ANALYSIS OF NUTRITIONAL COMPONENTS AND ACCUMULATION OF HEAVY METALS IN SELECTED BIOINDICATORS IN THERESPURAM AND VEMBAR COAST, THOOTHUKUDI, GULF OF MANNAR

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in partial fulfilment for the award of the degree of

Bachelor of Science in Zoology

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CERTIFICATE

This is to certify that the project entitled **ANALYSIS OF NUTRITIONAL COMPONENTS AND ACCUMULATION OF HEAVY METALS IN SELECTED BIOINDICATORS IN THERESPURAM AND VEMBAR COAST, THOOTHUKUDI, GULF OF MANNAR** is submitted to **St. Mary's College (Autonomous), Thoothukudi** in partial fulfilment for the award of the degree of **Bachelor of Science in Zoology** and it is a record of the work done during the year 2020-2021 by the following students

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INTRODUCTION

The human body needs nutrients to enable it function effectively and to maintain health. Fish have long been recognized as an important component of the diet of humans providing nutrients needed by the human body to function properly (Pigott and Tucker, 1990).

Knowledge of the proximate composition of fishes is essential to estimate their energy value and to plan the most appropriate industrial and commercial processing (Hanna, 1980). The differences in the chemical composition of fish meat for both the amount and the constituent components are due to the environmental and biological factors of the fish.

Biological factors which are commonly called intrinsic factors are factors derived from the type of fish such as the species or class, age and sex of fish (Muchtadi Tien, Sugiono and Ayustaningwarno, 2016).

The lack of sufficient protein is one of the most widespread nutritional deficiencies in many tropical countries (Eyo, 2001). All of the essential amino acids needed for good protein nutrition are present in fish meat. The protein content of fish is also important when considering quality and texture of the fish meat (Majid, Mokhlesi, Bastami, Khoshnood and Eshaghi, 2011). Fish having energy depots in the forms of lipids that indicates the quality of fish. The fish oil contains high amount of polyunsaturated fatty acid that reduce the serum cholesterol to prevent a number of coronary heart diseases.

Proximate analysis in fishes gives valuable information and helps to access the quality of the sample. The nutritional value of food depends on its biochemical composition.

Heavy metals are important component of pollution in the aquatic ecosystem. The sources of natural aquatic systems contamination of heavy metals are mostly household, manufacturing and man-made activities (Velez and Montoro, 1998).

Heavy metals are classified as essential and nonessential. Essential heavy metals are important for living organisms and may be required in the body in quite low concentrations. Non-essential heavy metals have no known biological role in living organisms.

The heavy metals Mn, Fe, Co, Ni, Cu, Zn and Mo are micronutrients. They are essential for growth. Either deficiency or excess of an essential heavy metal leads to diseases or abnormal conditions.

The contamination of heavy metals and metalloids in water and sediment, when occurring in higher concentrations, is a serious threat because of their toxicity, long persistence, bioaccumulation and biomagnification in the food chain (Eisler, 1993 and Has-schon, 2006). Fishes are considered to be most significant biomonitors in aquatic systems for the estimation of metal pollution level (Rashed, 2001 and Authman, 2008), they offer several specific advantages

in describing the natural characteristics of aquatic systems and in assessing changes to habitats (Lamas, 2007).

The accumulation of heavy metals in the aquatic environment has direct consequence to man and to the ecosystem. The impact of increasing concentration of such metal in the environment is further enhanced by their poor degradability, which results in bioaccumulation and transport along successive links of the food chain (Ciesielski *et al*., 2010).

Fishes are widely used to monitor the variations in marine environment of anthropogenic pollutants (Alemdaroglu and Erkakan, 2003). Fishes, crabs and shrimps form an important link in transferring the media to humans. Information on the level of heavy metal pollution in coastal origin is important because they cause serious environmental health hazards (Shukla, Rathi and Sastry, 2007).

The estimation of heavy metals in the food-chain will be used to know the heavy metal transfer to the human body through sea-food (Arun Kumar and Hema Achyuthan, 2005). The possible ways of heavy metal accumulation in fishes are through the direct uptake of water and food on the heavy metal polluted environment (Paquin, Farley, Santore, Kavvadas, Mooney, Winfield, Wu and Di Toro, 2003).

The heavy metals entering to the fish through gills and other organs have a chance to get accumulated in different parts of the body tissues and the

excessive amount can build up to a toxic level (Arun Kumar and Hema Achyuthan, 2005). Pollution by heavy metal has been the most widely studied and documented topic because of the disastrous effect and diseases that are produced in man after consumption of contaminated food. Coastal pollution due to chemicals is a global environmental problem.

This project was designed to determine the current level of protein, lipid and ash content and the effects of heavy metal contamination of selected bioindicators which are readily available and consumed in Thoothukudi Coast, Gulf of Mannar, Tamilnadu.

OBJECTIVES

- To analyse the physico-chemical parameters of the sea water in Therespuram coast and Vembar coast, Thoothukudi, Gulf of Mannar.
- To evaluate the proximate values of selected bioindicators such as *Sardinella longiceps, Lethrinus lentjan, Lethrinus microdon, Penaeus monodon* and *Penaeus semisulcatus.*
- To determine the concentration of heavy metals such as iron, copper, zinc in the water samples collected from Therespuram coast and Vembar coast, Thoothukudi.
- To determine the concentration of heavy metals such as iron, copper, zinc in the selected bioindicators from Therespuram coast and Vembar coast, Thoothukudi, Gulf of Mannar.
- To compare the concentration of heavy metal between the bioindicators of Therespuram coast and Vembar coast, Thoothukudi.

REVIEW OF LITERATURE

Presentation of research work depends on the comprehensive review of related topics in the selected subject. Study of literature in the relevant subject helps to analyze the data with clarity and clear presentation of observation.

Palani kumar *et al*., (2014) studied the proximate and major minerals composition of 23 medium sized marine fin fishes landed in the Thoothukudi coast of India and reported that the total calorific value of these food fishes are mainly influenced by the total fat content and also to a greater extent by the total protein content.

Roksana Huque *et al*., (2014) evaluated the comparative study of raw and boiled silver pomfret fish from coastal area and retail market in relation to trace metals and proximate composition and reported that in both areas the fish samples exceeded the standard limits set by FAO / WHO for Mn, Pb and Cr and boiling has no significant effects on these 3 metal concentrations.

Septina Mugi Rahayu *et al*., (2014) analysed the proximate, fatty acid profile and heavy metal content of selected by-catch fish species from Muara Angke, Indonesia and reported the heavy metal content Pb, Cd, Ni, Hg and As present in amounts below toxic levels. The proximate composition was found to be 15.00-14.70% protein, 0.44-2.78% fat, 69.01-76.61% water, 2.69-5.94% ash and 1.32-6.68% carbohydrate, whereas the fatty acid composition consists of

14.55-36.83% saturated fatty acid (SFA), 4.92-21.1% monounsaturated fatty acid (MUFA) and 10.9-23.06% polyunsaturated fatty acid (PUFAs).

Rahman *et al*., (2014) analysed the comparative study on proximate composition and heavy metal concentration of *Amblypharyngodon mola* and *Channa punctatus* collected from pond water and open water and reported that the level of proximate composition of *A.mola* and *C.punctatus* was as Moisture>Protein>Lipid>Ash and the study revealed that open water fishes accumulated more heavy metal in the muscle than pond water fishes.

Krishna *et al*., (2014) studied the Human health risk assessment of heavy metal accumulation through fish consumption from Machilipatnam Coast, Andhra Pradesh, India and reported the concentration of the metals in the fish muscle from Machilipatnam coast pose to health hazards to the consumers.

Concentrations of heavy metals Cu, Zn and Pb were estimated from Tuticorin, Tamilnadu (Isacc *et al*., 2014) in the collected marine fishes, *Pristis microdon* and *Scomberomorus guttats* and the results of the work recorded metal concentrations within the range or below the levels in similar species from global studies.

Imaobong (2015) evaluvated the proximate composition of the three commercial fishes commonly consumed in Akwa Ibon state, Nigeria and the data

showed that these species of fishes are rich in crude protein, lipid, moisture and ash and meet the requirement for human nutritional needs.

Chrisolite *et al*., (2015) evaluated the proximate and mineral composition of fifteen fresh water fishes of Thoothukudi, Tamil Nadu and reported that the fresh water fishes had protein and lipid content similar to marine fishes and can be used as a protein rich food relatively at a cheaper cost.

Victoria (2015) analysed the proximate composition of some tropical fish species and reported the four tropical fish species *Clarias gariepinus, Selar crumenoththalmus, Scomber scrombus* and *Pseudotolithus senegalensis* are high in protein and differed significantly in the moisture, protein and fat contents but the ash content were similar.

Rani *et al*., (2016) analysed the seasonal variation of proximate composition of tuna fishes from Visakhapatnam fishing harbor, East Coast of India and reported that the high moisture content was observed during pre-monsoon seasons in *E.affinis, A.thazard* while high protein concentration was seen in monsoon season in both *E.affinis* and *A.thazard* species and high mean concentration of fat was found in post monsoon season in the case of *E.affinis* and in monsoon season in the case of *A.thazard.* High ash percentage was noted in pre monsoon season in *E.affinis* and in post monsoon season in *A.thazard*.

Chrisolite Bagthasingh *et al*., (2016) analysed the seasonal variation in the proximate composition of sardine (*Sardinella gibbosa*) from Thoothukudi coast and the results showed on marked variation in the protein, carbohydrate and ash contents whereas greater seasonal variation in the lipid content of the sample.

Njinkoue *et al*., (2016) evaluated the proximate composition, mineral content and fatty acid profile of two marine fishes from Cameroonian Coast; Pseudotolithus types (Bleeker,1863) and Pseudotolithus elongatus (Bowdich,1825) and the results showed that the Na/k ratio values and ω3 fatty acid contents suggested that consumption of these two fish species could be recommended to prevent cardiovascular disease.

Fanuel Jim *et al*., (2017) analysed the comparative analysis of nutritional value of *Oreochromis niloticus* (Linnaeus), Nile Tilapia, meat from three different ecosystems and the results suggested that the effluent from sewage works and fertilizer industries caused pollution and proliferation of water hyacinth, contributing to pervasion of the chemical composition of fish.

Kaleshkumar Karunanidhi *et al*., (2017) studied the first report on distribution of heavy metals and proximate analysis in marine edible puffer fishes collected from Gulf of Mannar Marine Biosphere Reserve, South India and the proximate composition was determined in edible muscle tissues all the five species. The highest and lowest protein contents were observed *T. oblongus* (20.6±0.6%) and *C. patoca* (17.9±0.3%).

Syed Raffic Ali *et al*., (2017) studied the proximate composition of commercially important marine fishes and shrimps from the Chennai coast, India and the results showed that fishes and shrimps can serve as an alternative source of high-quality protein, energy and mineral supply for human consumption and feed formulation in animals.

Bilal hussain *et al*., (2018) recorded that the wild fish and farmed fish species had highest protein contents and amino acid profile and hence appeared to be the best for human consumption on the article named study on impact of habitat degradation on proximate composition and amino acid profile of Indian major carps from different habitats.

Hawaibam romharsha *et al*., (2018) evaluated the proximate composition, total amino acids and essential mineral elements of some cyprinid fishes of Manipur, India and reported that these fish species are significant sources of protein and essential mineral elements and they will provide good nutrition to the people of the region.

Issac (2019) studied the determination of selected heavy metal and analysis of proximate composition in some fish species from Ogun river, South western Nigeria and reported that the concentration of the metals in all cases were beyond regulatory limits by international standards and all the fishes had high moisture content between 75 and 80% while fat was the lowest nutrient 0.88-1.89% in all fish species.

Egerton *et al*., (2020) analysed the proximate composition of three marine pelagic fish, blue whiting (*Micromesistius poutassou*) boarfish (*Capros aper*) and Atlantic herring (*Clupea harengus*) and reported that these fishes contained significant amount of protein (16-17%) lipids (4-11%) and minerals (2-6%ash). The proteins, particularly from boar fish, had close to optimum amino acid profiles for human and fish nutrition.

Ahmed *et al*., (2020) studied the proximate composition and fatty acid profiles of selected fish species from Pakistan and reported that the fish species contained substantial amount of omega-3 PUFAs that suggest fish species especially rainbow trout as a good resources vigorous diet for human beings. Rainbow trout and thela fish are an excellent source of PUFAs total fatty acids whereas mahseer and rainbow trout were found to be an excellent source of protein with a low level of fat.

STUDY AREA

India is one among the 12 mega-biodiversity countries and 25 hotspots of the richest and highly endangered eco-regions of the world. Among the Asian countries, India is perhaps the only country that has a long record of inventories of coastal and marine biodiversity dating back to at least two centuries.

Tamil Nadu is endowed with rich biodiversity, right from marine coastal system in the Gulf of Mannar to Terrestrial evergreen forests in the Western Ghats. The Gulf of Mannar Biosphere Reserve (GOMBR) extending over 10,500 sq. km and includes 21 island of the national park (560 sq km) is the first Marine Biosphere Reserve in the country which is internationally recognized under the UNESCO-MAB programme.

Tuticorin is famous for pearl fishing and hence it is called Pearl City. It includes marine components such as coral reefs, seaweeds beds, sea grasses, salt marshes and mangroves. The length of the coast line of Tuticorin district is 163.5 km. The fishermen of Thoothukudi are mostly using gillnets and trawl nets in the motorized country craft and trawl boats, respectively. In Thoothukudi all FRP boats, wooden vallams and catamarans are motorized and totally there are about 4,200 traditional crafts altogether.

Therespuram is present in Tuticorin. There are 600-700 canoes and FRP boats and 1500 fishermen are involved in marine fishing. They are doing both

single and multiday fishing practices based on the seasons. They are using gill nets, bottom set gill nets, crab nets to catch fishes and other marine fauna.

Vembar is a village situated along the East Coast road at the north end of Tuticorin district. Main source of income are fishing and Palmyra tree climbing. There are 50 boats and 1000 fishermen are involved in marine fishing. The Vembar coast lies along the Gulf of Mannar.

MATERIALS AND METHODS

SAMPLE COLLECTION

The samples for the present study were collected from Therespuram coast and Vembar coast in Thoothukudi district, Gulf of Mannar. Therespuram is located at Lat.8.764166˚N, Long.78.134834˚E and Vembar is located at Lat.8.62035˚N, Long.77.97732˚E.

Water sample were collected in pre-cleaned, polyethylene bottles. Water sample is used for the analysis of physico-chemical parameters and heavy metal analysis.

ANALYSIS OF WATER SAMPLE

The physico-chemical parameters of the water samples were analysed by the following methods.

DETERMINATION OF pH

The pH of the water sample was recorded by using digital pH meter.

MEASUREMENT OF TEMPERATURE

The temperature of water sample was recorded by using mercury thermometer.

DISSOLVED OXYGEN (WINKLER'S METHOD)

Dissolved oxygen of water sample was recorded by Winkler's method.

BIOLOGICAL OXYGEN DEMAND

BOD was analysed by BOD analyser bottle.

SALINITY

Salinity was determined by using hand refractometer and the measurement was digitally read out.

DETERMINATION OF ALKALINITY

The water sample (50ml) was taken in a conical flask and 2-3 drops of phenolphthalein indicator was added titrated against 0.02N sulphuric acid to the colourless endpoint. Noted the reading as 'P'. Then to the same solution 2-3 drops of methyl orange indicator was added and continued the titration until the yellow colour turned into orange (end point). Noted the reading as 't' which was the volume of titrate used for both the titrations. Total alkalinity in water sample was calculated and expressed the value as mg/l.

DETERMINATION OF AMMONIA

25ml of water sample was taken in a conical flask and 1ml of citrate solution, 1ml of phenol nitroprusside solution and 1ml of bleaching powder solution were added. The contents were mixed well and the flask was covered with aluminium foil. The flask was then kept in dark place for about 2hrs. After 2hrs the OD of the blue coloured complex was measured at 630nm.

DETERMINATION OF SILICATE

To a 25m1 polypropylene measuring flask, transfer 20ml of sample solution. Add 1ml of 3.75 M sulfuric acid and 1.5ml of 0.2 M (as molybdenum) molybdate solution to it and mix thoroughly. After heating it in a boiling water bath for 10 min, cool to room temperature with water. Add 0.5ml of 5% PVA solution and 0.5ml of 4×10^{-3} M Malachite Green solution. Dilute to the mark with water and stand for 60 min after mixing. Measure the absorbance at 595 nm.

DETERMINATION OF PHOSPHATE

10ml of the fleshy prepared mixed reagent was added to 50ml of water samples. The contents of the flask were thoroughly mixed and allowed to stand for about 15 minutes at room temperature. The optical density was measured at 882nm. By comparing the absorbance of the sample with that of standard solutions the total phosphate was calculated and expressed in mg/l.

PROXIMATE COMPOSITION ANALYSIS

The process of proximate analysis for protein, lipid, ash of the dried samples were determined by the standard AOAC method. The protein content of samples was estimated by Lowry's method. All samples were analysed in triplicate interpretation.

ESTIMATION OF PROTEIN

The protein content was estimated using following method of Lowry et al., (1951). 10mg of sample was taken and homogenized with 5ml or 10% Trichloroacetic acid in a homogenizer. The homogenates were centrifuged and dissolved thoroughly. 5ml of 0.1N sodium hydroxide solution and kept in a water bath at 60˚C and 70˚C for 10 minutes. From the solution, 0.5ml was pipette out and poured in a clean dry test tube.

To this 4ml of copper solution was added. It was lateral shaking and kept in room temperature for 10 minutes. To this 0.4ml folin phenol reagent was added.

The test tube was shaken well for uniform mixing and kept in room temperature for another 30 minutes. The blue colour appeared at 640nm against a reagent blank in UV spectrophotometer.

The standard curve obtained using Bovine serum albumin expressed the protein content mg/g.

$$\% \text{ of protein} = \frac{\text{Standard value} \times \text{OD value} \times 100}{\text{Weight of the tissue}}$$

ESTIMATION OF LIPID

Lipid was estimated following the method of Bragdon (1951). A known weight about 10mg sample was grouped with a few ml of chloroform solution and was centrifuged at 3000 rpm for 15 minutes. The supernatant was evaporated to dryness. The 3ml of 2% potassium dichromate in concentrated sulphuric acid was added, which was followed by 3ml of distilled water. The developed colour was read in spectrophotometer using filter 640nm against a reagent blank. The standard curve was obtained by using cholesterol and the lipid was expressed in mg/g.

$$\% \text{ of lipid} = \frac{\text{Standard value} \times \text{OD value} \times 100}{\text{Weight of the tissue}}$$

ESTIMATION OF ASH

Ash content was determined based on the standard AOAC method. Approximately, 2±0.2 g of moisture free sample was taken individually in a silica crucible and placed in the muffle furnace set at 550°C for 12-15 hours. The difference between the initial and the final weight gave the ash content.

$$\% \text{ of ash} = (\text{Weight of ash} / \text{Weight of sample}) \times 100$$

HEAVY METAL ANALYSIS

Samples were collected from Therespuram coast and Vembar coast. The samples were dried in hot air oven at 80°C for 24 hours. The dried tissues were ground with mortar and pestle into fine powder. 1g of powder was digested with 9ml concentrated nitric acid and 1ml of perchloric acid. The sample was heated by keeping on a hot plate until evaporation of the sample. Then the digested sample was filtered through Whatmann No.1 filter paper and make up to 25ml in a volumetric flask using double distilled water. The digested sample was stored in pre washed polythene bottle until heavy metal analysis. The heavy metals such as Fe, Cu and Zn were analysed using atomic Absorption Spectrophotometer (AA SELI CO SD 194). Blank was also prepared by digesting the reagents without the samples.

RESULTS

PHYSICO-CHEMICAL PARAMETERS OF THE WATER SAMPLE

Physico chemical parameters of the water sample from the study areas were analysed and the data obtained were tabulated. (Table 1).

ECOLOGICAL CHARACTERISTICS

Ecological characteristics of the selected bioindicators from the study areas were recorded. (Table 2).

PROXIMATE COMPOSITION OF BIOINDICATORS IN THERESPURAM COAST, THOOTHUKUDI.

In the present study, protein, lipid and ash content were estimated and the values were tabulated (Table 3) (Fig. 1)

Sardinella longiceps

The protein content in *Sardinella longiceps* was 19.86%, the lipid content was 2.29% and the ash content was 2.33%.

Lethrinus lentjan

The protein content in *Lethrinus lentjan* was 18.84%. The lipid content was 3.67%. The ash content was 1.79%

Lethrinus microdon

The protein content in *Lethrinus microdon* was 21.25%. The lipid content was 1.52%. The ash content was 0.34%

Penaeus monodon

The protein content in *Penaeus monodon* was 8.98%. The lipid content was 6.38%. The ash content was 2.76%

Penaeus semisulcatus

The protein content in *Penaeus semisulcatus* was 19.56%. The lipid content was 6.65 %. The ash content was 3.30%

PROXIMATE COMPOSITION OF BIOINDICATORS IN VEMBAR COAST, THOOTHUKUDI

Proximate composition of protein, lipid and ash were estimated and the values were tabulated (Table 4) (Fig. 2)

Sardinella longiceps

The protein content in *Sardinella longiceps* was 19.90%. The lipid content was 3.02%. The ash content was 1.89%

Lethrinus lentjan

The protein content in *Lethrinus lentjan* was 21.75%. The lipid content was 3.78%. The ash content was 1.96%

Lethrinus microdon

The protein content in *Lethrinus microdon* was 21.20%. The lipid content was 1.68%. The ash content was 0.32%

Penaeus monodon

The protein content in *Penaeus monodon* was 8.72%. The lipid content was 6.45%. The ash content was 2.42 %

Penaeus semisulcatus

The protein content in *Penaeus semisulcatus* was 18.27%. The lipid content was 6.65%. The ash content was 3.10%

HEAVY METAL CONCENTRATION IN WATER SAMPLES FROM THERESPURAM COAST AND VEMBAR COAST, THOOTHUKUDI

The concentration level of iron, copper and zinc in the water sample were recorded and tabulated. (Table 5) (Fig 3). In Therespuram coast, water showed 28.57mg/l of iron, 5.33mg/l of copper and 39.14mg/l of zinc. In Vembar coast water showed 12.27mg/l of iron, 6.72mg/l of copper and 24.32mg/l of zinc.

ACCUMULATION OF HEAVY METALS IN BIOINDICATORS (µg/g) FROM THERESPURAM COAST, THOOTHUKUDI

The accumulation of heavy metals iron, copper and zinc in bioindicators were estimated and the results were tabulated (Table 6) (Fig 4)

Sardinella longiceps

In the present study *Sardinella longiceps* showed 48.22µg/g of iron, 12.28µg/g of copper and 18.24µg/g of zinc respectively.

Lethrinus lentjan

Lethrinus lentjan showed 34.53µg/g of iron, 22.65µg/g of copper and 15.35µg/g of zinc respectively.

Lethrinus microdon

Lethrinus microdon showed 26.22 µg/g of iron, 20.45µg/g of copper and 14.98µg/g of zinc respectively.

Penaeus monodon

Penaeus monodon showed 2.34µg/g of iron, 1.67µg/g of copper and 2.42µg/g of zinc respectively.

Penaeus semisulcatus

Penaeus semisulcatus showed 7.19µg/g of iron, 1.59µg/g of copper and 4.50µg/g of zinc respectively.

