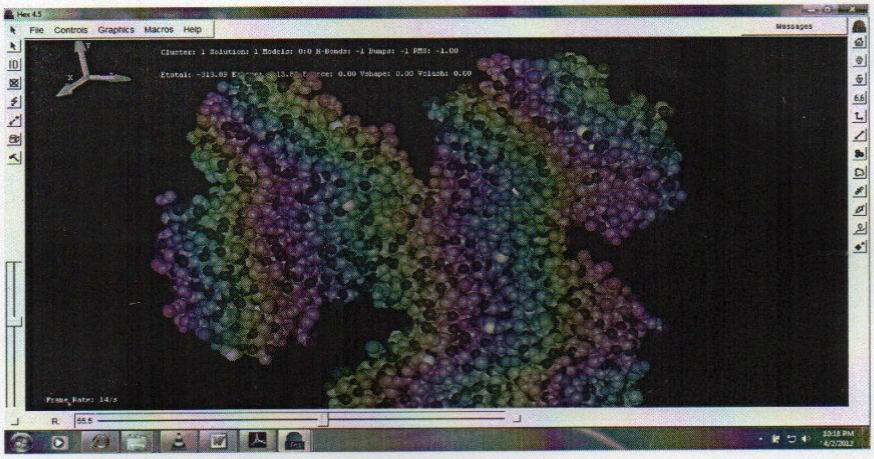

Fig 4: After docking the phytochemical compound and the Target Protein

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