ACCUMULATION OF HEAVY METALS IN BIOINDICATORS (µg/g) FROM VEMBAR COAST, THOOTHUKUDI

The accumulation of heavy metals iron, copper and zinc in bioindicators were estimated and the results were tabulated (Table 7) (Fig 5).

Sardinella longiceps

In the present study *Sardinella longiceps* showed 31.74µg/g of iron, 9.89µg/g of copper and 17.41µg/g of zinc respectively.

Lethrinus lentjan

Lethrinus lentjan showed 28.65µg/g of iron, 5.42µg/g of copper and 19.62µg/g of zinc respectively.

Lethrinus microdon

Lethrinus microdon showed 27.45 µg/g of iron, 5.32µg/g of copper and 19.43µg/g of zinc respectively.

Penaeus monodon

Penaeus monodon showed 3.48µg/g of iron, 1.38µg/g of copper and 3.64µg/g of zinc respectively.

Penaeus semisulcatus

Penaeus semisulcatus showed 6.79µg/g of iron, 1.63µg/g of copper and 2.11µg/g of zinc respectively.

Sardinella longiceps

Lethrinus lentjan

Lethrinus microdon

Penaeus monodon

Penaeus semisulcatus

TABLE 1: PHYSICO-CHEMICAL PARAMETERS OF WATER SAMPLES FROM THERESPURAM AND VEMBAR COAST, THOOTHUKUDI, 2020-2021

S.NO	PHYSICO-CHEMICAL PARAMETERS	THERESPURAM COAST	VEMBAR COAST
1	Temperature (˚C)	28	27
2	pH	8.30	7.8
3	DO (mg/l)	5.63	1.127
4	BOD (mg/l)	14.09	2.82
5	Salinity (ppt)	36	36
6	Alkalinity (mg/l)	150	135
7	Phosphate (µg/l)	0.16	0.13
8	Ammonia (µg/l)	0.497	0.456
9	Silicate (µg/l)	6.48	6.78

TABLE 2: ECOLOGICAL CHARACTERISTICS OF BIOINDICATORS FROM THERESPURAM AND VEMBAR COAST, THOOTHUKUDI, 2020-2021

SCIENTIFIC NAME	FAMILY	ENGLISH NAME	FEEDING HABIT	BIOTYPE COMPLEX	LENGTH (cm)	WEIGHT (g)
Sardinella longiceps	Clupeidae	Indian oil sardine	Omnivore	Pelagic zone	15	25
Lethrinus lentjan	Lethrinidae	Red spot emperor	Omnivore	Pelagic zone	27	420
Lethrinus microdon	Lethrinidae	Long face emperor	Omnivore	Pelagic zone	30	410
Penaeus monodon	Penaeidae	Black tiger shrimp	Omnivore	Benthic zone	11	14
Penaeus semisulcatus	Penaeidae	Green tiger prawn	Omnivore	Benthic zone	12	11

TABLE 3: PROXIMATE COMPOSITION (%) OF BIOINDICATORS IN THERESPURAM COAST, THOOTHUKUDI, 2020-2021

S.NO	SPECIES	PROTEIN (%)	LIPID (%)	ASH (%)
1	*Sardinella longiceps*	19.86	2.29	2.33
2	*Lethrinus lentjan*	18.84	3.67	1.79
3	*Lethrinus microdon*	21.25	1.52	0.34
4	*Penaeus monodon*	8.98	6.38	2.76
5	*Penaeus semisulcatus*	19.56	6.65	3.30

FIG 1: PROXIMATE COMPOSITION (%) OF BIOINDICATORS IN THERESPURAM COAST, THOOTHUKUDI, 2020-2021

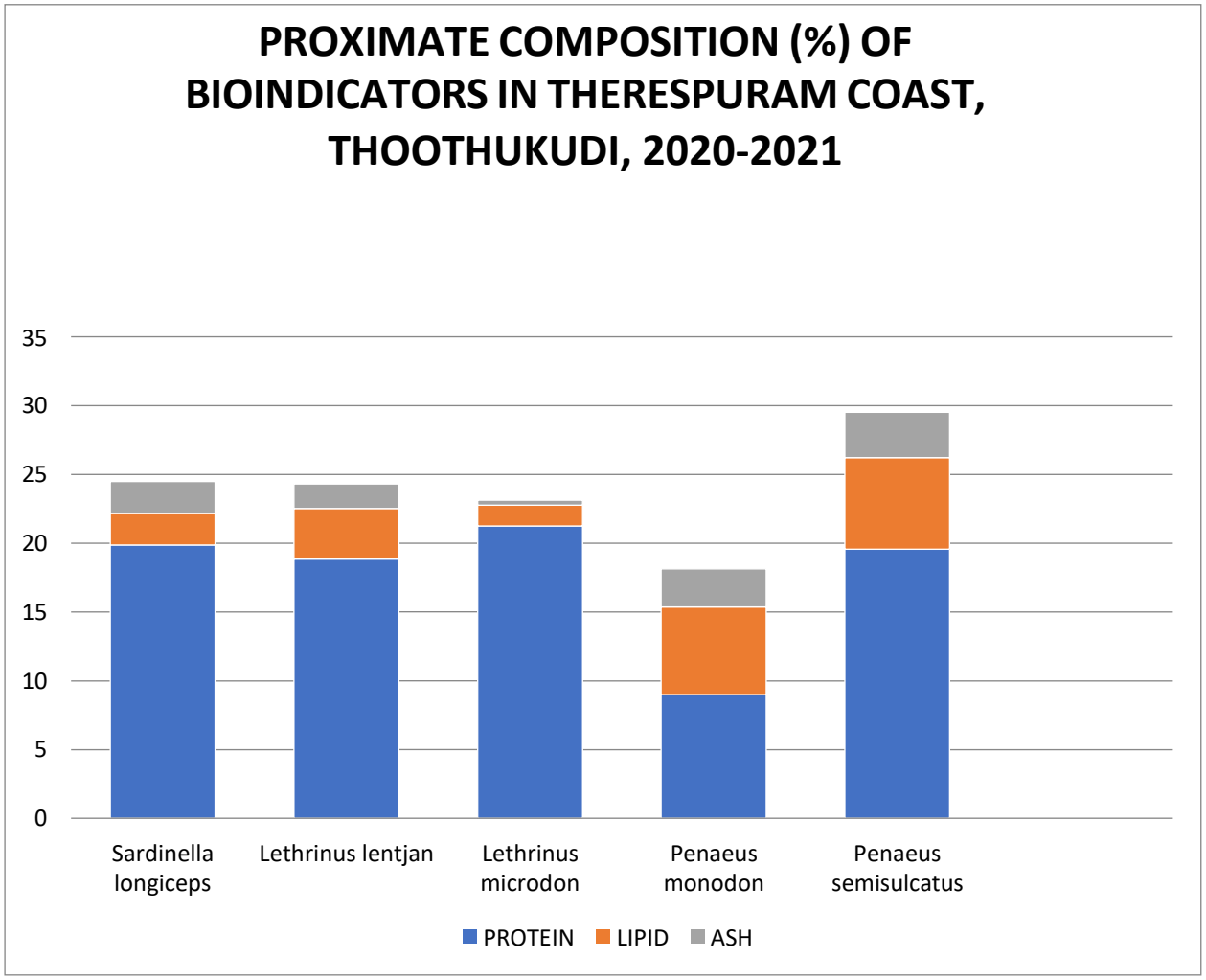


TABLE 4: PROXIMATE COMPOSITION (%) OF BIOINDICATORS IN VEMBAR COAST, THOOTHUKUDI, 2020-2021

S.NO	SPECIES	PROTEIN (%)	LIPID (%)	ASH (%)
1	*Sardinella longiceps*	19.90	3.02	1.89
2	*Lethrinus lentjan*	21.75	3.78	1.96
3	*Lethrinus microdon*	21.20	1.68	0.32
4	*Penaeus monodon*	8.72	6.45	2.42
5	*Penaeus semisulcatus*	18.27	6.65	3.10

FIG 2: PROXIMATE COMPOSITION (%) OF BIOINDICATORS IN VEMBAR COAST, THOOTHUKUDI, 2020-2021

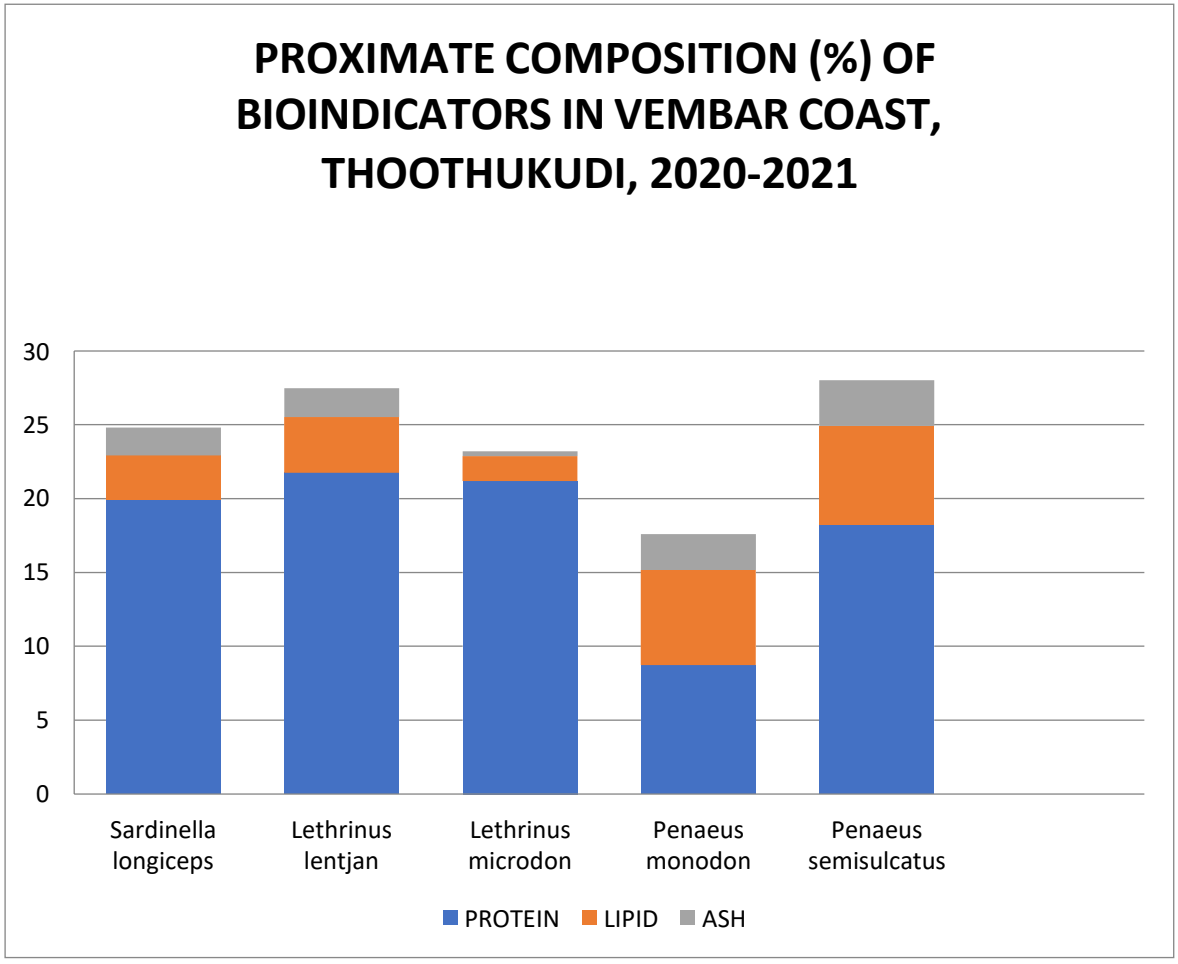


TABLE 5: HEAVY METAL CONCENTRATION (mg/l) IN WATER SAMPLES FROM THERESPURAM COAST AND VEMBAR COAST, THOOTHUKUDI, 2020-2021

S.NO	HEAVY METALS	THERESPURAM	VEMBAR
1	Iron(mg/l)	28.57	12.27
2	Copper(mg/l)	5.33	6.72
3	Zinc(mg/l)	39.14	24.32

FIG 3: HEAVY METAL CONCENTRATION (mg/l) IN WATER SAMPLES FROM THERESPURAM COAST AND VEMBAR COAST THOOTHUKUDI, 2020-2021

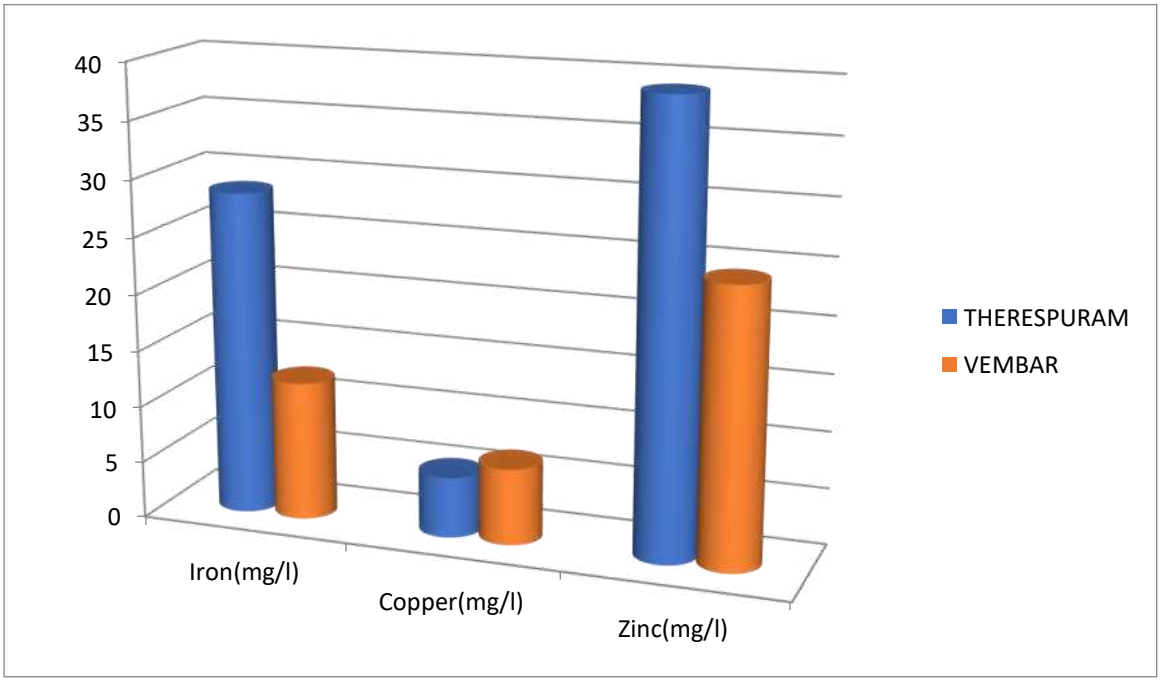


TABLE 6: ACCUMULATION OF HEAVY METALS IN BIOINDICATORS (µg/g) FROM THERESPURAM COAST, THOOTHUKUDI, 2020-2021

S.NO	SPECIES	IRON (µg/g)	COPPER (µg/g)	ZINC (µg/g)
1	*Sardinella longiceps*	48.22	12.28	18.24
2	*Lethrinus lentjan*	34.53	22.65	15.35
3	*Lethrinus microdon*	26.22	20.45	14.98
4	*Penaeus monodon*	2.34	1.67	2.42
5	*Penaeus semisulcatus*	7.19	1.59	4.50

FIG 4: ACCUMULATION OF HEAVY METALS IN BIOINDICATORS (µg/g) FROM THERESPURAM COAST, THOOTHUKUDI, 2020-2021

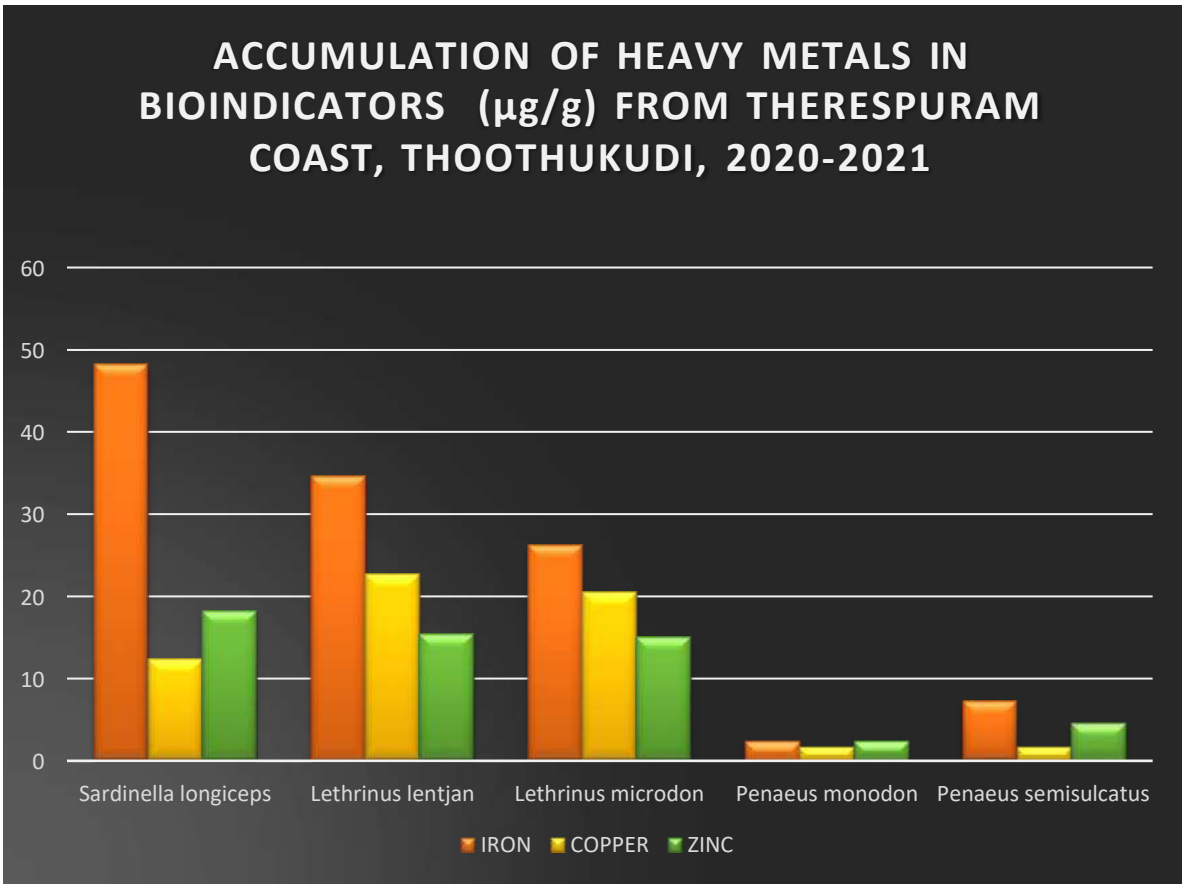
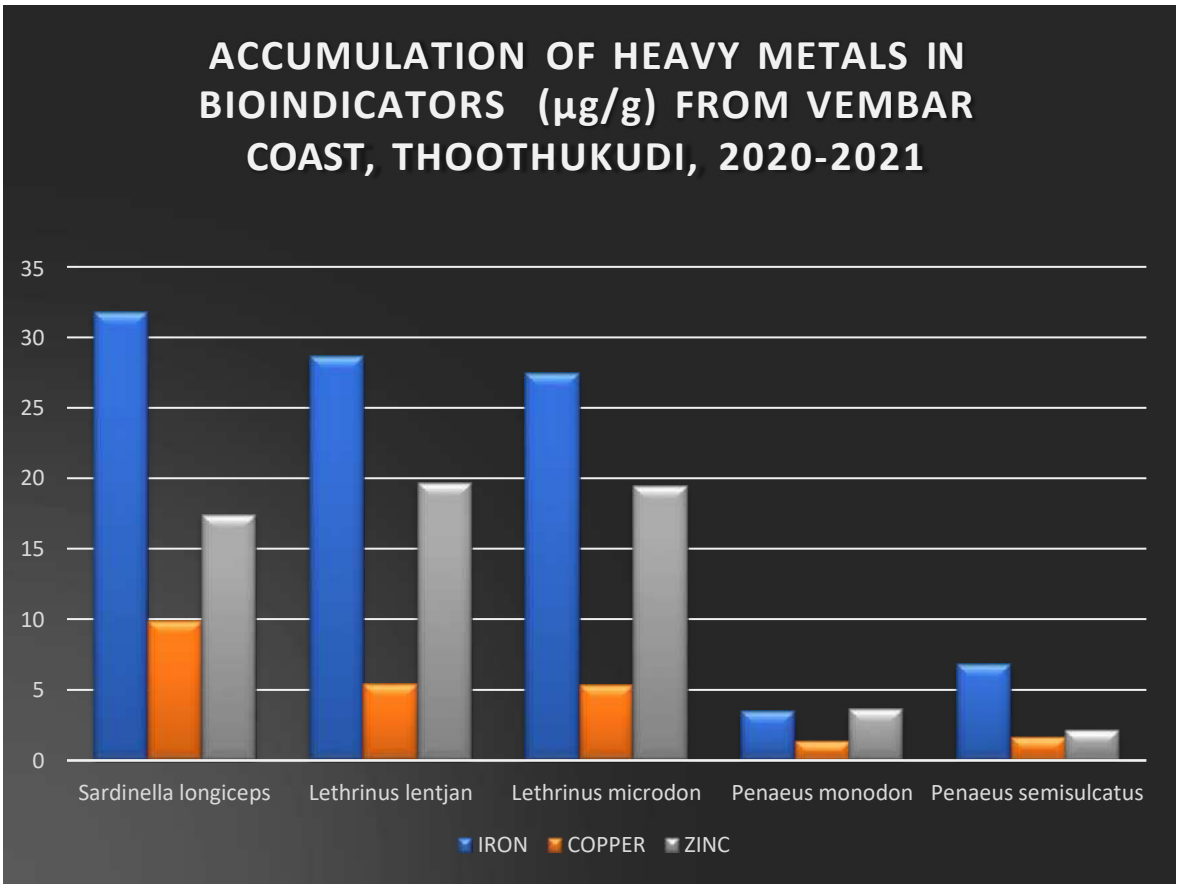


TABLE 7: ACCUMULATION OF HEAVY METALS IN BIOINDICATORS (µg/g) FROM VEMBAR COAST, THOOTHUKUDI, 2020-2021

S.NO	SPECIES	IRON (µg/g)	COPPER (µg/g)	ZINC (µg/g)
1	*Sardinella longiceps*	31.74	9.89	17.41
2	*Lethrinus lentjan*	28.65	5.42	19.62
3	*Lethrinus microdon*	27.45	5.32	19.43
4	*Penaeus monodon*	3.48	1.38	3.64
5	*Penaeus semisulcatus*	6.79	1.63	2.11

FIG 5: ACCUMULATION OF HEAVY METALS IN BIOINDICATORS (µg/g) FROM VEMBAR COAST, THOOTHUKUDI, 2020-2021



DISCUSSION

Consumption of fish and crustacean from polluted marine environment and exposure to heavy metals causes health problems in man. Fish and crustacean can be used as bio indicators of marine pollution and studying heavy metal accumulation in them will help us to take precautionary measures concerned with human health as well as to control and prevent aquatic pollution.

This study revealed that the sea water has prominent physico-chemical characteristics for aquatic life in Therespuram coast and Vembar coast in Thoothukudi, Gulf of Mannar, Tamil Nadu.

Biochemical studies are very important from the nutritional point of view. *Lethrinus lentjan* has high protein value in Vembar coast (21.75%) than in Therespuram coast (18.84%). It may be due to the omnivorous behaviour of *Lethrinus lentjan. Sardinella longiceps* (19.86%, 19.90%) *Lethrinus microdon* (21.25%, 21.20%) *Penaeus monodon* (8.98%, 8.72%) and *Penaeus semisulcatus* (19.56%, 18.27%) have more or less similar protein values in both stations. The variation of protein might be influenced by their feeding and breeding capabilities (Nazrul Islam and Abdur Razzaq Joadder, 2005).

Comparing human consumption preference in the selected organisms, *Lethrinus* species which had the highest protein value, is the most preferred.

The protein content of most fishes has been suggested to be in the range of 16 to 21% (Murray and Burt, 1969; Huss, 1995). More specifically the values ranged between 15.9-20.1% for oil sardine (Ali *et al*., 2013; Palani-kumar *et al*., 2014). The protein values ranged between 14.53–24.40% for *Lethrinus lentjan* (Chrisolite *et al*., 2016). Edah Bernard *et al*.,2016 and Merline, *et al*., 2020 reported the protein value for *Penaeus monodon* and *Penaeus semisulcatus* as 9.21±0.03 and 21.6±0.10 respectively.

Lipids are an alternative energy source in times of fasting and starvation. *Sardinella longiceps* (2.29%, 3.02%) *Lethrinus lentjan* (3.67%, 3.78%) *Lethrinus microdon* (1.52%, 1.68%) *Penaeus monodon* (6.38%, 6.45%) and *Penaeus semisulcatus* (6.65%, 6.65%) have more or less similar lipid values in both stations. Lipids are important in the structural and biological functioning of the cells. In crustaceans, not only do lipids function as the main organic reserve and source of metabolic energy but are also indispensable in maintaining cellular integrity. Generally, lipids act as major food reserve together with protein and may fluctuate periodically due to environmental variable like temperature (Varadharajan and Soundarapandian, 2014). Merline *et al*., 2020 reported the lipid value for *Penaeus semisulcatus* as 0.15±0.02.

Ash is the inorganic remnant of burnt organic matter. *Sardinella longiceps* (2.33%, 1.89%) *Lethrinus lentjan* (1.79%, 1.96%) and *Lethrinus microdon* (0.34%, 0.32%) have more or less similar ash values in both stations. High ash

content in prawns is due to the high level of chitin strengthened by a high level of calcium metal in the exoskeleton. Chitin is a linear polymer of acetyl D-glucosamine that has properties similar to cellulose in many respects (MacDonald *et al*., 1998). Palani-kumar *et al*., 2014, reported the ash value of *Sardinella longiceps* as 1.23±0.10. The ash values ranged between 1.24-2.70% for *Lethrinus lentjan* (Chrisolite *et al*., 2016). Edah Bernard *et al*.,2016 reported the ash value for of *Penaeus monodon* as 3.53 ± 0.06. Merline *et al*., 2020 reported the ash value for *Penaeus semisulcatus* as 2.40±0.07. The differences in the concentration of minerals may be influenced by different factors including seasonal changes, age, sex, size, and sexual maturity, food source, and availability in the respective habitat of organisms and other factors such as water chemistry, salinity, temperature, and contaminants (Hassan, 1996 and Kucukgulmez *et al*., 2006).

The proximate composition of the selected marine species did not show much variations. Though the pollution levels in both the areas vary considerably, the natural level of protein, lipids and ash contents in the species remained constant. The ecological characteristics of the species plays a vital role for this consistency.

Aquatic ecosystems are typically monitored for pollution of heavy metals using biological assays (Wong *et al*., 1975). Iron is prevalent component of industrial and mining effluents that are often discharged into aquatic

environments. The ingestion of large quantities of iron results in haemochromatosis, a condition in which normal regulatory mechanisms do not operate effectively leading to tissue damage as a result of the accumulation of iron. This condition rarely develops from simple dietary overloading (Watt & Merrill, 1963).

In the present work, *Sardinella longiceps* (48.22 µg/g, 31.74µg/g) and *Lethrinus lentjan* (34.53µg/g, 28.65µg/g) have more iron value in Therespuram coast than Vembar coast. *Lethrinus microdon* (26.22µg/g, 27.45µg/g) *Penaeus monodon* (2.34µg/g, 3.48µg/g) and *Penaeus semisulcatus* (7.19µg/g, 6.79µg/g) have more or less similar iron values in both stations. The values are below the maximum acceptable concentration limits (100mg/g) by WHO (1989). Zodape (2014) collected prawns from local markets of Malad suburban areas of Mumbai city and reported the iron value for *Penaeus monodon* and *Penaeus semisulcatus* as 2.648- 6.704 ppm and 6.121- 7.197 ppm respectively. Palani-kumar *et al*., 2014, reported the iron value for *Sardinella longiceps* as 4.31±0.28(mg/100g). Chrisolite *et al*., 2016, reported the iron value for *Lethrinus lentjan* as 4.20±0.05(mg%).

Copper is an essential trace element for living organisms which allows the critical enzyme to function properly and assists enzyme in transferring energy into the cells in humans (Prashanth *et al*., 2015). However, higher copper uptake than needed is a double-edged sword that causes adverse effects (Tvrda *et al*.,

2015). The effects include headache, vomiting, liver and kidney damage, and Wilson's disease (Mahurpawar, 2015).

Lethrinus lentjan (22.65µg/g, 5.42µg/g) and *Lethrinus microdon* (20.45µg/g, 5.32µg/g) have more copper value in Therespuram coast than Vembar coast. *Sardinella longiceps* (12.28µg/g, 9.89µg/g) *Penaeus monodon* (1.67µg/g, 1.38µg/g) and *Penaeus semisulcatus* (1.59µg/g, 1.63µg/g) have more or less similar copper values in both stations. The values are below the maximum acceptable concentration limits (30mg/g) by WHO (1989). Zodape (2014) collected prawns from local markets of Malad suburban areas of Mumbai city and reported the copper value for *Penaeus monodon* and *Penaeus semisulcatus* as 1.124-2.116 ppm and 1.765- 2.211ppm respectively.

Zinc is considered as an essential trace element for living organisms which is relatively less toxic compared to other metals. It assists in metabolism, enzyme catalytic activity, and immune system functioning and possesses antioxidant properties (Bhattacharya *et al*., 2016). Excess Zn uptake damages the brain, respiratory tract, gastrointestinal tract and prostate gland (Plum *et al*., 2010). Besides, high Zn intake disrupts homeostasis for other essential elements and suppresses the Cu and Fe absorption (Osredkar *et al*., 2011).

Lethrinus lentjan (15.35µg/g, 19.62µg/g) and *Lethrinus microdon* (14.98µg/g, 19.43µg/g) have high zinc value in Therespuram coast than Vembar coast. *Sardinella longiceps* (18.24µg/g, 17.41µg/g) *Penaeus monodon*

(2.42µg/g, 3.64µg/g) and *Penaeus semisulcatus* (4.50µg/g, 2.11µcceg/g) have more or less similar zinc values in both stations. The values are below the maximum acceptable concentration limits (100mg/g) by WHO (1989). Zodape (2014) collected prawns from local markets of Malad suburban areas of Mumbai city and reported the zinc value for *Penaeus monodon* and *Penaeus semisulcatus* as 1.865-3.428 ppm and 2.121-4.806 ppm respectively.

The water from Therespuram coast has high iron and zinc concentrations. One of the reasons for the occurrence of this heavy metal in high concentration is due to untreated sewage and other domestic wastes including plastics and solid wastes.

Though the level of heavy metals did not exceed the acceptable levels, regular monitoring and assessment of heavy metals in bioindicator should be carried out to ensure the food safety and to protect the coastal environment from heavy metal contamination.

CONCLUSION

Humans require adequate food for growth and development and to lead an active and healthy life. Intake of sea food is one of the nutritionally superior animal protein sources accessible to man. Consumption of fish and other marine products has always been a major factor in the economy and nutrition of the coastal inhabitants. India with its immense coastal line has tremendous potential in terms of marine food capital.

The chemical composition of fish varies greatly from one individual to another depending on age, sex, environment and season. The prawns and fishes have high protein content and can be utilized maximally by food processors and other value-added products. Hence, they are suitable as potential industrial material for possible utilization for different products.

Heavy metal contamination affects the fish and other aquatic life as well as human consumers. Level of heavy metals in the coastal marine environment is the indicator of anthropogenic activity and its potential risk to the organisms. Therefore, it becomes essential to evaluate the presence of heavy metals in water, sediments and fish from the coastal environment.

Heavy metals, Fe, Cu and Zn in the marine species collected from Therespuram coast and Vembar coast did not exceed the recommended safety limits for food specified by international standards. Therefore the selected

commercially important marine species from this region are safe for human consumption.

Since heavy metal accumulation in fish depends on pollution, installation of domestic sewage treatment system and proper treatment of industrial effluents before its discharge into any water body is required to reduce the contamination of the marine environment. Monitoring of water quality parameters is necessary to manage the aquatic environment and to restore polluted environments.

SUGGESTIONS

- The waste water such as domestic sewage and industrial effluents should be treated before discharge into the sea.
- Strict method of waste disposal control should be adopted to ensure the safety of the environment and to safeguard the aquatic life.
- Awareness should be created among the people about heavy metal accumulation of marine organisms.
- To educate people about danger related to metal bioaccumulation in marine organisms.
- To provide quality conservation and research report opportunities.

SUMMARY

- Therespuram coast and Vembar coast, Thoothukudi was selected as study area.
- *Sardinella longiceps, Lethrinus lentjan, Lethrinus microdon, Penaeus monodon, Penaeus semisulcatus* were selected as study species.
- Physico-Chemical parameters of the study area was found to be prominent.
- The species were enriched with essential nutritional components.
- The pattern of heavy metal concentration in water was Zn> Fe> Cu in Therespuram coast and Vembar coast.
- The heavy metal concentration in marine species were not exceeded the recommended safety limits for food specified by international standards. So they are safe to consume.
- Regular monitoring of marine resources is essential to improve the quality of sea food against contamination.

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AN ANALYSIS OF BIOACCUMULATION OF HEAVY METALS IN THE SELECTED SHELL AND FINFISHES OF THOOTHUKUDI COAST

A project submitted to

ST.MARY'S COLLEGE (Autonomous), THOOTHUKUDI

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

in partial fulfillment for the award of the degree of

Bachelor of Science in Zoology

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CERTIFICATE

This is to certify that the project entitled "**AN ANALYSIS OF BIOACCUMULATION OF HEAVY METALS IN THE SELECTED SHELL AND FINFISHES OF THOOTHUKUDI COAST**" is submitted to **St. Mary's College (Autonomous), Thoothukudi** in partial fulfillment for the award of the degree of **Bachelor of Science in Zoology** and it is a record of the work done during the year **2020-2021** by the following students

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CONTENTS

INTRODUCTION

1. INTRODUCTION

The world is mostly covered with water, three fourth of it is marine water and the remaining is in the form of brackish and fresh water. The release of substances into subsurface groundwater or into lakes, streams, rivers, estuaries and oceans to the point where the substances interfere with beneficial use of the water or with the natural functioning of ecosystems is called as water pollution. Like climate change, biodiversity loss, and depletion of the world's fresh water supply, pollution endangers the stability of the earth's support systems and threatens the continuing survival of human societies (Rockstrom *et al*., 2009).

Pollution is a great and growing threat to human health. It is the largest environmental cause of disease in the world today, responsible for an estimated 9 million premature deaths per year. It causes enormous economic losses, undermines national trajectories of economic development, and impedes attainment of the sustainable development goals (Landrigan *et al*., 2018).

Water bodies can be polluted by a wide variety of substances, including pathogenic microorganisms, putrescible organic waste, plant nutrients, toxic chemicals include improperly disposed wastewater from industrial plants and chemical process facilities (lead, mercury, chromium) as well as surface runoff containing pesticides used on agricultural areas and suburban

lawns (Jerry *et al*., 2014) sediments, heat, petroleum (oil), and radioactive substances.

Metals are mainly released from industrial operations, domestic sewage discharges, atmospheric release from fossil fuel burning and land run off. In aquatic environment metals are being formed as conservative pollutants because once added to the environmental they prevail forever. These metals cannot be broken down to harmless substances by bacterial action. Most of them are biomagnified in water and get accumulated in higher tropic levels. Though there are many pollutants reported, the heavy metals have recognized as one of the major pollutants of the aquatic environments.

Heavy metals are considered the most important form of pollution of the aquatic environment because of their toxicity intrinsic persistence, non-biodegradable nature, and accumulative behaviors (Islam *et al*., 2018). These metals differ from other toxic materials in a way that they are neither created nor destroyed by human. The rapid industrialization, urbanization, population growth, agricultural and other human activities have resulted in severe pollution by heavy metals globally, especially in developing countries. Significant quantities of heavy metals from such activities are discharged into rivers, which can be strongly accumulated, and biomagnified along water, sediment, and aquatic food

chain, resulting in sublethal effects or death in local fish population (Tao *et al*., 2004). Some heavy metals are necessary for life and are called essential elements which are required for a variety of biochemical and physiological functions. However, they can be toxic when present in large amounts (Duffus *et al*., 2002, Wang *et al*., 2001, Tchounwou *et al*., 2008 and Gautam *et al*., 2007).

Seafood is significant in human nutrition because of its unique nutritive value related to the presence of proteins, fats, vitamins and minerals. Fish are excellent source of protein and its low – calorific value makes it a healthier alternative to red meats or poultry; it is also rich in omega-3 polyunsaturated fat which has been known to reduce cholesterol levels in man (Kaushik., 2009).

Thus, the human body is largely susceptible to enriched heavy metal concentration in fishes (Ali *et al*., 2018). Pollution enters fin and shell fishes through five main routes namely food or non-food particles, gills, oral consumption of water and the skin (Amin *et al*., 2011, Mitra *et al*., 2012). As fishes occupy higher tropic level in the food chain, they are considered one of the most common bioindicator for pollutants (Idriss *et al*., 2015 and Authman *et al*., 2015).

Some metals (copper, iron and zinc) at low concentrations are of nutritional importance and are essential for healthy living. In contrast, metals such as

mercury, cadmuim and lead have not been known to play beneficial role in human metabolism and are considered as chemical carcinogens even at very low levels of exposure (Luch, 2005).

Mercury is widely distributed around the earth. Mercury cycles in the environment as a result of natural phenomena and human activities (Pirrone *et al*., 2010) Natural phenomena such as volcanoes cause mercury to be released in the air. Mercury has been widely used in industrial processes because of its chemical and physical properties (for example, it conducts electricity, it response to temperature and pressure changes, and it forms alloys with many metals). Industrial processes and combustion of mercury-containing wastes and fuels also release mercury.

Mercury that is released into the air is mercury vapor or inorganic mercury. Once in the atmosphere as a gas ultimately it is redeposited on the earth with precipitation or in the waterways, it is incorporated into sludges or sediments, where it is methylated. The plant and sedimentary materials containing methylmercury are consumed by small fish that are consumed by progressively larger fish and finally by humans. During the course of this progression a great increase in concentration occur known as bioaccumulation. This increase can result in concentrations of methylmercury in fish tissues that are hundreds or thousands

of times higher than the levels of inorganic mercury in the water (Mahaffey *et al*., 2005). Chronic exposure to Hg and Hg compounds is harmful to human health, especially to the fetuses and children at early stages of development (Chahid *et al*., 2014). This metal can cause most damage and dysfunction of the central nervous system.

Bioaccumulation of heavy metals (Zn, Pb, Cd, and Cu) was determined in the liver, gills, and flesh from benthic and pelagic fish species. The levels of the heavy metals varied significantly among fish species and organs. Lead occurs naturally combined with two or more other elements to form lead compounds sources (Gorer *et al*.,2012). Mining and smelting, soldering, battery manufacturing, ammunition, metal water pipes; paint and petrol are reported as anthropogenic sources (Gorer *et al*., 2012). Pb is a nonessential element for living organism and also it possess various adverse effects such as neuro and nephro toxicity, rapid behavioral malfunction, and decreases the growth, metabolism, and survival rate, alteration of social behavior in some mammals (Ekundayo *et al*., 2014). Chronic exposure to Pb is deleterious for the hematological system, the CNS, and the renal system.

Copper is an essential mineral that supports various body functions, such as enzyme production and neurological functions. However, exposure to high levels

of copper in water or food can lead to copper toxicity. Too much copper in the body can damage the liver, kidney, heart, and brain. If left untreated, copper toxicity can have severe health effects and even result in death (Alana Biggers., 2020).

Zinc was found to be the second most abundant metal in the crustaceans sampled. Zinc is an essential mineral that is naturally present in some foods, added to others, and available as a dietary supplement. Zinc is also found in many cold lozenges and some counter drugs sold as cold remedies. Zinc is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of approximately hundred enzymes (Sandstead ., 1994) and it plays a role in immune function (Solomons,1998 and Prasad *et al*.,1995), protein synthesis, wound healing, DNA synthesis and cell division. Zinc also supports normal growth and development during pregnancy, childhood, and adolescence (Simmer, *et al*., 1985) and is required for proper sense of taste and smell (Prasad *et al*., 1995). A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system (Prasad *et al*., 1995).

Acute Cd toxicity by ingestion of contaminated seafood can cause increased salivation, choking or vomiting, abdominal pain, vertigo, loss of consciousness, painful spasm of the anal sphincter and impairment of renal function for severe

toxicity (Nordberg.,2009). Additionally, Cd chronic toxicity affects bones, causing fractures, severe pain, malformations (Nogawa *et al*.,1975), hypercalciuria and impaired vitamin D metabolism (Bhattacharyya *et al*.,1992). Cd long-term exposure impairs kidney's normal functioning (Jinadasa *et al*., 2015).

The numerous health benefits provided by fish consumption may be compromised by the presence of toxic metals and metalloids such as lead, cadmium, copper and mercury, which can have harmful effects on the human body if consumed in toxic quantities. The monitoring of metal concentrations in fish meat is therefore important to ensure compliance with food safety regulations and consequent consumer protection. The estimation of heavy metals in the food-chain will be used to know the heavy metal transfer to the human body through sea-food. Fish can concentrate very high levels of these contaminants, sometimes exceeding authorized limits. This bioaccumulation is closely related to the place of some fish species at the top of the aquatic food chain. For this reason, determination of the chemical quality of aquatic organisms, particularly their heavy metals contents, is important for human health. (Bosch., 2016).

REVIEW OF LITRATURE

2. REVIEW OF LITERATURE

The development of urban infrastructure, fisheries, maritime cultivation, transport and tourism, deficient wastewater treatment has caused major disturbance to the coastal environment. (Khan and Homaid, 2011; Alyahya *et al*., 2011). Bioaccumulation level of heavy metals in marine organism is very important, with many implications in various domains, like environment protection, public health, control of standards compliance or risk assessment. The ability of marine biota to accumulate metals from their environment, their utilization as marine pollution bio indicators has been confirmed by numerous examples. (Sallam *et al*., 2006; Abdel-Salam *et al*., 2011 and Viswanathan *et al*., 2013). Heavy metal concentration was determined by variables such as water contamination, mining activities and effluent treatment activities in the fishing region (Kulkarini, 2005).

The use of marine organism as bioindicators of metal pollution of aquatic environments and suitability for human use from toxicological point has been documented by Amin *et al*. 201I; Mitra *et al*., 2012; Viswanathan *et al*., 2013: Yilmaz *et al*, 2007 and Abdel Salam *et al*., 2011). Humood *et al*., (2013) assessed the heavy metal pollution in the marine environment of the Arabian Gulf and confirmed that heavy metal concentrations in marine organisms were generally within allowable concentrations and pose no threat to public health.

Ganesan *et al*. (1995) measured higher concentration of heavy metals like Mn, Fe, Cu and Zn in the seaweeds off Tuticorin. Senthilnathan *et al*., (1998) showed seasonal variation with an increased metal load during monsoon period in the mussel and oysters from the southeast coast of India. Monawwar and Kazi (1998) investigated the concentration and distribution of heavy metals (lead,cadmium,copper,zinc) in Karachi shore and off shore sediments. The measured heavy metal pollution level in the sediment of coastal and offshore area indicated that high concentration of heavy metal were found around Manora channel and Eastern Coastal of Karachi.

Palanichamy and Rajendran (2000) indicated high concentration of Cd and Pb in the bottom waters than the surface waters off Tuticorin. Baskaran *et al*., (2002) observed relatively higher concentration of Fe, Cu, Zn and Al in the fly ash dumping area than in the deeper waters off Tuticorin. Kaladharan *et al*., (2005) determined heavy metal concentrations in sediment, fin fishes and shellfishes in inshore waters of Cochin, southwest coast of India. Bindu *et al*., (2007) studied the trace metal contamination of the marine environment in Palk Bay and Gulf of Mannar. Comparative study of heavy metal concentrations in razor clam (Solenregularis) in Moyan and Serpan, Sarawak by Devagi ., 2008. Monikh *et al*., (2011) measured the heavy metal levels in the sediments and ray fish Dasyatis bennettii from Persian Gulf.

Asha *et al* ., (2010) studied the concentration of heavy metals Cd, Cu, Fe, Mn, Ni, Pb and Zn in sea water, sediment and bivalve samples from three stations for one year along Tuticorin coast. The concentration was in the order of Fe>Mn>Zn>Cu>Pb>Cd>Ni. Generally the concentration of Fe was very high in the sediment and bivalves High concentration of Fe, Mn, Cu, Pb and Zn was observed during monsoon season. Lourenco *et al*., (2009); Ayas and Ozogul, (2011) measured concentration of both essential and non essential metals in the digestive gland and mantle of female cephalopods *Sepia officinalis* from two coastal lagoons of Portugal

Fathi Alhashmi Bashir *et al*.,(2012) evaluated of trace metal levels in tissues of two commercial fish species in Kapar and Mersing Coastal Waters, Peninsular Malaysia and the study revealed that the studied metals concentrations are generally low in the tissues of the examined fish in the two study areas. Although the levels of these heavy metals are not high, a potential danger may emerge in the future depending on pollution sources. Yu Wenjin *et al*., (2013) examined the distributional characteristics of heavy metal in Jiangsu Province Shoal Sea and they reported that although there are some heavy metal enrichment and pollution in the core area, no ecological hazard has been produced yet, and overall environment quality in Xin yang gang core area was excellent. Distribution of heavy metal pollution in Vaipar coastal sediments,

southeast coast of India was reported by Abukashim *et al*., (2014).

Jinadasa *et al*., (2015) determined the total mercury, cadmium and lead levels in main export fish of Sri Lanka. Results show that swordfish contained the highest total Hg and Cd levels, whereas yellowfin tuna contained the highest Pb levels. Heavy metal content in marine fish collected from the outlets of Hyderabad and Secunderabad, Andhra Pradesh, India was studied by Rao *et al*., (2014). Krishna *et al*., (2014) studied the Human health risk assessment of heavy metal accumulation through fish consumption from Machilipatnam Coast, Andhra Pradesh, India and reported the concentration of the metals in the fish muscle from Machilipatnam coast pose to health hazards to the consumers. Isacc.,2014 estimated the concentrations of heavy metals Cu, Zn and Pb from Tuticorin, Tamilnadu in marine fishes.

Biochemical composition and heavy metal content of selected marine fishes from the Gulf of Mannar (Ajeeshkumar *et al*., 2015). James and Mary, 2015 determined the heavy metal distribution in the coastal waters and sediments of Mandapam, Thoothukudi, Arumuganeri and Kanyakumari coasts. The enrichment in the concentration of heavy metals in the samples that are close to the coastal areas indicated that higher concentration was due to the anthropogenic activities in the coastal area.

Sanchari *et al*., (2017) investigated heavy metals concentration namely

Mn, Ni, Co and Cu in different tissues (Gills, Muscle and Liver) of three selected edible fishes of Visakhapatnam namely Indian Mackerel (Rastrelliger kanagurta), Yellow tail Scad (Atule mate) and Pink Perch (Nemipterus japonicas) in the vicinity of Visakhapatnam coast. The extent of metal concentration in the tissues ranged from Ni>Cu>Mn>Co. The levels of heavy metals varied among the various tissues in the fish species studied and the concentrations of the metals found in gill tissues were high.

Kaleshkumar *et al*., (2017) analyzed the distribution of heavy metals and proximate composition in marine edible puffer fishes collected from Gulf of Mannar Marine Biosphere Reserve, South India. Fetta Mehouel *et al*., (2019) evaluated the heavy metals (mercury, lead, and cadmium) contamination of sardine (*Sardina pilchardus*) and swordfish (*Xiphias gladius*) fished in three Algerian coasts. Edgar Mapunda *et al*., (2017) estimated the concentrations of metallic elements in kidney, liver, and lung tissue of Indo-Pacific bottlenose dolphin *Tursiops aduncus* from coastal waters of Zanzibar, Tanzania and found that *Tursiops aduncus* dolphins from coastal waters around Zanzibar carry low concentrations of metals compared with dolphins from other areas.

Pragnya *et al*.,(2020) reported the bioaccumulation of heavy metals in different trophic levels of aquatic ecosystems with fish as a bioindicator in Visakhapatnam, India.

OBJECTIVE

3. OBJECTIVES

Tuticorin coast is situated along the Gulf of Mannar Biosphere reserve. This coast is known for pearl fishing. Now it is flourished with petrochemical industries namely Thermal Power Station, Heavy Water Plant and Tuticorin Alkali Chemical (TAC) pouring heavy metals and other contaminants into the sea. All the wastes from these factories reach the coastal system. As a consequence of these processes, severe damages have already come to light on the Ecosystem along the coast of Gulf of Mannar. In the view of fast deterioration of coastal environment, a careful pollution monitoring is required to stabilize and save the coastal ecosystem before any harmful effects are carried to human beings. So the present study has been carried out to investigate the heavy metal concentration in the commercially important species of marine fishes.

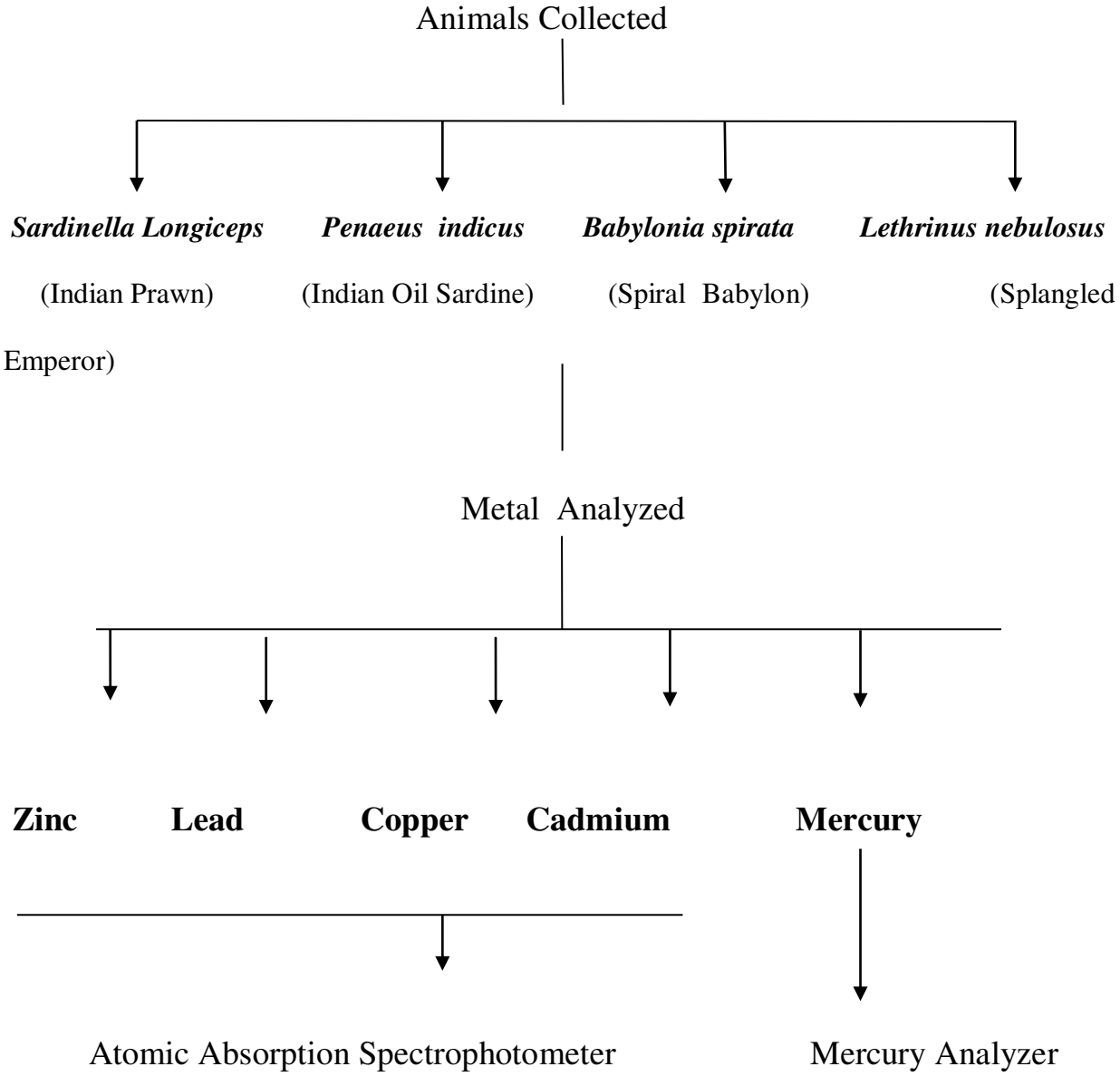
The objectives of the present study are:

- To determine and compare concentration levels of heavy metals (Zn, Pb, Cu, Cd and Hg) in commercially important species of fin and shell fishes.
- To use these organisms as bioindicators of pollution of Gulf of Mannar.
- To ensure the seafood safety from this region.

EXPERIMENTAL DESIGN

4.EXPERIMENTAL DESIGN

Flow chart displaying the detailed Methodology



MATERIALS AND METHODS

5. MATERIALS AND METHODS

5.1.Description of the study area

The Gulf of Mannar is located between India and Srilanka (Long.78'8 " to 79'30 " E and Lat 8 ' 35 " to 925 " N). It is a part of the southward extension of the Bay of Bengal and it meets in the Indian Ocean. This geographical area runs from Pamban Island including Rameshwaram to Cape Comarin along the Southeast coast of India to a distance of about 170 nautical miles. This coast maintains a rich biological diversity of flora and fauna largely due to diversified microhabitats such as mangroves , corals , seaweed beds , sea grasses , sandy , rocky and muddy shore etc.. For the present study the animals were collected from the fishing trawlers operated for crabs and prawns from the Thoothukudi coastal region (Fig 1).

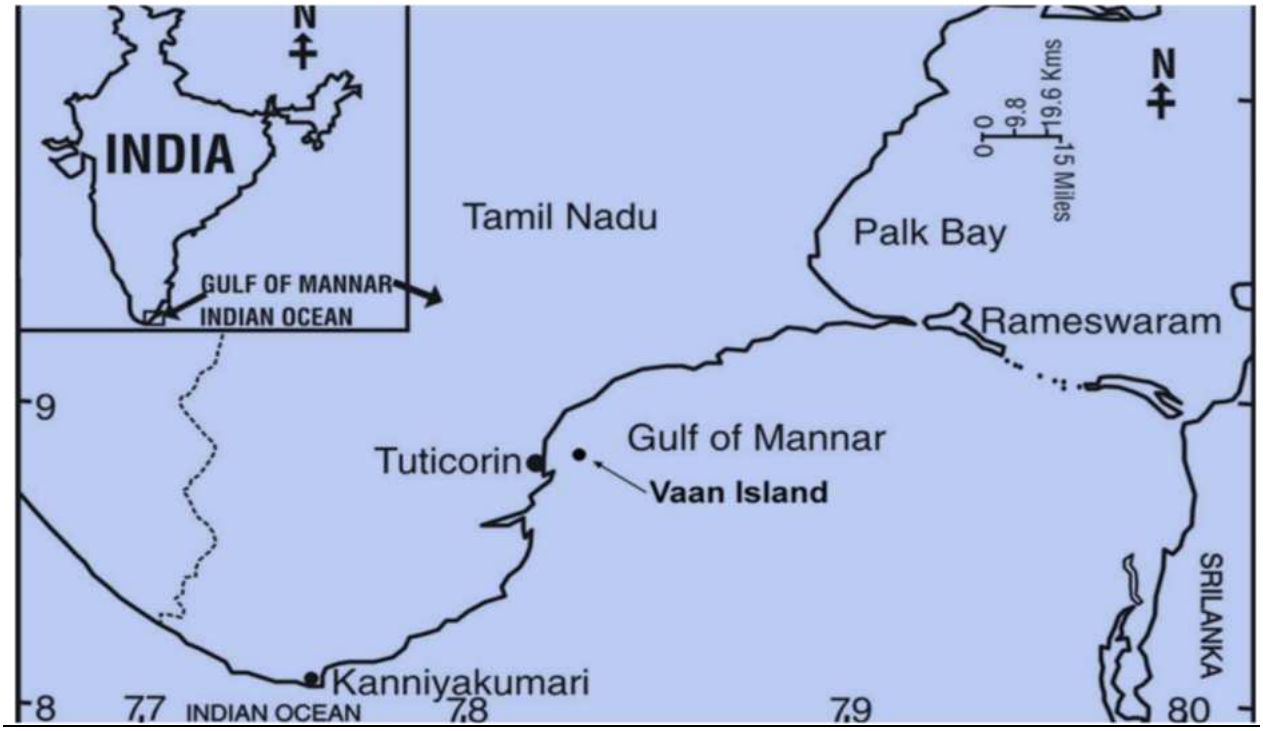


Fig.1.**MAP SHOWING THOOTHUKUDI COAST, THE GULF OFMANNAR**

5.2. Sample collection and Preparation:

Fresh samples were purchased directly from fishermen with their commercial captures landed at the fishing harbor. Samples were then immediately iced and brought to the laboratory in insulated boxes. Specimen was identified by using FAO Fishery identification key. At the laboratory, they are cleaned with water to remove all impurities. Samples were carefully dissected in order to separate the flesh. Then the samples were dried in the oven at 100°C; they were crushed into a fine powder by using a porcelain motor and pestle and analyzed for metals.

5.3.Experimental Animal

Systematic position

Sardinella longiceps (Valenciennes, 1847)

Phylum	:	Chordata
Class	:	Actinopterygii
Order	:	Clupeiformes
Family	:	Sardinella
Genus	:	Clupeidae
Species	:	*S. longiceps*
Common Name	:	Indian Oil Sardine

The body of *Sardinella longiceps* is particularly elongated even to the point of being sub cylindrical. They have a slightly rounded belly and have eight rays on their pelvic fins. They have a very large number of gill rakers and a faint golden spot behind the gill opening. They also have a faint golden midlateral line, as well as a black spot on the hind border of their gill covers. *S. longiceps* attains sexual maturity around 15 cm and 1 year of age. The lifespan is about 2.5 years.

Penaeus indicus (H.Milne-Edwards,1837)

Phylum	:	Arthropoda
Class	:	Malacostraca
Order	:	Crustacea
Family	:	Penaeidae
Genus	:	Penaeus
Species:	:	*P.indicus*
Common Name	:	Indian Prawn

It is found at depths of 2 to 90 m, inhabiting bottom mud or sand. It is most abundant in shallow waters of less than 30 m depth, on sand or mud (FAO, 1984). The adults are marine and breed offshore, while post larvae and juveniles are estuarine (FAO, 1980). They are euryhaline and live in brackish, estuarine and marine environments with temperature ranges between 18 and 34.5 °C and salinities of from 5 to 50 ppt. Shrimp have an exoskeleton (the "shell") that is periodically shed during moulting to allow further growth. Shrimp have a head (thorax) and a tail, and an abdomen with six segments. The last abdominal segment is the telson. The thorax has a spine called the rostrum, one pair of eyes, two pairs of antennae, three pairs of maxillipeds for feeding and five pairs of walking legs. Each abdominal segment except the telson has a pair of fins called pleopods on the

ventral side. Shrimp use the pleopods for forward swimming and the telson and pleopods to propel backwards rapidly when the abdomen is flexed. The maximum length of *P. indicus* is about 184 mm for males and 228 mm for females, although adult shrimp are usually much smaller (170 mm). The maximum carapace length is 56 mm (FAO, 1980).

The eyestalks and antennal scales are bluish and the margins of the uropods are blue with a bright red fringe. The antennae are not banded, and the antennules are spotted (Racek, 1955). The body is semi-translucent, with olive green to grey-blue speckles. The pereopods are generally the same colour as the body. Pleopods are pink or red and the distal part of the uropods green or red, with the fringe of setae usually red. Juveniles are whitish, with specks of the same colour as adults (FAO, 1984)(Plate-2).

Plate.1.*Sardinella longiceps* (Indian Oil Sardine)

Plate 2. *Penaeus indicus*(Indian Prawn)

Babylonia spirata (Linnaeus, 1758)

Phylum	:	Mollusca
Class	:	Gastropoda
Order	:	Neogastropoda
Family	:	Babyloniidae
Genus	:	Babylonia
Species	:	*B. spirata*
Common Name	:	Spiral Babylon

Body pale, with a long muscular foot that is dark with an orange rim, short tentacles and long siphon. Shell thick, conical, smooth with distinctive spiral. Shell colour and pattern variable, from plain brown to white with orange or brown spots. There is notch at the tip of the shell where the long siphon emerges. Operculum thin and flexible, made of a horn-like material.

Shell large upto 70mm in height, smooth and heavy, body whorl inflated, spire high and elongate, sutures deep and channeled. Shoulders prominent; whorls inflated; columella smooth and heavily calloused; umbilicus broad, deep, and heavily calloused, fasciole wide, anterior canal in the form of oblique notch at the base of aperture, posterior canal well developed, aperture large, ovate, outer lip sharp and strongly flexed at the top, interior of aperture smooth and thickened; Colour white with prominent light brown blotches, oblique streaks and spots;

aperture,outer lip and columellar callus white, fasciole orange brown, tip of apex and aperture tinged blackish; fresh shells covered by light brown periostracum.

Lethrinus nebulosus (Forsskal,1775)

Phylum	:	Chordata
Class	:	Actinopterygii
Order	:	Perciformes
Family	:	Lethrinidae
Genus	:	Lethrinus
Species	:	*L. nebulosus*
Common Name	:	Spangled Emperor

Lethrinus nebulosus inhabits both marine and brackish waters at depths of between 10 and 75 meters. It is a non-migratory species, and is found on coral and rocky reefs, sea grass beds, mangrove swamps, as well as over sandy substrates. Juveniles may be found in large schools. They are reef dwellers, ranging from shallow coral reefs and sea grass beds to rocky reefs more than 200 meters deep. Grow up to 100cm, they look like snapper, deep bodied and not as elongated. Most species inhabit hard bottom of sandstone and coral, or coral and sandstone. Live in large schools. They feed on mollusks, bivalves or oysters. It has large head. Single continuous dorsal fin with 10 spines and 9-10 branched soft rays pectoral fins moderately long and pointed, caudal fin emarginated to forked lateral lines continuous with simple tubes.

Plate:3 *Babylonia spirata* (Spiral Babylon)

Plate :4 *Lethrinus nebulosus* (Spangled Emperor)

5.4. Determination of Total mercury content using mercury analyzer :

Principle

The mercury analyzer MA5800A is a sensitive instrument designed for the practice of precise determination of minute trace of mercury at nanogram of samples. Basically it is a cold Vapor atomic absorption Spectrophotometer bared on the principle that mercury Vapor (atoms) absorbs resonance radiation at 253.7nm. The MA 5800B mercury analyzer consists of a low mercury lamp as radiation source in an absorption cell, a filter, a detector with associated electronics and a vapor generation system. The carrier gas (air from mercury) bubbles through the vapor generation system, Carries elemental mercury from the solution and then pause through the absorption cell.

Reagents required:

1. 500ml of 1 % w / v $KMnO_4$ in 10 % H_2SO_4: Dissolve 5g of $KMnO_4$, in water and carefully add to it 50ml of H_2SO_4. Make up the volume to 500ml using distilled water.

2. 250ml of 20 % W / V NaOH: Dissolve 50g of NaOH pellets in distilled water and make up to a volume of 250ml.

3. 100ml of 20 % $SnCl_2$ (W / V) in 10 % HCL 20 % (W / V) : Dissolve 20g in 100ml of hot HCL acid and make up to 100ml with distilled water .

4. Sulphuric acid (1 + 1)

5. Diluting acid solution: Mix 100ml or 16M HNO3 and 50ml of 18M H_2SO_4, with 850ml of water .

6. Standard mercury Solution: 100µg / ml. Dissolve 0.135g HgCl +2 in 1 lit of IN HCL. Dilute Iml of this stock solution to 100ml with IM HCL concentration of this solution is 10mg / ml

7. Standard mercury working solution: (100ng / ml) - Dilute I ml of the above solution to 100ml with IM HCL.

Procedure

Digestion of sample

Weigh approximately Ig of homogenized *Sardinella longiceps, Penaeus indicus,* , *Babylonia spirata* and *Lethrinus nebulosus* sample into a 125 ml concical flask, and add 5ml of 16m HNO_3, 2.5 ml of 18M H_2SO_4 and I ml of 10m HCI. Then, cover the flask with a glass marble and when initially reaction subsides (after approximately 15 minutes places the flask in a boiling water bath for 40 minutes. Then remove the flask from the water bath and cool. Make up to 50ml with distilled water. A suitable aliquot of this solution is used for mercury determination.

Analysis of sample

Pour 20ml of digested sample into the reaction vessel and add 3ml of stannous chloride solution and stir the combined solution and allow to reaction for 5 minutes. The generated mercury vapour is the passed through an absorption cell and detected at 253.7nm by Atomics Absorption Spectrophotometer.

5.5 Analysis of Lead,Cadmium,Copper and Zinc

Digestion of Samples

The samples were subjected to acid digestion following the method of FAO (1975). Total Lead,Cadmium,Copper and Zinc were estimated. The samples were digested with a mixture of concentrated nitric acid (HNO_3) and perchloric acid ($HCIO_4$) in the ratio of 1: 2 until the formation of white residue at 100°C in a water bath. The cooled residue was dissolved completely by adding I N HCL and made upto 25ml with distilled water. The content was filtered by cotton wool and the filterate was subjected to metal analysis in Atomic Absorption Spectrophotometer (GBC Avanta Ver 2.02). The instrument was calibrated using standards.

RESULTS

6. RESULTS

The results on the concentration of heavy metals namely mercury, cadmium ,Copper, Lead and Zinc in *Sardinella longiceps,Penaeus indicus,Babylonia spirata* and *Lethrinus nebulosus* are represented in the Fig 2. The concentration of mercury varies from 0.006 µg/g to 0.095µg/g. Maximum concentration of 0.095µg/g. was observed in *Babylonia spirata* and the minimum of 0.006µg in the *Sardinella longiceps* followed by 0.044 µg/g in *Penaeus indicus* and 0.067 µg/g in *Lethrinus nebulosus*. The order of abundance of mercury could be arranged in the following order

Babylonia spirata>*Lethrinus nebulosus*>*Penaeus indicus* > *Sardinella longiceps*

The concentration of Cd ranged from 0.17 µg/g to 4.72 µg/g in the marine collected samples. The *Lethrinus nebulosus* showed the maximum concentration of 4.72µg/g followed by *Babylonia spirata* (3.27 µg/g), 0.23 µg/g (*Sardinella longiceps*) and 0.17 µg/g in *Penaeus indicus*. The Cd content in the samples could be arranged in the following ascending order.

Lethrinus nebulosus<*Babylonia spirata*<*Sardinella longiceps*< *Penaeus indicus*

Concentration of Pb in the samples are presented in the Fig 2. *Lethrinus*

nebulosus was the most contaminated species compared to the other species. There were significant differences among the concentrations of heavy metals in the other three species. Maximum concentration of 2.33 µg/g was observed in *Babylonia spirata* and the minimum of 0.067 µg/g in the *Sardinella longiceps*. Accumulation of lead in *Penaeus indicus* as 1.10 µg/g and 2.29 µg/g in *Lethrinus nebulosus*. The order of abundance of Pb in the samples could be arranged in the following order

Babyloniaspirata>*Lethrinus nebulosus*> *Penaeus indicus*> *Sardinella longiceps*

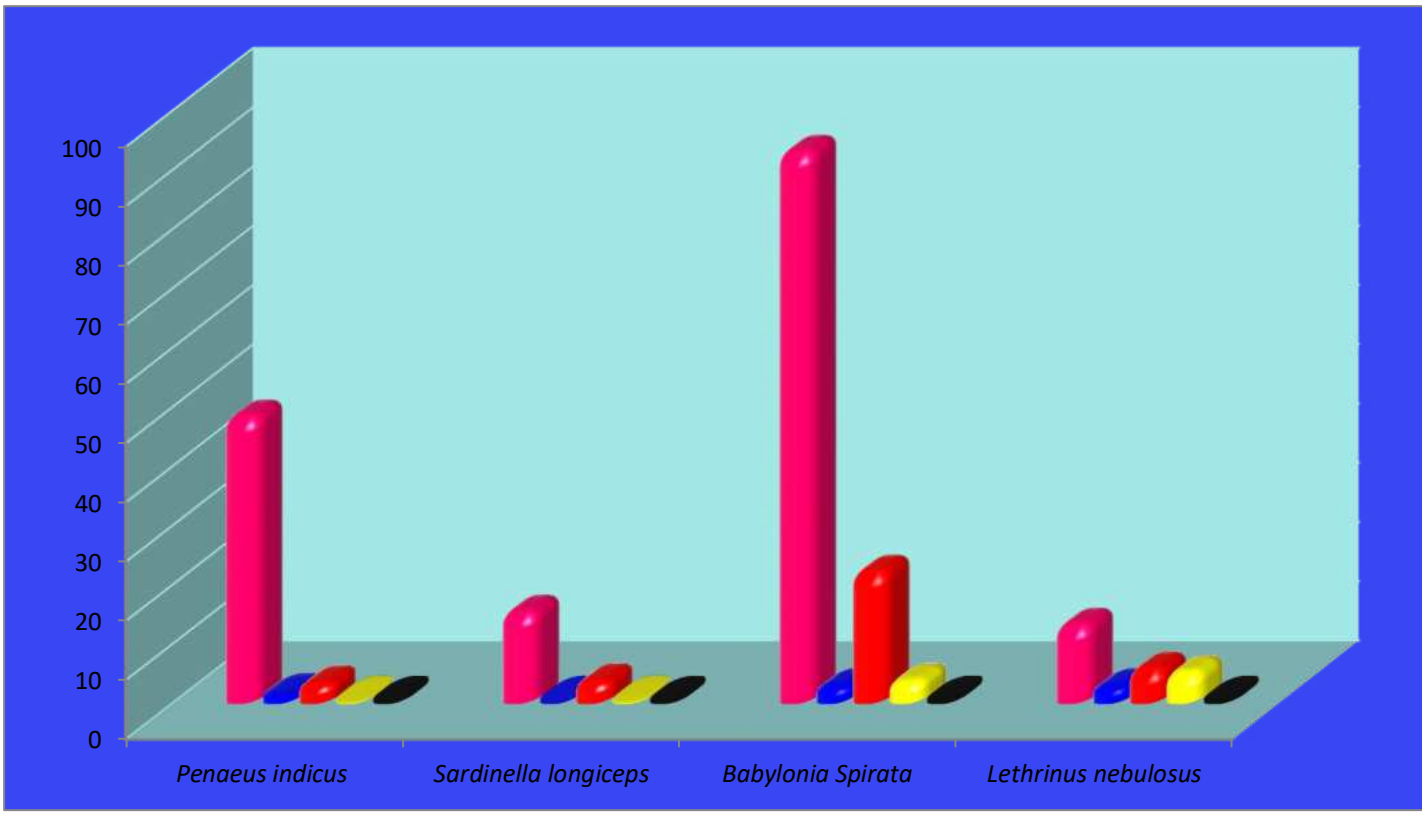
In the present study, Zinc was found to be abundant in all the collected samples compared with all the other metals. *Babylonia spirata* contain maximum concentration of 92.57 µg/g zinc and a minimum concentration of 12.75 µg/g occurred in *Lethrinus nebulosus*. Concentration of 47.89 µg/g was observed in *Penaeus indicus and* 14.96 µg/g in *Sardinella longiceps.*

Accumulation of copper in the samples were represented in the Fig:2 There were significant differences in the concentrations of copper in the different species. Concentration of 2.81 µg/g was observed in *Penaeus indicus* 3.25 µg/g, 5.35 µg/g and 21.71 µg/g in *Sardinella longiceps, Lethrinus nebulosus* and *Babylonia spirata* respectively.

The order of the levels of the elements obtained from the different species

$$Zn > Cu > Cd > Pb > Hg.$$

Figure 1-Accumulation of Heavy Metal in Fin and Shell Fishes



DISCUSSION

7. DISCUSSION

Levels of contaminants in marine animals are of particular interest because of the potential risk to humans who consume them. Accumulation of metals in marine animals is the function of their respective membrane permeability and enzyme system, which is highly species specific and because of this fact different metals accumulated in different orders.

Mercury is one of the most toxic elements among the studied heavy metals and exposure to high level of this element could permanently damage the brain kidneys and developing foetus. In the present investigation concentration of mercury varies from 0.006 µg/g to 0.095µg/g. Maximum concentration of 0.095µg/g. was observed in *Babylonia spirata* and the minimum of 0.006µg/g in the *Sardinella longiceps* followed by 0.044 µg/g in *Penaeus indicus* and 0.067 µg/g in *Lethrinus nebulosus*. In contrast to the present study Fetta Mehouel *et al*., (2019) evaluated the concentration of heavy metals in Sardine pilchardus (0.62 mg/Kg) and *Xiphias gladius* (0.56 mg/Kg). Hg contamination level was slightly higher in Sardine than in *Xiphias gladius*. Amani *et al*., (2012) reported the concentration of heavy metals (Fe,Mn,C,Zn,Pb,Cd,and Hg) in certain common fish species such as blackspot emperors, grouper and sardines. The concentrations of heavy elements in the selected species are varied quietly such as, Fe (44.87–250.23), Mn (7.72–

13.99), Cu (2.3–12.05), Zn (16.79–49.43), Pb (3.24–9.17), Cd (1.17–4.25) and Hg (0.014–0.055 µg/g dry wt.). The order of the levels of the trace elements obtained from the three different fish species in three different sites Fe > Zn > Mn > Pb > Cu > Cd > Hg. Highest concentration of iron,manganese and zinc were detected in sardine but minimum values were recorded in the case of Hg. (concentration of Hg was in the range of 0.014 - 0.055 µg/g).These results were in agreement with the present study. Concentration of Zinc was high 14.96 µg/g followed by copper (3.25 µg/g), cadmium (0.23 µg/g), lead(0.067 µg/g) and mercury (0.006 µg/g). The distribution of Hg in the tissue may be linked to the preponderance of this element in methylated forms allowing movements of mercury across cell membranes (Pinho *et al*., 2002). The maximum mercury contents of sardine in the literature was reported as 0.05 mg/kg (Bordajandi *et al*.,2004) and 0.09 mg/kg (Falco *et al*., 2006, Shiber 2010), respectively.

The mollusc`s shells and tissues are good indicator of metal pollution as they are sessile and sedentary and they reflect the heavy metals concentration of that particular area (Brugman 1981). Heavy metal analyzed in the present study showed that in Babylonia spirata Zn contamination level was slightly higher 92.57µg/g followed by Cu -21.71 µg/g , Cd- 3.27 µg/g , Pb- 2.33 µg/g and Hg -0.095 µg/g. This study is almost similar with a previous study (Jamila

Patterson et al.,1997) on edible gastropods *Babylonia spirata*, *Hemifusus pugilinus, Rapana rapiformis, Xancus pyrum* and *Melo melo* at four sites along the southeast coast of India.

High concentrations of all metals namely Zn,Mn, Fe and Cu were recorded during the rainy monsoon whereas concentrations were low during the dry summer. At Tuticorin and Madras metal concentration were high in all the gastropods confirming that the harbour as well as the industrial wastes pollutethese coastal waters. Similar to the present study Rasyid, 2018 evaluated the concentration of heavy metals lead, cadmium and mercury in the dried marine gastropod *Laevistrombus turturella*. The result showed that the lead content was 0.61 mg/kg and the cadmium 0.23 mg/kg, while the mercury content was not detected.

Primost *et al*.,(2017) reported that cadmium and lead concentration detected in digestive gland and gonad complex of three marine gastropods from Nuevo gulf,namely *Adelomelon ancilla,Buccinanops deformis* and *Trophon geversianus* was 8.02 mg/kg and 1.81 mg/kg, 38.86 mg/kg and 1.95 mg/kg, 104.24 mg/kg and 3.51mg/kg respectively. Habitat and anthropogenic activities near the habitat of marine gastropods are crucial in order to identify the accumulation basis of heavy metals in marine gastropods (Hajeb and Jinap 2009; Zhang *et al.,* 2007). Sharif *et al*., (2016) reported that the weather

changes or climate changes could be contributory factors to heavy metal concentration in gastropods.

Mariam *et al*., 2016 investigated the concentration levels of heavy metals in bivalve mollusks Oysters, Mussels and donax at Tudor Creek Mombasa Kenya. Concenteration varied from species to species and size of the bivalves. Fe and Zn were the most abundant heavy metals in all the molluscs while Mn and Cr concentration levels for most of the organisms were very low. Donax had the lowest concentration of Fe while oysters and mussels had the highest concentration levels of Zn. Zn and Fe had the highest concentration levels in all bivalves. The concentration levels for Zn ranged 13.80±3.23 in the donax and 2504.92±6.96 in mussels between 9.15±2.57 µg/g to 440.13±22.39 µg/g.

Cadmium is a very toxic metal and has been responsible for a number of deaths. The most serious situation being the disease called Itai Itai disease. The presence of Cd could be due to the byproduct of the reaction between Zn, Cu andPb. Cd also released from biogenic detritus in order to regenerate phosphate and nitrate. The threshold for acute cadmium toxicity would appear to be a total ingestion of 3–15 mg. Severe toxic symptoms are reported to occur with ingestions of 10– 326 mg. Fatal ingestions of cadmium, producing shock and acute renal failure, occur from ingestions exceeding 350 mg (NAS-NRC,

1982).

Accumulation of cadmium in living organisms is a major ecological concern, especially because of its ability to accumulate very quickly (Besirovic *et al.,* 2010). Furthermore, seafood is the main source of cadmium for people. In the present study concentration of Cd ranged from 0.17 µg/g to 4.72 µg/g in the marine collected samples. *Lethrinus nebulosus* showed the maximum concentration of 4.72µg/g followed by *Babylonia spirata* (3.27 µg/g), 0.23 µg/g (*Sardinella longiceps*) and 0.17 µg/g in *Penaeus indicus* . This study corroborate with the result of Canli and Atli, 2003.

Cadmium content (1.25 mg/kg) in the muscle tissue of the sardine is higher than the recommended limit for cadmium in fish (0.05 mg/kg) according to the Commission of The European Union (Anonymous 2006) and Turkish Food Codex(Anonymous 2008). But, the maximum allowed cadmium doses for an adult are 0.5 mg/week (Anonymous 1976). The mean level of cadmium in our samples. Mercury, a very toxic metal, is present at trace levels in living organisms. It is generally accepted that seafood represents one of the major sources of mercury in the human food chain (Plessi *et al.* 2001).

Lead constitutes a serious health hazard to both children and adults that may be directly ingested by man or indirectly through aquatic animals like fish and shellfish (Olaifa *et al.,* 2003) and is one of the most important heavy metal

pollutant found in the environment, including the aquatic environment (Cigerci *et al.,* 2010). The high blood lead level can cause kidney dysfunction, brain damage,anemia and can inhibits the normal functioning of many enzymes. Pb present in the marine environment through atmospheric deposition and soil erosion as well as vehicle exhaust and industrial discharge. Lead is a potentially toxic chemical.

In the present study maximum concentration of 2.33 µg/g was observed in *Babylonia spirata* and the minimum of 0.067 µg/g in the *Sardinella longiceps*. 1.10 µg/g and 2.29 µg/g of lead content was observed in *Penaeus indicus* and *Lethrinus nebulosus*. Celik *et al.* (2004) has reported a mean lead concentration of 0.05 mg/kg in the sardine from İzmir Bay (Turkey)

Copper is an essential element. However, it can be potentially toxic to aquatic organisms when in excess in water (Martins *et al.* 2011). In the Turkish Food Codex (Anonymous, 2002) the recommended limit for copper in fish is 20 mg/kg. In this study the maximum copper content for sardine was 2.04 mg/kg and the obtained copper levels in the sardine samples were found to be lower than the set limits. The mean concentration of copper reported in this study (1.00 mg/kg) was lower than the values reported by several investigators (Tarley *et al.,* 2001, Canli and Atli, 2003).

Lethrinidae fish are widely consumed and are considered among the favourite dish. International Standard Statistical Classification for Aquatic Animals and Plants (ISSCAAP) has represented Lethrinidae fish family as demersal fishes and classified them as bottom-feeding (bottom-dweling) carnivorous fish. Due to their feeding habits and long life (30 years), Lethrinids can be used as an indicator of heavy metal bioaccumulation in the aquatic environment since they feed on mainly molluscs, crustaceans, sea urchins, hard-shell invertebrates and sometimes fishes (Carpenter and Allen, 1989).

Analysis of metals Zn, Hg, Cu, Cd, and Pb in the tissue of *Lethrinus nebulosus* in the present study shows the presence of 12.75 µg/g of Zn 2.29 µg/g of Lead 5.35 µg/g of Cu 4.72 µg/g of Cd and 0.067 µg/g of Hg. Concentration of Zn, Lead and Copper were higher compared with the cadmium and and mercury. Similary, Bhanoo Saulick *et al*.,(2017) confirmed the presence of trace elements primarily of Cu, Ni and Zn and heavy metals Hg and Pb in muscle tissue of the four fish species namely the sky emperor (*Lethrinus mahsena*, Bank), sky emperor(*Lethrinus mahsena*, coastal), blackspot emperor (*Lethrinus harak*) and the spangled emperor (*Lethrinus nebulosus*) in different region of Mauritius. Uptake of heavy metals and trace elements is related to fish age, mass and length and aquatic environment. This clearly supports the fact that higher concentration of zinc, lead and mercury

were recorded in Lethrinus nebulosus and lowest concentration of lead in Lethrinus mahsena Coastal. Kumar *et al.* (2013) also supported the fact that there are significant differences between species and heavy metals accumulation in fish.

Several factors may be involved in explaining the differences between our results and those reported by other authors: Intrinsic factors: Fish size, age, sex, reproductive cycle, diet, and metabolic activity. The latter is proportional to heavy metals' accumulation (Kim and Kang, 2015) and extrinsic factors: The environment whose fish live, which significantly affects the rate of contaminant accumulation by different organisms, as well as the concentrations of contaminants in the water column of fishing areas, handling, and processing fish during transportation and storage. The fishing season is also an important factor to consider as well as temperature, salinity, pH, and the presence of ligands in the marine environment (Guner, 2008). The periodical control of heavy metals in the is needed both for the assessment of toxic metal intake from these fish by humans and for generating data for further studies.

These values are fortunately below the permitted limit of 1.0 mg/kg (EC, 2001) in the edible portion

SUMMARY

8. SUMMARY

The present investigation was carried out to analyze the heavy metal concentration in Fin and Shell Fishes namely are *Penaeus indicus* (Indian Prawn),*Sardinella longiceps*(Sardine), *Babylonia Spirata*(Spiral Babylon) and *Lethrinus nebulosus* (Splangled Emperor).The data obtained from this study suggest that the accumulation of heavy metal like zinc,Lead,Copper,Cadmium and Mercury in these marine species were below the permissible level.Hence ,the utilization of these Fin and Shell fishes along the Thoothukudi coast are safe.

These five metals of tissues of four species collected from Gulf of Mannar were measured to investigate their potential sources, bioaccumulation rate and human health risk. Among the marine species studied, *Babylonia Spirata* had high concentration of Zinc compared with other samples.This may due to the higher level of heavy metal in the bottom water or sediment compared with surface water. Accumulation of heavy metals in *Babylonia spirata* could be resulted from surface contact with the water, by breathing, and by the food chain. Heavy metals in the sediment enter the food chain through the feeding of benthic animals.

Factors like seasonability, metabolic and trophic changes during it life stages may alter the accumulation of lead which needs further

investigations. The knowledge of heavy metal concentrations in the edible organism is very important with respect to nature management. Though the input of anthropogenic pollutants into the system has not affected the levels of metals in the muscle to an alarming extent, this baseline data can be used for regular ecological monitoring considering the industrial growth around this coast of Thoothukudi.

CONCLUSION AND SUGGESTIONS

9.CONCLUSION AND SUGGESTION

The present study shows that , Zinc was found to be abundant in all the collected samples compared with all the other metals of studied species. The data in this study may form baseline values for metals. *Sardinella Longiceps, Penaeus indicus, Babylonia spirata* and *Lethrinus nebulosus* could be used as a good indicator organism due to its short life span and also rapid growth.

Stringent pollution abatement measures have to be followed to safeguard the diversified fishery resources of Tuticorin bay. Since Tuticorin bay is a part of the Gulf of Mannar, which is the first Marine Biosphere reserve in the South East Asia, the biodiversity of this region needs to be conserved.

As a measure of conservation of fauna and flora of this bay, the following pollution abatement measures are recommended.

- An effective treatment and measures for industrial effluents and other anthropogenic discharges into the coastal waters so as to reduce the heavy metal.
- Programmes on the protection of Gull of Mannar biosphere reserve may be periodically broadcast and telecast to raise awareness among the public.
- To avoid dumping of fly-ash in the near shore areas, new industries should be started in this coastal town for the utilization of fly-ash waste for the

production of useful materials like concrete slabs and fish aggregating devices.

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EVALUATION OF ANTIMITOTIC ACTIVITY OF *DONAX VARIABILIS* (SAY,1822) ON THE ROOT TIP OF *ALLIUM CEPA*

A project submitted to

ST.MARY'S COLLEGE (Autonomous), THOOTHUKUDI

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

in partial fulfillment for the award of the degree of

Bachelor of Science in Zoology

by

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(Re-accredited with 'A+'grade by NAAC)

April 2021

CERTIFICATE

This is to certify that the project entitled **Evaluation of antimitotic activity of *Donaxvariabilis*(Say,1822) on the root tip of *Allium cepa*** is submitted to **St.Mary's College (Autonomous), Thoothukudi** in partial fulfillment for the award of the degree of **Bachelor of Science in Zoology** and it is a record of the work done during the year 2020-2021 by the following students.

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CONTENTS

INTRODUCTION

1. INTRODUCTION

Marine organisms are used as nutritious foods, animal feed, ornamental and recreational items and also as potential source of marine natural products in health care since ancient times. Exploration and exploitation of sea based resources have witnessed a paradigm shift in recent years. The rapid increase in human population, change in their life style and climate change impacts have propelled the origin and spread of many incurable and fatal diseases like influenza, diabetes, coronary disorder, AIDS and cancer globally. Again, many disease causing pathogens became drug resistant giving rise to their mutant forms. Coupled with such threat perceptions and rapid fall in the availability of land based natural resources, the scientists have shifted their research to marine environment which has been found as the hidden ground for a plethora of bio-molecules which can be used for discovery of new drugs (Premalata Pati *et al*., 2015).

The extrapolation of natural products for developing novel drugs has been highly appreciated. Although these biochemicals face certain obstacles in bioavailability and isolation, they can overcome most of the difficulties evoked by synthetic drugs and therapeutics (da Rocha *et al.,* 2001*).* For instance, around 60% of the anticancer drugs are of natural derivatives and several are in clinical and pre- clinical trials (Patwardhan and Vaidya, 2010).

The antimitotic activity was screened using *Allium cepa* root meristematic cells which have been used extensively in screening of drugs with antimitotic activity (Aunti *et al*., 2010 and Angayarkanni *et al.,* 2007). The roots of all plants have distinguished regions, one of them being the region of cell division that lies beyond the root cap and after this it even extends a mm. Cells of this region undergo repeated divisions, and the fate of cell division is higher in this region compared to that of the other tissues; hence, this region is called the meristematic region (Jordan *et al.,* 2002).

This rapid division of meristematic cells is similar to that of the cancer cell division in humans. Hence, these meristematic cells can be used for preliminary screening of drugs with anticancer activity. Even though doubts can be raised about extrapolation of results from plant tissue to animals and finally to humans, plant cells are 1000 times more resident. to colchicine's which are a potent anticarcinogen and act by inhibiting the microtubule formation. It is inferred that the chemicals which affect plant chromosomes will also affect animals (Saxena *et al.,* 2005).

Allium cepa bioassay can be used to assess cytotoxic and genotoxic endpoints such as chromosomal aberrations, nuclear alterations, root growth inhibition, and mitotic index alterations (Sudhagar *et*

al., 2001; Gana *et al*., 2008; Samuel *et al*., 2010; Antonise – Weiz 1990 and Roa *et al*., 2012).

Oxidative stress, due to the overproduction of reactive oxygen species (ROS) and reactive nitrogen species, can cause damage to nucleic acids, proteins, or lipids which eventually can lead to cardiovascular and neurodegenerative diseases, Alzheimer's and cancer (Dayem *et al.,* 2010). The most destructive one among these is cancer. These highly reactive species cause impairment in replication, transcriptional and translational errors and genomic instability, all of which finally result in cancer (Ogasawara and Zhang, 2009). Antioxidants are competent in scavenging these reactive species to prevent cancer development (Bennett *et al.,* 2012). Cancer is essentially a problem of abnormal cell growth. Under the influence of chemicals, viruses, and free radicals, normal cells are converted to tumour masses that divided in an uncontrolled manner (Nwafor *et al.,* 2001).

The general principles of the mechanisms of mitosis are best and most easily studied in the actively growing regions of plants such as a shoot or root apex. Frequently, such studies involve the use of chemicals, which modify the normal course of mitosis (Nwakanma *et al., 2010).* A wide variety of secondary metabolites obtained from molluscs

are tested for their ability to treat cancer. Various anticancer drugs from molluscs are known to be effective against proliferating cells.

They exhibit cytotoxic effect by interfering with cell-cycle kinetics. These drugs are effective against cells that are proliferating and produce cytotoxic effect either by damaging the DNA during the S-phase of the cell cycle or by blocking the formation of the mitotic spindle in M-phase. However, most of the cytotoxic drugs exhibit side effects, and hence, there is a need for drugs that are efficient and have less side effects (Sehgal *et al.,* 2006).

In *Allium cepa* L. root tip model root system of plant cells is commonly used as a test for investigating environmental pollution factors, toxicity of chemical compounds and evaluating potential anticancer properties It has been used since 1938. It is very comfortable as it is easy to make preparations of onion roots. They contain rather homogenous meristematic cells, having only 16 chromosomes, which are very long, well visible and get stained easily. It is a fast and inexpensive method, allowing the investigation of universal mechanisms for meristematic plant cells and extrapolation on animal cells (Kuras *et al.,* 2006).

The chemical investigation of marine molluscs has led to the isolation of a wide variety of bioactive metabolites, which evolved in marine

organisms as favourable adaptations to survive in different environments. However, the chemical investigation of the phylum Mollusca has provided many compounds of interest as potential anticancer drugs that assume particular importance in the light of the growing literature on cancer biology and chemotherapy (Ciavatta *et al*., 2016).

Bivalves are a relevant ecological group, widespread in freshwater, estuarine and marine ecosystems, with many edible species, such as oysters mussels and clams. Bivalves are the most widely used bio indicators, as they accumulate pollutants, present a low rate of regulatory mechanisms of internal concentrations of chemicals, and are able to concentrate both inorganic and organic contaminants. So, the present study has been carried out with a view to evaluate the antimitotic activity of *Donax variabilis.*

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The chemical investigation of marine molluscs has led to the isolation of a wide variety of bioactive metabolites, which evolved in marine organisms as favourable adaptations to survive in different environments. Most of them are derived from food sources, but they can be also biosynthesized de novo by the molluscs themselves, or produced by symbionts. The "promise" of a mollusc-derived natural product as an anticancer agent is evaluated on the basis of its ability to target biological characteristics of cancer cells responsible for poor treatment outcomes.

Celik and Aslanturk (2010) evaluated cytotoxicity and genotoxicity of *Inula viscosa* leaf extract with *Allium* test. Samuel *et al.*, (2010) showed the cytogenotoxicity evaluation of two industrial effluents using *Allium cepa* assay. Olorunfemi *et al*., (2011) studied the cytotoxic and genotoxic effects of *Cassava* effluents using the *Allium cepa* bio assay. Thenmozhi *et al.*, (2011) reported the cytotoxic and antimitotic activities of *Solanum nigrum* by using *Allium cepa* root tip assay and cancer chemopreventive activity using lines .

Singh (2012) investigated antimitotic activity of new compound isolated from the flower of *Prosopis juliflora*. Bhattacharya and Halder (2012) evaluated antimitotic and genotoxic effects of the triterpenoid enriched extract from *Trichosanthes dioica* root. Roa *et al.,* (2012) studied the

genotoxicity and evaluations of ECF cellulose bleaching gluents using the *Allium cepa* L test. Saboo *et al.,* (2012) revealed *in vitro* evaluation of antimitotic, antiproliferative, DNA fragmentation and anticancer activity of chloroform and ethanol extracts of *Revia hypocrateriformis.*

Yuet *et al.,* (2012) showed the genotoxicity of *Euphorbia hirt*: an *Allium cepa* assay. Periyanayagam *et al*., (2013) observed *Vitis vinifera* leaves towards antimitotic and anti-proliferative activity of anticancer drug discovery. Hemachandra and Pathiratne (2013) found the toxicity and genotoxicity of complex effluents discharged from two industrial zones in Srilanka using *Allium cepa* (Common onion) bioassay .

Kirankumar Shivasharanappa and Ramesh Londonkar (2014) showed the clot lysis and antimitotic study of a *Ficus glomerata* roxb fruit extracts. Alamgir *et al*., (2014) investigated the phytochemical characteristics, antimitotic, cytotoxic and antiflammatory activities of *Coccinia grandis*. James *et al.,* (2014) studied the cytotoxic effects and genotoxic screening of pharmaceutical effluents using onion bulbs (*Allium cepa* L.) Geena Mariya Jose *et al*., (2015) analysed antioxidant and antimitotic activities of sulfated polysaccharide from marine brown algae *Padina tetrastromatica* .Pathiratne *et al*., (2015) reported the efficacy of *Allium cepa* test system for screening cytogenotoxicity and genotoxicity of industrial effluents originated from different industrial activities. Mesi and Kopliku (2015) found the use of

Allium cepa L. assay for toxicity bio - monitoring of hospital effluents - an Albanian case .

Hemachandra and Pathiratne (2016) investigated the combination of physico - chemicals analysis, *Allium cepa* test system and *Oreochromis niloticus* erythrocyte based comet assay and nuclear abnormalities tests for cyto - genotoxicity assessments of treated effluents discharged from texile industries. Boumaza *et al.,* assessed the cytotoxic and genotoxic effects of clodinafop - propargyl commercial formulation on *Allium cepa* L . Shalini and Velavan (2017) evaluated antimitotic activity of *Aplotaxis auriculata* rhizomes using root meristematic cells.

Patil *et al.*, (2018) studied the antimitotic activity of *Murraya Koenigii* by using *Allium cepa* root assay. Dimuthu Nilmini Wijeyaratne and Wadasinghe (2019) used *Allium cepa* bioassay to assess the water and sediment cytogenotoxicity in a tropical stream subjected to multiple points and no point source pollutants.

From the above review it is pronounced that knowledge on the antimitotic activity is lacking for marine bivalves. Considering the importance of this species as a novel bioactive agent, the present work on *Donax variabilis* is planned and carried out to study the antimitotic activity.

OBJECTIVES

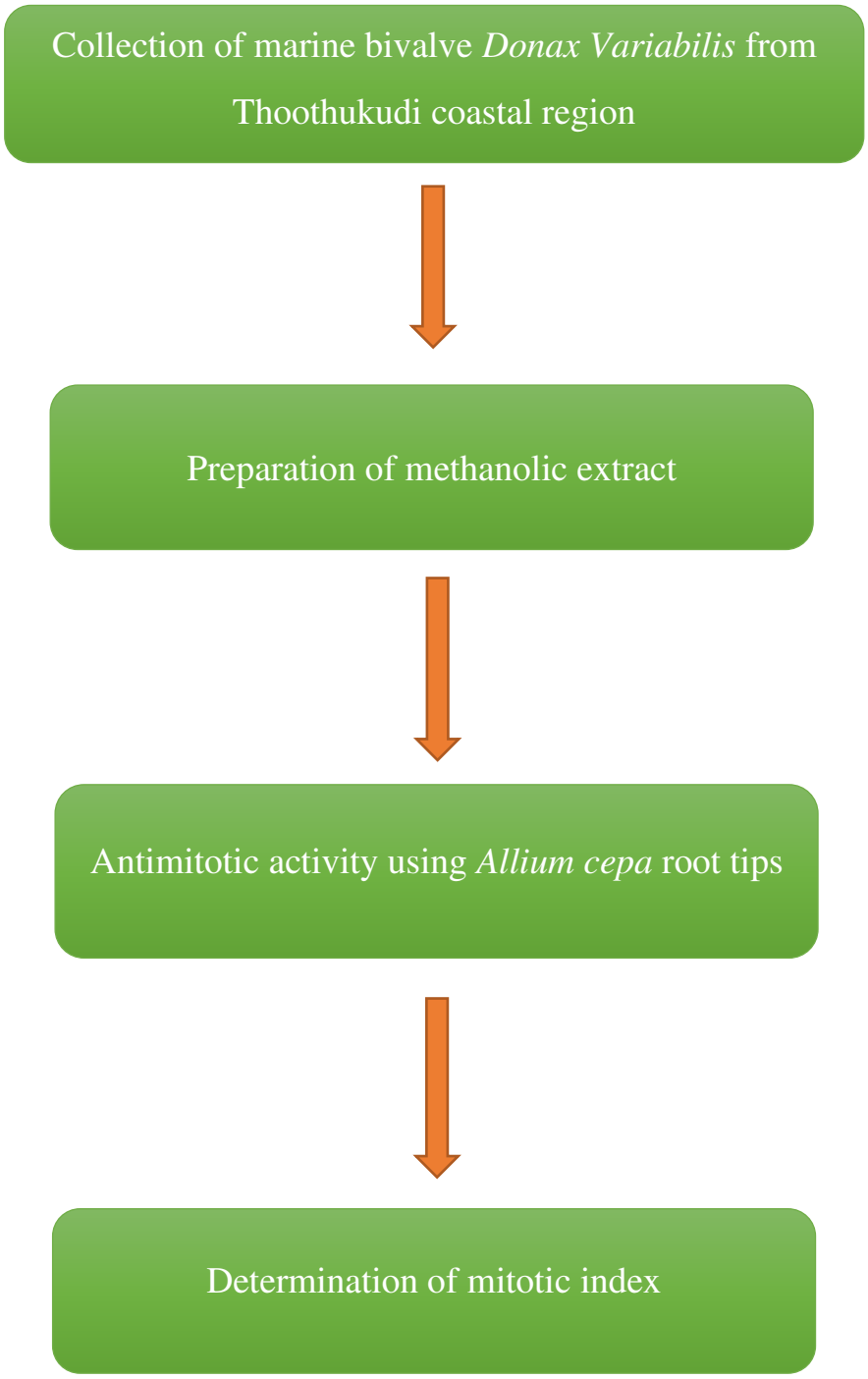
3. OBJECTIVES

The antimitotic activity was screened using *Allium cepa* root meristematic cells which have been used extensively in screening of drug with antimitotic activity. Cells of this region undergo repeated divisions, known as meristematic region, which is similar to cancer division in human. Hence, *Allium cepa* meristematic cells can be used for preliminary screening of drug with anticancer activity. The present study has been carried out with the following objectives.

- To investigate the antimitotic activity of marine bivalve *Donax variabilis*.
- To compare the mitotic index of different concentrations of the methanolic extract of *Donax variabilis*.

EXPERIMENTAL DESIGN

4. EXPERIMENTAL DESIGN



MATERIALS AND METHODS

5. MATERIALS AND METHODS

5.1 DESCRIPTION OF THE STUDY AREA

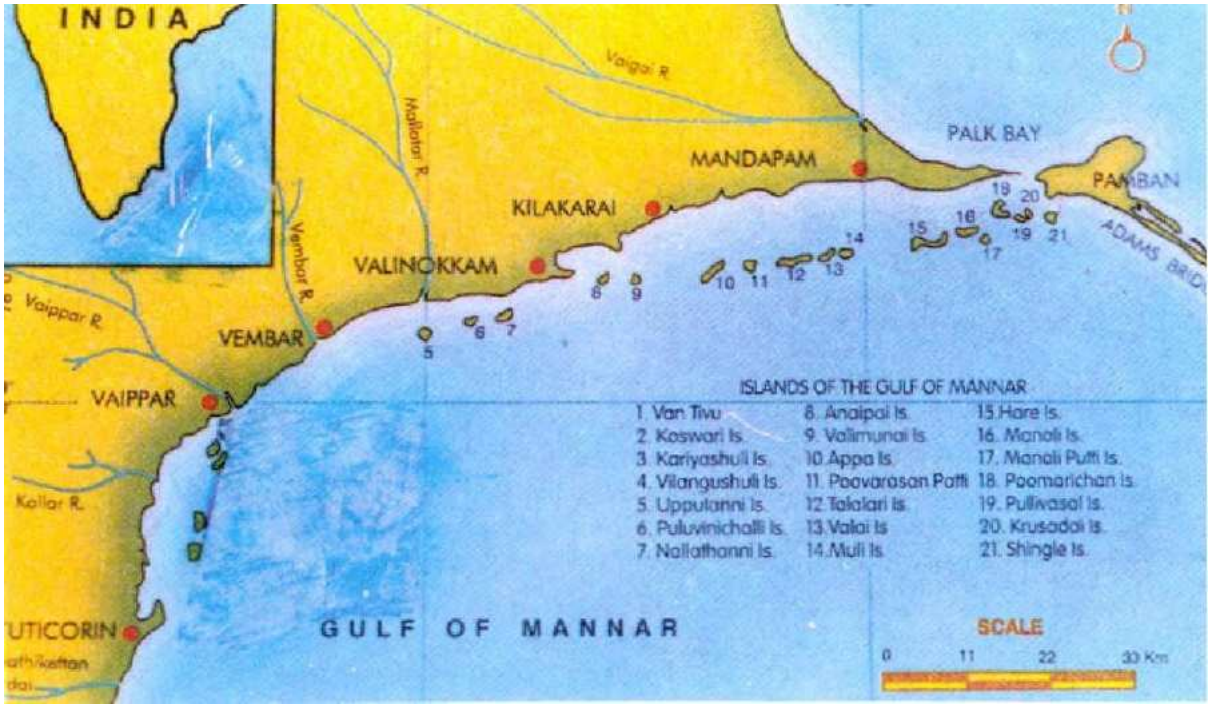
Specimens of *Donax variabilis* used in the present study were collected from Gulf of Mannar Coastal region. Gulf of Mannar is situated on the South-east Coast of India. This area is remarkable for its richness and variety of fauna and the inshore sea bottom forms an ideal habitat for the growth of the shell fishes which sustain a good fishery. The Indian part of Gulf of Mannar covers approximately an area of 10,500 km^2 along lat 8°35′-9°25′ N and long 78°08′ - 79°30′E (Figure.1).

It is a part of the southward extension of Bay of Bengal, it meets in the Indian ocean. This geographical area runs from Pamban island including Rameshwaram to Cape Comarin along the South east coast of India to a distance of above 170 nautical miles. This coast contains a rich biological diversity of flora and fauna largely due to diversified micro habitats such as mangroves, corals, seaweed beds, sea grasses, sandy, rocky and muddy shore etc. The fauna diversity is also well pronounced with reference to different molluscan groups.

5.2 COLLECTION OF EXPERIMENTAL ANIMAL

Specimens of *Donax variabilis* were collected during low tides from the sea in their natural habitat that is intertidal zone and from seashore.

Figure 1: Map showing the study area Gulf of Mannar Thoothukudi coastal region



These bivalves were collected during the month of December 2020. The freshly collected samples were brought to the laboratory, cleaned and washed with fresh sea water to remove all impurities. The shells were broken, tissues were removed and then dried in hot air oven at 56ºC for 48 hours and used for further studies.

5.3 DESCRIPTION OF EXPERIMENTAL ANIMAL

5.3.1 Systematic position of *Donax variabilis* (Say, 1822)

Phylum	:	Mollusca
Class	:	Bivalvia
Order	:	Cardiida
Super family	:	Tellinoidea
Family	:	Donacidae
Genus	:	*Donax*
Species	:	*variabilis*

Donax variabilis, known by the common name coquina, is a species of small edible saltwater clam, a marine bivalve mollusc in the family Donacidae, the bean clams. The maximum reported size is 19 mm. The exterior of the small shell of this species can have any one of a wide range of possible colors, from almost white, through yellow, pink, orange, red, purple, to brownish and blueish, with or without the presence of darker rays. This

species lives from the intertidal zone of sandy beaches to a depth of 11 m. Coquinas are eaten and used as decoration because of their colourful markings. The shells are also used in ornamental landscaping.

5.4 PREPARATION OF METHANOLIC EXTRACT

The collected bivalve *Donax variabilis* were washed several times with distilled water to remove the traces of impurities. The shells were broken and the tissues were shade dried at room temperature for about 10 days and ground into fine powder using mortar and pestle. 25gms of the powder was transferred into different conical flask (250 ml). The conical flask containing different concentrations of methanol (10,20,30,40mg/ml) were taken. The conical flask containing bivalve tissue powder and solvent was shaked well for 2 hours by free hand. After 1 day, the extracts were filtered using Whatmann filter paper No.1 and was transferred into china dish. The supernatant was completely removed by keeping the china dish over water bath at 45°C. The obtained extracts were stored at 4°C in air tight bottle until further use.

5.5 EVALUATION OF ANTIMITOTIC ACTIVITY USING *ALLIUM CEPA* ROOT TIPS

Antimitotic activity study was conducted as per the methods reported by previous workers with modifications (Grant, 1982; Fiskesjo, 1988; Shweta *et al.,*2014).

5.5.1 *ALLIUM CEPA* BULBS

Approximately equal size bulbs of the onions (*Allium cepa* L.) were obtained from the local vegetable market at Thoothukudi, TamilNadu, India. Any onions that were dry, moldy or have started shooting green leaves were discarded.

5.5.2 *ALLIUM CEPA* MERISTEMS

The outer scales were removed from the healthy onion bulbs leaving the root primordial intact. These bulbs were grown in dark for 48 h over 100ml of tap water at ambient temperature until the roots have grown to approximately 3 cm. The water was changed daily during this period. The viable bulbs were then selected and used for subsequent studies.

5.5.3 EXPOSURE TO TEST SAMPLES

The bulbs with root tips grown up to 2-3 cm were removed from the water and placed on a layer of tissue paper to remove excess of water. The bulbs were divided into four groups. The first group served as control (water). Second group : *Allium cepa* root were dipped in the methanolic tissue extract of *Donax variabilis* (10mg/ml). Third group : *Allium cepa* root were dipped in the methanolic tissue extract of *Donax variabilis* (20mg/ml). Fourth group: *Allium cepa* root were dipped in the methanolic tissue extract of *Donax variabilis* extract (30mg/ml). Fifth group: *Allium cepa* root were

dipped in the methanolic tissue extract of *Donax variabilis* (40mg/ml). All the groups were incubated at 25±2°C for 96 h away from direct sunlight. The test samples were changed daily with fresh ones. The length of roots grown during incubation (newly appearing roots not included), and the mitotic index were recorded after 96 h.

5.6 DERMINATION OF MITOTIC INDEX

After 96 h , the root tips were fixed with fixing solution of acetic acid and alcohol, (1:3) squash preparations were made by staining the treated root with acetocarmine stain (Badria *et al.,* 2001). The slide was then squashed and observered under microscope. The numbers of cells in each stage of cell division were counted in four fields for each group. Mitotic index was calculated using the following formula

$$Mitotic\ Index = \frac{Number\ of\ dividing\ cells}{Total\ number\ of\ cells}$$

RESULTS

6. RESULTS

ANTIMITOTIC ACTIVITY OF METHANOLIC EXTRACT OF *DONAX VARIABILIS* USING *ALLIUM CEPA* ROOT MERISTEMATIC CELLS

The antimitotic activity was screened using *Allium cepa* root meristamatic cells which have been used extensively in screening of drugs with antimitotic activity. The mitotic activity methanolic tissue extract of *Donax variabilis* was evaluated on *Allium cepa* root meristems.

The percentage of non dividing cells in water control was found to be 40% and the percentage of different concentrations of methanol extract was found to be 45% at 10mg/ml, 52 % at 20 mg/ml, 58% at 30 mg/ml and 67% at 40 mg/ml respectively.

In water (control) the percentage of dividing cells in prophase, metaphase, anaphase and telophase was found to be 51, 10, 1 and 1 respectively. In the methanolic tissue extract of 10 mg/ml, the percentage of telophase was found to be 42,12, 2 and 1 respectively. In the methanolic tissue extract of 20 mg/ml, the percentage of dividing cells in prophase, metaphase, anaphase and telophase was found to be 39, 4, 1 and 1 respectively. In the methanolic tissue extract of 30 mg/ml concentration, the percentage of dividing cells in prophase, metaphase, anaphase and telophase

was found to be 37, 3, 1 and 1 respectively. In the methanolic concentration of 40 mg/ml the percentage of dividing cells in prophase, metaphase, anaphase and telophase was found to be 26, 7, 2 and 1 respectively.

Plate 1: Showing the marine bivalve *Donax variabilis*

Table 1: Antimitotic activity after treatment of *Allium cepa* roots with different concentrations of methanolic extract of *Donax variabilis*

S.No.	Different solution used for treatment	% of Non dividing cells	% of dividing cells				Mitotic Index (%)
			P	M	A	T	
1.	Group I (water control)	40	51	10	1	1	63
2.	Group II (methanol - 10 mg/ml)	45	42	12	2	1	57
3.	Group III (methanol - 20 mg/ml)	52	39	4	1	1	45
4.	Group IV (methanol - 30 mg/ml)	58	37	3	1	1	42
5.	Group V (methanol - 40 mg/ml)	67	26	7	2	1	36

Figure 2: Percentage of dividing cells after treatment of *Allium cepa* roots with methanolic tissue extract of *Donax variabilis*

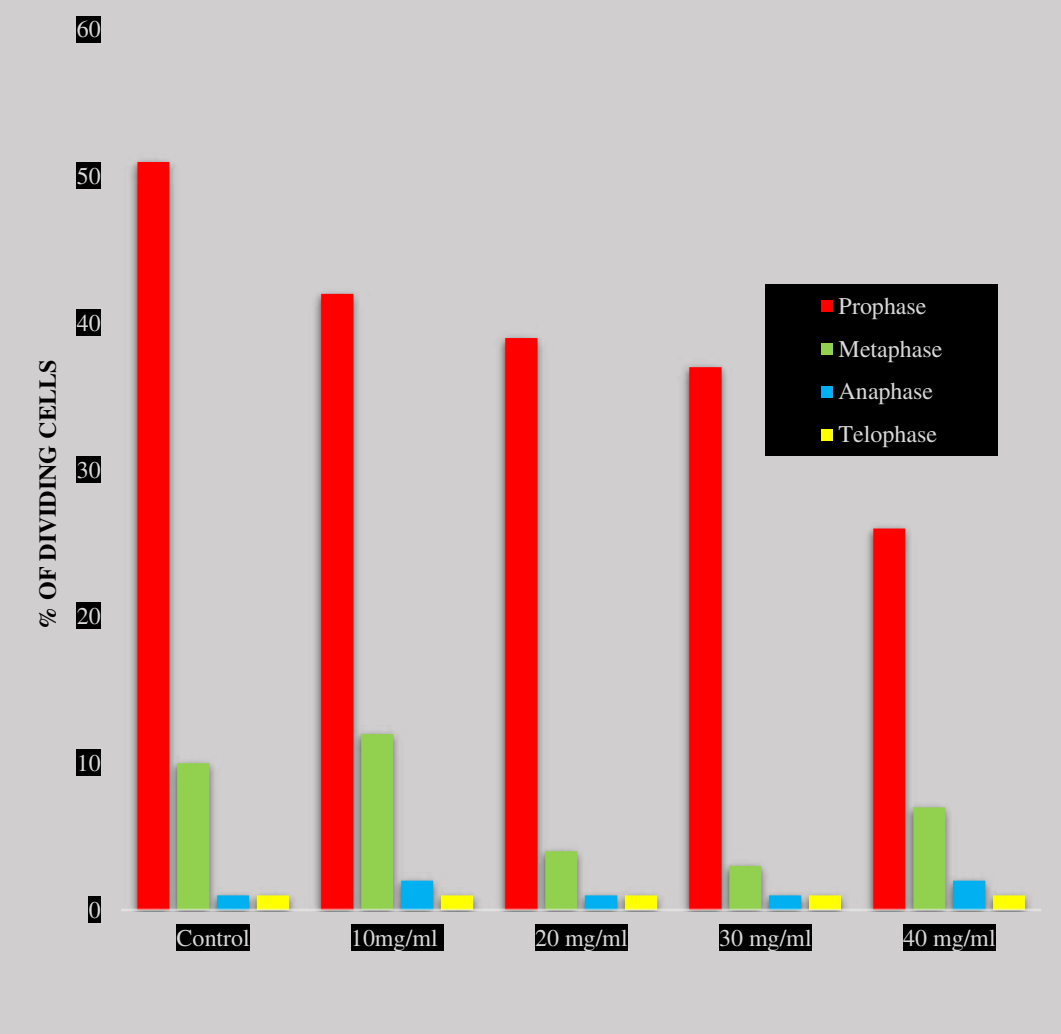


Figure 3: Mitotic index of methanolic tissue extract of *Donax variabilis*

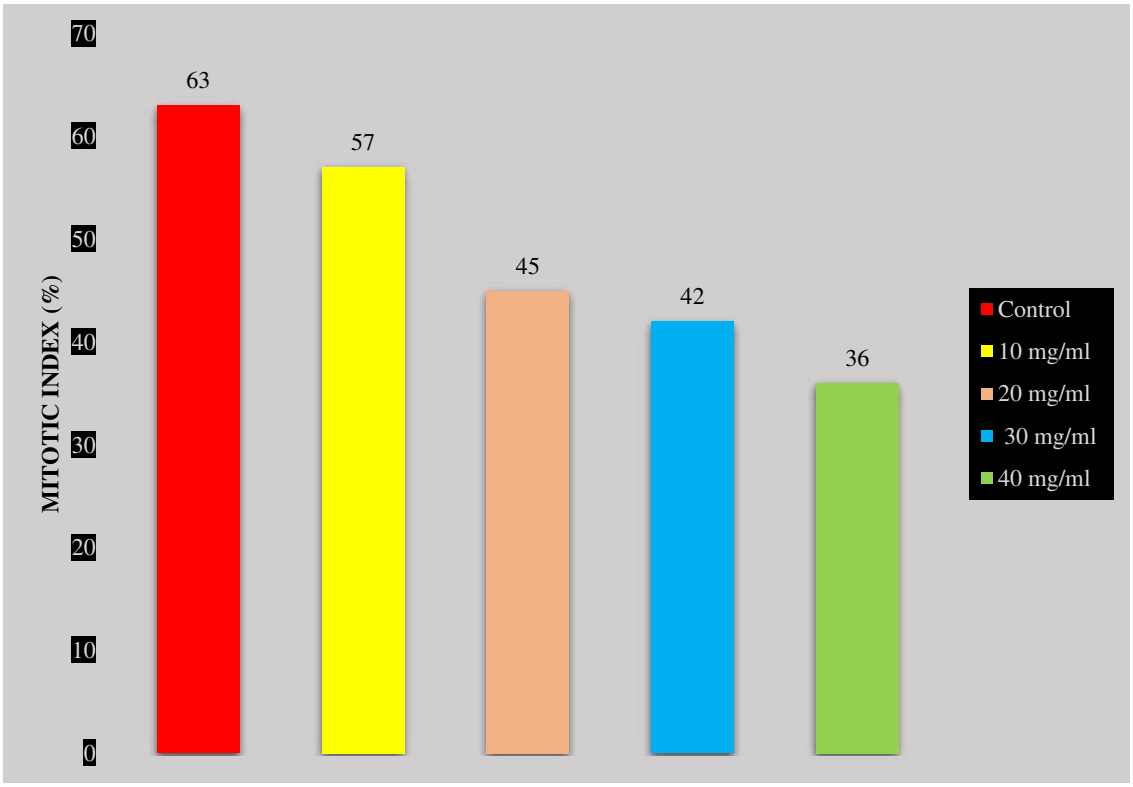
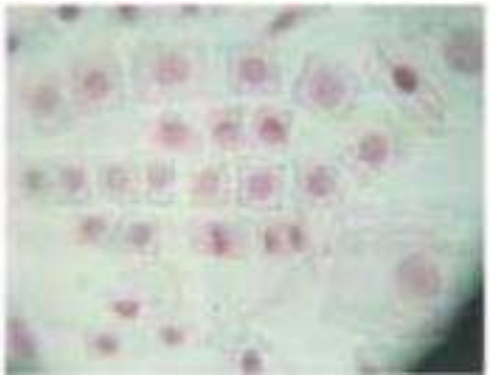
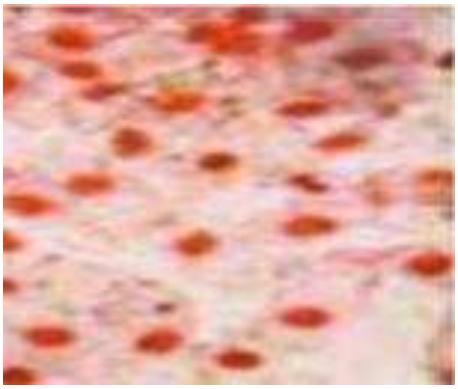


Plate 2: ANTIMITOTIC ACTIVITY OF *DONAX VARIABILIS* ON *ALLIUM CEPA* ROOT TIP

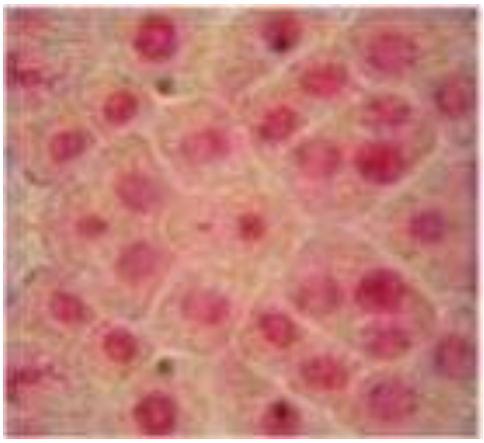
Group I (Control)



Group II (10 mg/ml)

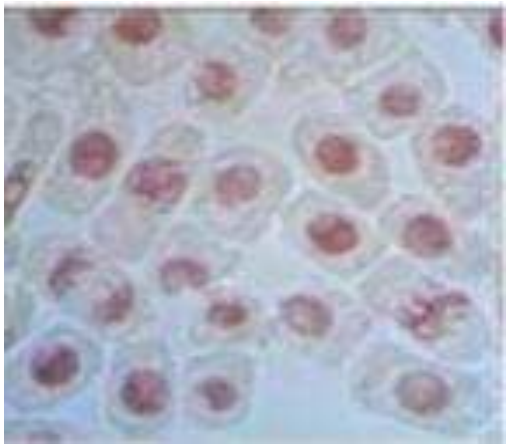


Group III (20 mg/ml)



Group IV (30 mg/ml)

Group V (40 mg/ml)



DISCUSSION

7. DISCUSSION

The antimitotic activity was screened using *Allium cepa* root meristematic cells which have been used extensively in screening of drugs with antimitotic activity (Abhang *et al.*, 1991; Latha *et al*., 1998). The antimitotic activity was screened using *Allium cepa* root meristematic cells which have been used extensively in screening of drugs with anticancer compounds. Cytotoxicity at all concentrations test extract were evidenced by evaluating macroscopic parameters, i.e., reduction in root number and root length both of which were indicative of root growth inhibition.

In the present study mitotic index of different concentrations of extract clearly indicates the efficiency in the inhibition of growth of cancer cells either by affecting microtubules or encouraging microtubule formation, and thus stopping the microtubules from being broken down. This make the cell become so clogged with microtubules that they cannot continue to grow and divide.

The rate of tumor growth is dependent upon a balance between the rates of cell proliferation and apoptosis. Apoptosis is a programmed cell death, as influenced by sterol (Awad *et al*., 2000). The result from the present study showed that the methanol extract of *Donax variabilis* had excellent antimitotic activity. Maximum numbers of non-dividing cells were observed. As a result of this cells arrest in mitosis and eventually die by apoptosis.

Similar reports were observed by Shweta *et al.*, (2013 and 2014) and Thenmozhi and Mahadeva Rao (2011).

Kirankumar and Ramesh (2014) reported that the extracts of *Ficus glomerata* have have mild to moderate antimitotic and clot lysis activity. The significant average percent of clot lysis is observed in methanol extract (47.23%), moderate in chloroform extract (27.89%), and least in petroleum ether extract (23.11%) treatment, where as the standard thrombolytic drug streptokinase treatment has showed 74.65% of clot lysis. On the other hand methanolic extract has stopped the root growth significantly, followed by petroleum ether and chloroform extract. These antimitotic activities were supported by mitotic index. This effect may be possible due to phytoconstituents present in the plant extracts affecting the cytoskeleton or inhibiting the activity of one or more components of the cell cycle, thereby providing clear information regarding cytotoxic action of fruit extracts as reported by Bhattacharya and Halder (2010). Growth inhibition effect may be due to diminished cell division (Fiskesjo, 1988).

Onion roots have been used as a model for the study of phytotoxic activity as per Williams and Omoh (1996) who studied other similar systems. *Allium cepa* has been used for evaluating cytotoxicity since the early 1920s (Grant, 1982). This method is an easy and sensitive tool for measuring the total toxicity caused by chemical treatments as expressed by growth

inhibition of the roots of onion bulbs. Levan (1938) has reported that *Allium* test is a rapid, highly sensitive, and reproducible bioassay for detecting cytotoxicity of phytochemicals and results of *Allium* test fit well for prokaryotes and other eukaryotes.

Dimuthu and Wadasinghe (2019) assessed the root growth inhibition of the *Allium cepa* bulbs exposed to water and sediment samples collected from study sites were compared with that of the reference site. The results indicated a root growth inhibition in all sites compared to the reference site. However, the percentage root growth inhibition trends in the *Allium cepa* bulbs exposed to water samples were different from that of those exposed to sediment elutriates. In the *Allium cepa* bulbs exposed to water samples, significant root growth variations were not observed within the first 48 hours of exposure and significant changes of root growth were observed after 7 days of exposure. However, significant root length variations were observed in the *Allium cepa* bulbs exposed to sediment elutriates within the first 48 – hour exposure and the percentage root growth inhibition of all the sites, except site D, increased with increase of exposure time. A similar trend was observed with the mitotic activity as well, indicating significantly lower mitotic indices (compared to that of reference site) in the *Allium cepa* root tip cells exposed to sediment elutriates of study sites than those exposed to water samples.

The significant reductions of root length and mitotic activity are considered as indicators of rhizotoxicity, being a general phenomenon caused by most pollutants (Fiskesjo 2006., Mesi and Kopliku, 2015). In the aquatic environment, sediments play a key role as they provide the basis of aquatic ecosystems by providing a substrate for many aquatic biota and being a deposition substrate for many suspended and dissolved matter. The results of *Allium cepa* bioassay indicated higher significant reductions in root length and mitotic activity of *Allium cepa* bulbs exposed to sediment elutriates compared to water samples indicating high phytoxicity in sediments .

Therefore, these results indicate that the sediments of *Dandugan oya* are acting as storage of cytotoxic and genotoxic compounds to overlying water column and biota. Sediments in the stream ecosystems are characterized by presence of fine particles and many aquatic pollutants are predominantly associated with fine deposits that are rich in organic matter (da Costa *et al.,* 2012*).* Further, the mitotic index of the *Allium cepa* root tip cells of the ranged from mitotic index less, than 22% is recorded to be lethal to the organism (Antonise – Wiez, 1990). Therefore, the mitotic indices recorded in the present study was 63% in group I followed by 57% in group II, 45% in group III, 42% in group IV and 36% in group V.

The occurrence of nuclear abnormalities in *Allium cepa* root tip cells indicates the possibility of occurrence of genotoxic compounds in

the exposed medium (Kannangara and Pathiratne, 2015), (Boumaza *et al.,* 2016). The industrial wastes may be rich with cytotoxic and genotoxic compounds including heavy metals, poly aromatic hydrocarbons, and other organic and inorganic compounds that can trigger cellular and genetic variations in biota in these waste water receiving areas of the stream. Studies have proven that compounds such as poly aromatic hydrocarbons, copper, arsenic, and other industrial effluents can display cytotoxic and genotoxic effects in the *Allium cepa* root tip cells (Kannangara and Pathiratne, 2015), (Samuel *et al.,* 2010), (da Costa *et al.,* 2012; Boumaza *et al.*, 2016; Olorunfemi *et al.*, 2011). The present study concludes that the marine bivalve *Donax variabilis* can be used as an antimitotic or anticancer agent in the Indian system of medicine.

SUMMARY

8. SUMMARY

- The antimitotic activity was screened using *Allium cepa* root meristematic cells which have been used extensively in screeing of drugs with antimitotic activity.
- The mitotic activity methanolic tissue extract of *Donax Variabilis* was evaluated on *Allium cepa* root meristems.
- The percentage of non dividing cells in water control was found to be 40% and the percentage of different concentrations of methanol extract was found to be 45% at 10mg/ml, 52% at 20 mg/ml, 58% at 30 mg/ml and 67% at 40 mg/ml respectively.
- In water (control) the percentage of dividing cells in prophase, metaphase, anaphase and telophase was found to be 51, 10, 1 and 1 respectively.
- In methanol (10 mg/ml) the percentage of telophase was found to be 42, 12, 2 and 1 respectivey.
- In the methanol (20 mg/ml) the percentage of dividing cells in prophase, metaphase, anaphase and telophase was found to be 39, 4, 1,and 1 respectively.

- In the methanol (30 mg/ml) concentration the percentage of dividing cells in prophase, metaphase, anaphase and telophase was found to be 37, 3, 1 and 1 respectively.
- In the methanol concentration of 40 mg/ml the percentage of dividing cells in prophase, metaphase, anaphase and telophase was found to be 26, 7, 2 and 1 respectively.

CONCLUSION AND SUGGESTIONS

9. CONCLUSION AND SUGGESTIONS

The result from the present study showed that the methanolic tissue extract of *Donax variabilis* had excellent anti mitotic activity. Maximum numbers of non-dividing cells were observed. As a result of this cells arrest in mitosis and eventually die by apoptosis.

From the present study, it could be suggested that *Donax variabilis* is a promising source of natural drug which has the ability to modify the physiological function of cells and hence acts as anticancer drugs to arrest the proliferation of cancer cells. Therefore, it can be concluded that the methanolic tissue extract have shown a commendable antimitotic activity.

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Antioxidant Activity of Methanolic Extract of Puffer fish *Lagocephalus inermis* from Thoothukudi Coast

A Project submitted to

ST. MARY'S COLLEGE (Autonomous), THOOTHUKUDI

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

In partial fulfilment for the award of the degree of

Bachelor of Science in Zoology

By

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Re-accredited with 'A^{+}' Grade by NAAC

April 2021

CERTIFICATE

This is to certify that the project entitled **Antioxidant Activity of Methanolic Extract of *Lagocephalus inermis* from Thoothukudi coast** is submitted to **St. Mary's College (Autonomous), Thoothukudi** in partial fulfillment for the award of the degree of **Bachelor of Science in Zoology** and it is a record of the work done during the year 2020 - 2021 by the following students.

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CONTENTS

1. INTRODUCTION

The sea is immense almost unexploited source of new potentially useful biologically active substance (Faulkner, 1999). Recently marine microorganism have been found to produce a variety of chemically interesting and biologically significant secondary metabolites. Some of them being expected to serve a lead compound for drug development or pharmacological tools for basic studies in life science (Kobayashi and Jensen 1994). In terms of marine environment, India has a coastline of about 800 km adjoining the continental regions and the off shore islands and a very wide range of coastal ecosystem such as estuaries, lagoon, mangroves, backwater, salt marshes, rocky coast, sandy stretches and coral reefs which are characterized by unique biotic and abiotic properties and processes. The Indian coastal length measures about 8, 129 km and the Exclusive Economic Zone extends up to about 2.0 million km^2 (Ramani *et al.,* 2010). The Gulf of Mannar, situated in the south eastern coast of India extending from Rameshwaram in the north to Tuticorin in the south along with its marine environment has been declared as India's first Marine Biosphere Reserve. Marine Biosphere Reserve was established during the year 1989. The Gulf of Mannar has an area of about 10,500 km^2. In this region, totally 3600 species

of fauna and flora have been identified. It is one of the most biologically diverse coastal regions in the planet earth (Venkataramani *et al.,* 2007).

Ocean offers a large biodiversity of fauna and flora which is estimated to be over 5,00,000 species and more than double that of the land (Anand *et al.*, 1997). This rich diversity of marine organisms assumes a great opportunity for the discovery of new bioactive substances. Thus the marine environment is an exceptional reservoir for bioactive natural products, many of which exhibit structural features that are not found in terrestrial natural products (Johansson and Soderhall, 1985). In the recent past, several pharmacological substances of marine origin have been developed (Kamboj, 1999).

Marine organisms are a rich source of structurally novel and biologically active metabolites. Primary and secondary metabolites produced and stored by these organisms may be potential bioactive compounds of interest in the pharmaceutical industries. The number of natural products isolated from marine organism increases rapidly (Faulkner, 2002; Proksch and Muller, 2006). More than 10,000 compounds have been isolated from marine organisms (Proksch *et al.,* 2002) with hundreds of new compounds still being discovered every year.

Apart from human medicines, the research on marine natural products in the last three decades have also brought to the discoveries of many chemically and biologically interesting molecules, that have become indispensable tools in biochemical research and played significant role in the recent advancement of life science (Umaya Parvathi *et al*., 2012).

Like all aerobic organisms, fish are susceptible to the attack of reactive oxygen species and have developed antioxidant defences demonstrated by research primarily dating to the 1980s. Specially adapted enzymes, such as catalase (CAT), super oxide dismutase (SOD) and enzymes dependent on glutathione (glutathione peroxides-GPX, glutathione reductase-GR) have been detected in most fish species investigated to date (Rudneva, 1997).

Fishes are one of the diverse sources of natural products and bioactive compounds with over 40,000 known species. They combat infections caused by viruses, bacteria, fungi and parasites that are similar to those of humans and other vertebrates. Many species of marine fish have been reported as ithyocrinotoxic (Halstead, 1978), releasing into the water toxic secretions. Fish live in intimate contact with an environment containing both saprophytic and pathogenic microbes, capable of digesting and degrading fish tissues (Ellis, 2001; Plouffe *et al*., 2005). The slow adaptive immune response of fish

makes innate immunity, which is fast acting and temperature independent (Ellis, 2001) the predominant system of fish host defense. This innate immune response is essential for the survival of this whole class of animals. The defence includes many elements such as antimicrobial peptides (Cole *et al*., 1997) and polypeptides (Fernandes and Smith, 2002) non classical complement activation, release of cytokines, inflammation and phagocytosis (Ellis, 2001; Magnadottir, 2006). Concisely, fish have evolved a number of innate immune responses to defend themselves against infection.

Tetraodontidae is diverse with species such as Puffer fish, Balloon fish, Blowfish, Bubble fish, Globe fish, Swell fish, Toad fish, Toadies, Honey Toads and Squab (Mills and Passmore, 1988; Ramaiyan and Senthil kumar, 1998; Froese and Pauly, 2007). They are commonly distributed in the tropics, but are relatively uncommon in temperate regions and completely absent from cold water. There are 189 species of puffer fishes and 28 genera in the family Tetraodontidae (Oliveira *et al*., 2006). Puffer fishes are the second most poisonous vertebrate in the word, the first being a "Golden Poison Frog" (Keiichi *et al*., 1998). The skin and certain other internal organs of puffer fish are highly toxic to humans. Puffer fish poisoning is considered to be the common cause of fish poisoning along the Asian coast (Chew *et al.*, 1983).

Puffer will eat all type of food such as shrimp, fish, clams, molluscs and crustaceans etc. It is also important that they consume hard shelled crabs, mussels and shell fish in their diet to wear down their teeth and prevent them from overgrowing. Furthermore toxicity changes with age, sex, season and geographical variations (Homaira *et al*., 2010). Puffers are able to move their eyes independently and many species can change the colour or intensity of their patterns in response to environmental changes. They are somewhat similar to the terrestrial chameleon. Although most puffers are dark, many have bright colours and distinctive markings and make no attempt to hide from predators (Keiichi *et al.,* 1998). Puffer fish are able to produce toxin with associate microbes on the mucus of their body.

They are named after their habit of inflating themselves with water or air when threatened making it difficult for a predator to swallow them. This fish is known to carry tetrodotoxin (TTX) (Bilecenoglu *et al*., 2006; Kasapidis *et al*., 2007 ; Sabrah *et al*., 2006) which is known a non-protein organic compound (amino perhydroquinazoline) and one of the strongest marine paralytic toxins today. The toxin has occasionally been detected in the muscles of these fishes. The toxin is produced by several bacteria species including *Mycobacterium arabinogalatanolyticum*, *Serratia mascescens, Vibrio alginolyticus* and *Bacillus spp*. (Yu *et al*., 2001; Wu *et al*., 2005).

Tetrodotoxin is a low molecular weight with 319 small molecules with a unique cage structure. The basic molecule for TTX consists of a positively charged Guanidium group. The source of tetrodotoxin is accepted that bacteria in the fish's intestinal tract (Shibamoto and Bjeldanes, 2009). Saxitoxin, the cause of paralytic shell fish poisoning and red tide, can also be found in certain puffers (Lehman, 2006). It is also important to note that puffer fish toxin is 100 times more potent than cyanide (Alipala, 2012).

Antioxidant means "against oxidation". Antioxidants work to protect lipids from peroxidation by radicals. Antioxidants are effective because they are willing to give up their own electrons to free radicals. When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken (Dekkers *et al.,* 1996). The role of antioxidants has received increased attention during the past decade. However, the use of synthetic antioxidant have potential health hazard (Park *et al*., 2001). Antioxidants can be derived from the daily diet, including fruits, vegetables, nuts and fish (Anderson *et al.,* 2001; Pellegrini *et al.,* 2006; Rajaram *et al.,* 2009). Antioxidants lower the level of low - density lipoprotein cholesterol, thus preventing plaque deposition in the blood vessels. It is beneficial in cancer prevention (Bartlett and Eperjsei, 2003). Many naturally occurring antioxidant compounds in main ingredients used

for the preparation of Traditional Chinese medicine have been identified as free radical or active oxygen scavengers (Duh, 1998; Kumaravel *et al*., 2012; Pan *et al*., 2007). Antioxidants absorbed from food have been shown to be effective scavengers of active oxygen (Havsteen, 2002).

Oxidative stress is an imbalance between generation of reactive oxygen species (ROS) and antioxidant defense capacity of the body. A variety of anti-oxidant compounds have been used to control free radicals produced by the oxidation process which include sources from synthetic chemicals, chelating agents, vitamins, plants and bioactive peptides (Hinneberg, 2006). Recently ,bioactive peptides were widely studied and have been a focus area due to increasing demand for natural and safe source of antioxidants (Hagen and Sandnes, 2004; Pena-Ramos and Xiong, 2003; Sakanaka and Tachibana, 2006).

Enzymatic hydrolysis has been widely used to improve the functional properties of proteins, such as solubility, emulsification, gelation, water and fat-holding capacities, and foaming ability, and to tailor the functionality of certain proteins to meet specific needs (Panyam and Kilara, 1996).

Lipid oxidation is of great concern to the food industry and consumers because it leads to the development of undesirable odours and flavours and

potentially toxic reaction products (Lin and Liang, 2002). Moreover, the production of free radicals from oxidation may be associated with the onset of many diseases such as cancer, neuro degenerative and coronary diseases (Halliwell and Gutteridge, 1984; Diaz *et al.,* 1997). Many synthetic antioxidants such as Butylated Hydroxyanisole (BHA), Butylated Hydroxytoluene (BHT), tert-Butylhydroquinone (TBHQ) and Propyal Gallate (PG) are used as food additives to prevent lipid peroxidation in many fields, especially in food (Wanita and Lorenz, 1996). However, the use of these antioxidant chemical compounds is restricted because of potential risks to health (Park *et al*., 2001). Therefore the search for natural antioxidants as alternatives to synthetic ones is of great interest to researches.

The antioxidant activity of proteins and peptides may be result of scavenging of specific radicals formed during peroxidation, scavenging of compounds containing oxygen, or the chelating capacity of metals (Kristinsson and Rasco, 2000).

Fish tissues contain large quantities of polyunsaturated fatty acid (PUFAs) essential for membrane function. However, PUFAs are also highly susceptible to oxidative attack (Hsieh and Kinsella, 1989). To prevent oxidative damage, fish must possess effective antioxidant defenses that, in

part, depend on dietary supply of essential antioxidants, such as vitamin E. High dietary lipid levels, i.e., PUFA levels, increase vitamin E requirement in fish (Watanabe *et al*., 1997; Cowey *et al*., 1981). Many studies have shown that high dietary-PUFA levels increase lipid peroxidation in fish tissues (Stephan *et al*., 1995; Olsen and Henderson, 1997; Olsen *et al*., 1999; Tocher *et al*., 2002a). Vitamin E requirements depend not only on dietary lipids, but also on vitamin C (Olsen *et al*., 1999). Vitamin C regenerates vitamin E from the vitamin E radical (Tappel, 1962).

The human body is constantly being exposed to reactive oxygen species, which are characterized by the presence of molecules that carry unpaired electrons that may damage cellular molecules and structures. These reactive oxygen species can trigger the reactions in the cells and result in significant damage to the entire tissue, which is known as oxidative stress (Banach *et al.,* 2009; Lehucher-Michel *et al.,* 2001; Niki, 2000). Anti – oxidant compounds play an important role in various fields such as medical (to treat cancer, cardiovascular disorders and chronic inflammations), cosmetics (antiaging process) and others (Ganapathy *et al*., 2007).

Body processes, such as metabolism, as well as environmental factors, like excess exposure to the sun, cigarette smoke and air pollution, excess

alcohol and even X-rays can produce free radicals. The formation of free radicals and other reactive oxygen species is unavoidable during the oxidative metabolic process (Shailaja *et al.,* 2012). These free radicals may oxidise nucleic acids, proteins, lipids or DNA which start chain reactions that damage cells and it can initiate degenerative disease such as cancer and other diseases like stroke, diabetes, Alzheimer's disease (Devasagayam *et al*., 2004).

Puffer fishes common in Thoothukudi water are often caught by trawlers. The potential of puffer fishes as a source of biologically active products is largely unexplored. Hence a broad based screening of puffer fishes for bioactive compounds is necessary. The study aims to evaluate the antioxidant activities of skin and muscle extracts of *Lagocephalus inermis* collected from Thoothukudi Coast.

OBJECTIVES

The objectives of the present study are

- To extract the chemical compounds using methanol from skin and muscle of puffer fish *L inermis.*
- To analyze the antioxidant activity of methanol extract of skin of *L inermis.*
- To analyze the antioxidant activity of methanol extract of muscle of *L inermis.*

2. REVIEW OF LITERATURE

The marine biota is the largest source for novel discovery of natural products such as pharmacological metabolites and medicines. There has been an extensive research showing that vast bio active substances were identified and characterized from marine organisms, indeed several of them showed promising results to treat human and animal diseases (Sato, 1996; Grabley and Thiericke, 1999).

When compared with terrestrial, marine biodiversity are larger, older and have a huge impact on global climate (Norse, 1993). Bioactive natural products derived from marine resources were reported over the last 20 years (Faulkner, 2000) and have expanded significantly in recent years. Around 200 species of marine fish, including stingrays, scorpion fish, zebra fish, stone fish, weever fish, toadfish, stargrazers and some species of shark, ratfish, catfish, sugeon fish and blenny which are known or suspected to be venomous (Russell, 1996). The complexity of fish venoms are evident from a number of different components found mainly proteins and peptides (Sivan, 2009) which are responsible for a variety of pharmacological activities.

Marine food derived functional ingredients as potential antioxidants in the food industry (Ngo *et al*., 2011). The reducing power of a sample is an indicator of its antioxidant activity. The reducing power assay is used to

evaluate the ability of an antioxidant to donate an electron or hydrogen (Yildirim *et al*., 2001). DPPH is a stable free radical has been used to evaluate the ability of compound as free radical scavengers or hydrogen donars and to evaluate the antioxidant activity. It is a compound with a characteristic absorption at 517 nm, using a Shimadzu UV spectrophotometer (Wu *et al*., 2003).

Many human diseases are known to be caused by free radicals and the natural antioxidants can act as free radical scavengers. Protein hydrolysates with antioxidant properties, in particular have become a topic of great interest for the pharmaceutical industries (Alasalvar *et al*., 2002; Hagen and Sandness, 2004). There is also a growing interest in antioxidant from natural sources, which may have less potential health hazard compared with synthetic antioxidants. Water soluble proteinous substances may contribute to the scavenging activity of fish. Antioxidative activity of peptides produced from protein hydrolysis has been reported by numerous studies (Srinivas *et al.,* 1992; Chen *et al.,* 1995; Park *et al.,* 2001). Sampath kumar *et al*., (2012) reported that purification and identification of antioxidant peptide from the skin protein hydrolysate of two marine fishes, horse mackerel (*Magalaspis cordyla*) and croaker (*Otolithes ruber)*. Studies showed that fish and fishery products have a high radical – scavenging activity comparable to that of some

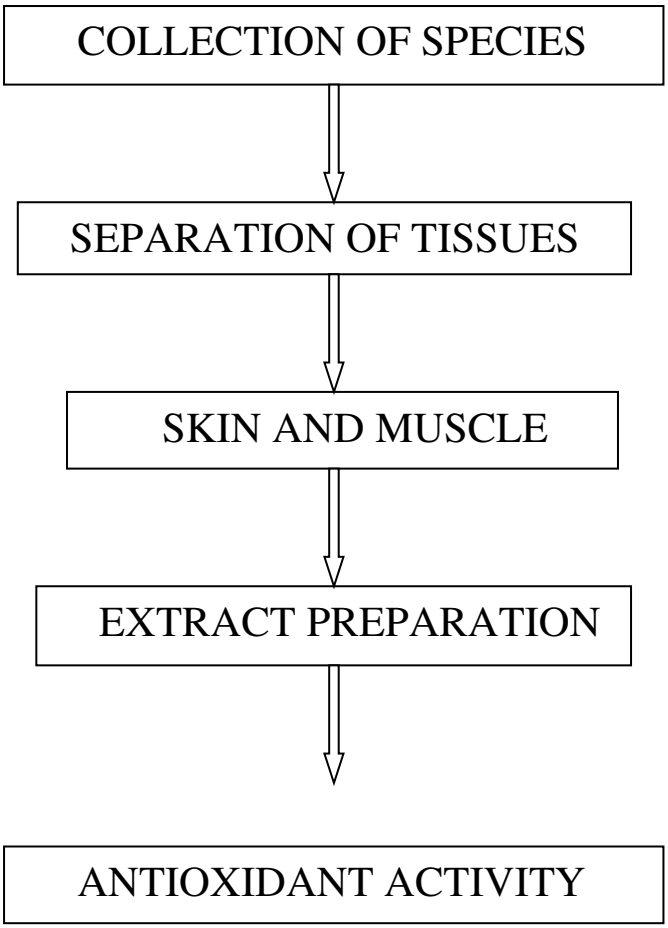
vegetables (Khanum *et al*., 1999 and Bhadra *et al*., 2004).

Oxidative stress is recognized as the major cause of chronic disease progression, such as cancer, hypertension, cardiovascular disease, stroke, arteriosclerosis, diabetes and neuro generative disorders. This leads to the generation of highly reactive molecules which are responsible for the development of such disease. Studies have revealed that the ingestion of antioxidant supplements or food containing antioxidants may reduce oxidative damages (Samadi and Ismail, 2010; Ratnam *et al*., 2006; Duthie *et al.,* 1996). Natural antioxidants such as polyphenols, phytosterols, carotenoids, vitamins C and E, tocopherols, herbal extracts like rosemary, sage and tea extracts have been commercialized as alternatives to synthetic antioxidants. In addition, proteins and protein hydrolysates derived from milk, soy, egg and fish have also been shown to exhibit antioxidant activity (Samaranayaka and Li-Chan , 2011; Elias *et al.,* 2008).

Dietary levels of lipids and some vitamins have been reported to influence antioxidant defense and oxidative status of fish. (Mourente *et al*., 2002) It showed that diets containing oxidized oil significantly affected the activities of liver antioxidant defense enzymes of gilthead sea bream and that dietary vitamin E partially abrogated these effects.

Most of the research on oxidative stress in fish focuses on toxicological aspects, such as the effects of different xenobiotics on antioxidant - enzyme activities and on the intensity of lipid peroxidation (Di Giulio *et al*., 1989; Bainy *et al*., 1996; Zikic *et a*l., 1996; Hai *et al*., 1997). Daniel *et al*., (2020) reported that application of pulsed electric fields for obtaining antioxidant extracts from fish residues. Xiaogang *et al*., (2020) investigated the chemical and cellular antioxidant activities of in vitro digesta of tilapia protein and its hydrolysates. These parameters have been proposed as biomarkers for contaminants.

EXPERIMENTAL DESIGN



3. MATERIALS AND METHODS

3.1 SYSTEMATIC POSITION OF EXPERIMENTAL ANIMAL:

Kingdom	:	Animalia
Phylum	:	Chordata
Class	:	Actinopterygii
Order	:	Tetraodontiformes
Family	:	Tetraodontidae
Genus	:	*Lagocephalus*
Species	:	*inermis*

3.2 DESCRIPTION OF EXPERIMENTAL ANIMAL:

Class: Actinopterygii

The actinopterygii or ray finned fishes constitute a class of the bony fishes. The ray finned fishes are so called because their fins are webs of skin supported by bony or horny spines (“rays”), as opposed to the fleshy, lobed fins that characterize the class Sarcopterygii (lobe - finned fish). These actinopterygian fin rays attach directly to the proximal or basal skeletal elements, the radials which represent the link or connection between these fins and the internal skeleton (e.g., pelvic and pectoral girdles). Numerically,

actinopterygians are the dominant class vertebrates, comprising nearly 99% of the over 30,000 species of fish. They are ubiquitous throughout freshwater and marine environment from the deep sea to the highest mountain streams. Traditionally they have been divided into the sub-class Chondrostei and Neopterygii. Neopterygii in turn have been divided into two infra class. Holestei and Teleostei, Ray-finned fishes constitute a major source of food for millions of people (Helfman *et al*., 1997).

Order: Tetraodontiformes

The Tetraodontiformes are an order of highly derived ray – finned fish, also called the Plectognathi. Sometimes these are classified as a suborder of the order Perciformes. The Tetraodontiformes are represented by 10 extant families and at least 349 species overall; most are marine and dwell in and around tropical coral reefs, but a few species are found in freshwater streams and estuaries (Tyler, 1980).

Family: Tetraodontidae

The species that come under this family are well known for its unique and distinctive adaptations that the puffer fish has to defend itself. They are the second most poisonous creature on the planet. The puffer's remarkable

ability to expand its body extremely quickly when forced with danger, unavailing its long poisonous spikes that cover its body. They can be found in a variety of colour but can sometimes be hard to identify when they are not inflated. The puffer normally has appearance of a large tadpole with bulging eyes and an elongated snout. They are omnivorous and they mainly feed on the algae that grow on the rocks and corals (Gladfelter *et al*., 1980).

Lagocephalus inermis **(Temminck and Schlegel, 1850) (Plate-1)**

L inermis is commonly known as smooth blaasop fish or puffer fish or blow fish or shell fish or maki maki. The vernacular name of this species is Bandi Peytheya or Pippa Peytheya. It belongs to the family Tetraodontoidae due to the presence of four strong teeth that are useful for them to crunch the shell and coral to the food.

This fish was considered as a menace by fishermen during the previous year (2006) as it caused damage to other species landed and the net. It is ray-finned fish depth range 10 - 200 m. Its maximum length varies from 45.0 cm. It is greenish or dark grey above, silvery white below. Dorsal part of body without prickles, belly covered with prickles. Teeth are four in number and form a beak like structure which is quite useful for it to eat its prey. The pectoral fin is bright yellow and the anal fin is white. It is oviparous.

Lagocephalus inermis **(Temminck and Schlegel, 1850) (Plate-1)**

3.3 Collection of Specimen:

Specimens of the puffer fish *L. inermis* were collected from fishing harbour Thoothukudi. They were kept in ice-box and transported to the laboratory. They were maintained in a deep freezer at -20^{o}C until use.

3.4 Preparation of Methanol extract:

The preparation of methanol extract was followed by Chellaram *et al.,* (2014). 10 g of dry powdered tissue was soaked in methanol and kept in an orbital shaker for 72 hours. The extract was filtered through Whatman No.1 filter paper, centrifuged at 15,000 rpm for 30 minutes and the solvent was concentrated by rotary evaporator (VC 100 A Lack Rotavapor at 30^{o}C) with reduced pressure to give a dark brown gummy mass. The resultant residue was stored at 4^{o}C for further analysis.

3.5 Antioxidant Activity:

Measurement of DPPH Radical scavenging activity:

The ability of the samples to annihilate the DPPH radical (1,1-diphenyl-2-picrylhydrazyl) was investigated by the method described by Blois, 1958. Stock solution of compound was prepared to the concentration of 10 mg/ml. Different concentration of the extracts (1 µg, 2.5 µg & 5 µg) of samples were added, at an equal volume to methanolic solution of DPPH (0.1

mM). The reaction mixture is incubated for 30 minutes at room temperature and the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid was used as standard control. The capability of scavenging DPPH radical was calculated using the following equation.

$$\% \text{ of Inhibition} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

IC_{50} value:

For each concentration of sample solution, the IC_{50} values reflecting the concentration that produced 50% inhibition of DPPH radicals were calculated by dividing (Blank Absorbance – sample absorbance) with blank absorbance, then times 100%. The equation y= a+bx was determined from linear regression, where, X was the concentration (µg/mL) and y was the percentage inhibition (%). Antioxidant activity was expressed as the IC_{50}, previously defined. The $IC_{50}$was obtained from the x value after setting y= 50 from the equation y= a+bx, we calculated the value of IC_{50} by dividing (50-a)w.

4. RESULTS

The antioxidant activity of the extracts (skin and muscle) and positive control (ascorbic acid) was assessed based on their ability to scavenge the DPPH free radicals. The free radical scavenging activity of methanol extracts of skin and muscle of *L.inermis* was evaluated. The skin exhibited the strongest antioxidant activity with 18.421 %, 26.842 % and 39.684 % at 1 µg, 2.5 µg and 5 µg respectively. The muscle extract showed antioxidant activity with 8.571 %, 13.292% and 25.342% at1 µg, 2.5 µg and 5 µg respectively *(*Figure 1). The DPPH radical scavenging activity of positive control was found to be 14.534%, 17.391% and 21.118% at 1 µg, 2.5 µg and 5 µg respectively. The IC_{50} values indicated the concentration required to inhibit 50% of the DPPH free radicals. The IC_{50} value of positive control was 22.662 µg/ml. The IC_{50} values of the methanol extract of skin, and muscle of *L.inermis* were 6.928µg/ml and 10.884 µg/ml respectively (Figure 2).

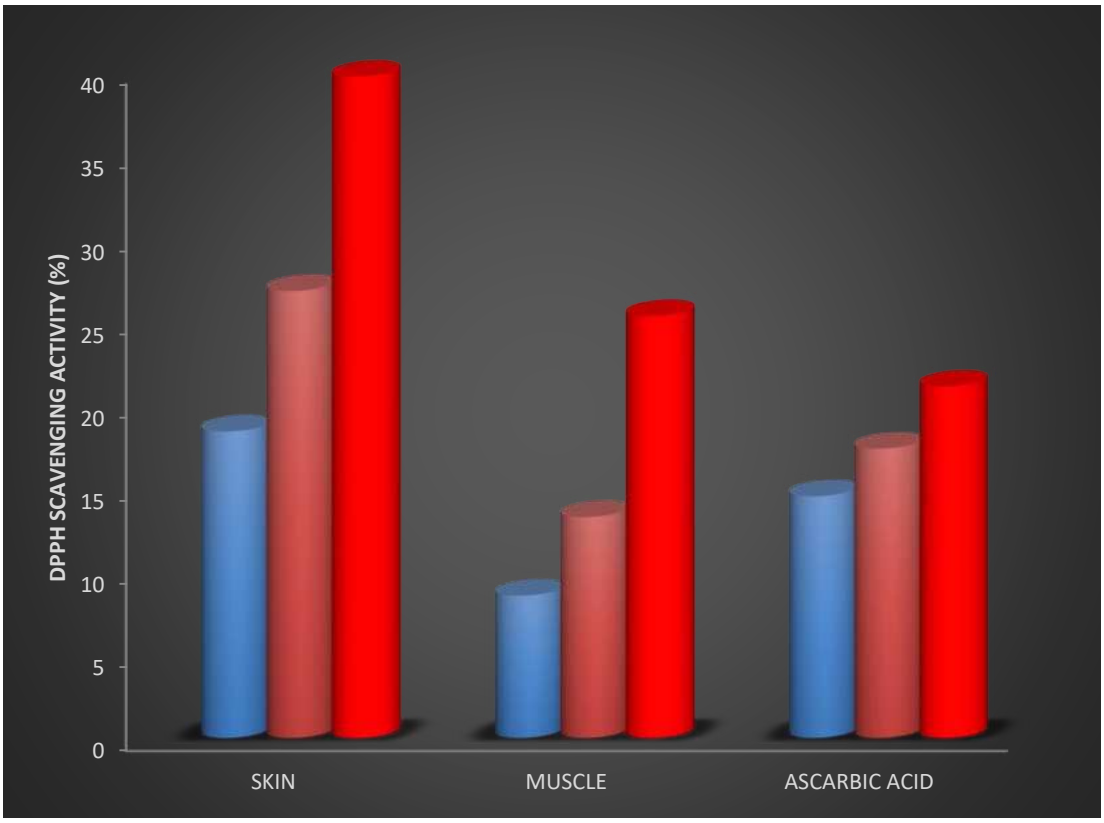


Figure 1 : DPPH Scavenging activity of methanol extracts of various tissues of Lagocephalus inermis

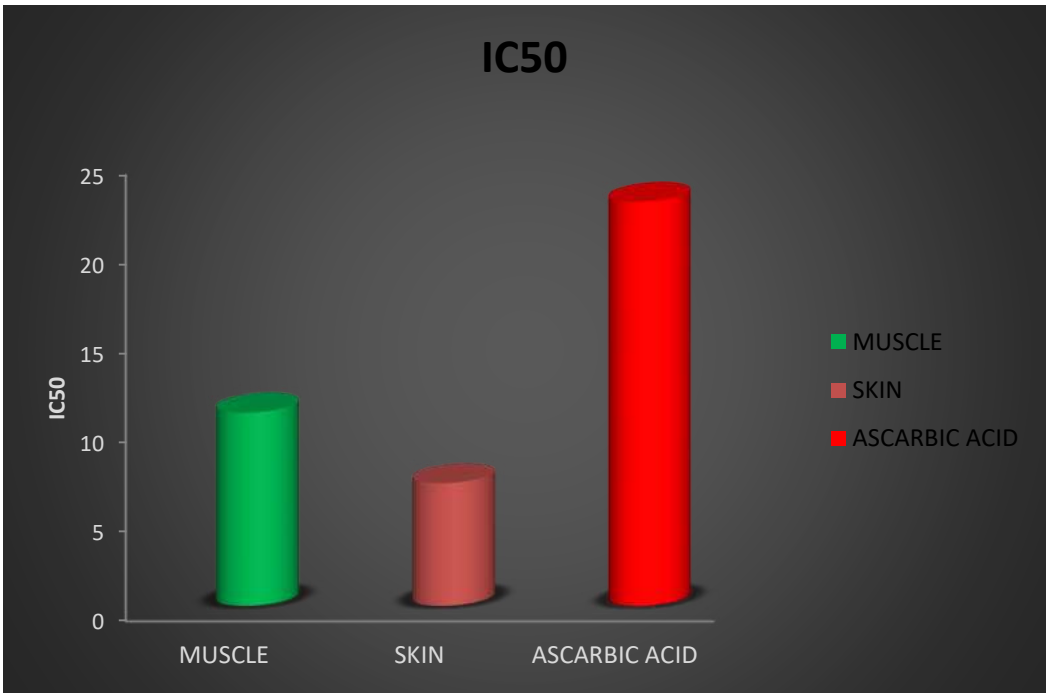


Figure 2: IC_{50} Values of DPPH Scavenging activity of methanol extracts of various tissues of Lagocephalus inermis

5. DISCUSSION

Marine ecosystem has been drawn attention of many scientists due to its biological and chemical diversity (Chew *et al.,* 2007). The bio resources present in the marine ecosystem have potent biomolecules which includes many natural organic compounds. These compounds are reported to have biological activities like antitumor, antiviral, analgesic etc. (Raja Manikandan *et al.,* 2011). Because of so many toxic molecules have been known from marine organisms, it has become evident that ocean is a likely source of pharmaceuticals and interesting biochemicals useful to biotechnology and its researches (Colwell 2002; Rajeev and Xu 2004; Faulkner and Fenical 2005). According to Arakawa *et al*., (2010) many marine puffer fish possess a potent neurotoxin, which is named as tetrodotoxin (TTX) is originally produced by marine bacteria. It can be found in liver, gonads, intestine and skin of puffer fishes can cause death in persons who ingest it (Ellenhorn and Barceloux, 1988).

Oxidation stress is closely related to all aspects of cancer, from carcinogenesis to the tumour bearing state and from treatment to prevention (Noda and Wakasugi, 2001). Epidemiologic studies have suggested that some antioxidants of dietary constituent exhibit antioxidant properties may be

acting as naturally occurring anticancer agents and may explain some of the differences in cancer incidence seen in populations with varying dietary intake (Greenwald *et al.,* 2001). Synthetic antioxidants are suspected of being toxic upon long – term exposure (Kahl and Kappus, 1993).

The methanol extract of puffer fish *L.inermis* was examined for its antioxidant activity using DPPH free radical scavenging activity. As DPPH picks up one electron in the presence of a free radical scavenger, the absorption decreases and the resulting decolouration is stechiometrically related to the number of electrons gained (Silva *et al .,* 2005*).* Thus the production of such antioxidant compounds and their DPPH scavenging effect expected to be highly variable. Such a large variability in the DPPH radical scavenging activity may possibly due to struggle faced by wild animals for accessing food and acclimatizing to the dynamic conditions. Among the genders, the activity levels were higher in females than in males (Sanaye *et al*., 2014). Hung *et al.,* (2008) who found relatively higher scavenging activity in belly portions compared to other body portions. Crude extracts of skin and muscle of *L.inermis* were subjected for the evaluation of antioxidant activity using DPPH radical scavenging activity test. Results of the present study showed that the crude methanol extracts of skin and muscle tissues of *L.inermis* exhibited antioxidant activity.

Kazuki *et al*., (2010) studied the antioxidant activity of fish sauces including puffer sauce measured by the oxygen radical absorbance capacity method. Antioxidant defences in fish: Biotic and Abiotic factors have also been reported by Rosa *et al*., (2005). These findings are also supporting the present study. In the present study the crude extracts of puffer fish scavenged DPPH radical in a concentration dependent manner. Among the skin and muscle tissues tested, skin extract showed strong scavenging activity of DPPH radical and clearly suggested that the antioxidant activity of skin extract was related to its ability to scavenge DPPH radical.

6. SUMMARY

Biological oxidation is a primitive process and, in the face of the inevitable consequences of O_2 toxicity, evolution has provided appropriate defensive strategies. The present study has been carried out to establish the occurrence of antioxidant activities of skin and muscle extracts of *L. inermis.*

Methanol extract of skin exhibited maximum DPPH free radical scavenging activity of 39.684 %.

Methanol extract of muscle showed antioxidant activity with 25.342%.

7. CONCLUSION and SUGGESTIONS

The sea possesses plenty of metabolites which constitutes a potential source of new drugs for fighting antibiotics resistant infections and other deadly diseases. Currently the many number of natural products are increasing, however very few compounds have reached the market. Bioactive compounds from various marine sources have often been found to be promising pharmaceutical agents. It is worthy to note that the product from nature source is good for health and devoid of side effects. In the present study, the skin and muscle of puffer fish *L. inermis* has been examined for antioxidant activity.

Free radicals are known to play a definite role in a wide variety of pathological manifestations of pain, inflammation, cancer, diabetes, hepatic damage etc. Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. Among the extracts of skin and muscle tissues studied, methanol extract of skin showed potent scavenging activity on DPPH free radical compounds with the standard antioxidant ascorbic acid.

In conclusion, it can be stated that the puffer fish *L. inermis* extracts have strong antioxidant activity. On the basis of these results, *L. inermis* appears to be good candidate species for the extraction of safe natural antioxidant agents and also could be used in human therapy.

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INVITRO ANTIOXIDANT ACTIVITY OF *PHALLUSIA NIGRA*AND *DIDEMNUM PSAMMATHODES*

A project submitted to

ST. MARY'S COLLEGE (Autonomous), **THOOTHUKUDI**

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In partial fulfilment for the award of the degree of

BACHELOR OF SCIENCE IN ZOOLOGY

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(Re-accredited with 'A+' Grade by NAAC)

ARRIL- 2021

CERTIFICATE

This is to certify that the project entitled "***Invitro* Antioxidant activity of *Phallusia nigra* and *Didemnum psammathodes***" is submitted to **St.Mary's College (Autonomous), Thoothukudi** in partial fulfilment for the award of the degree of **Bachelor of Science in Zoology** and it is a project work done during the year 2020-2021 by the following students.

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CONTENTS

Oxygen is an essential element of the aerobic organisms for the production of energy. It is the key element in the human body, capable of combining with every other element leading to the formation of essential components necessary for maintaining its regular metabolic activities. Oxygen regulates about 90% of the body function and plays a pivotal role in the respiration (Severyn, *et al*., 2009), gets absorbed by the blood stream in the lungs, transported to the cells and participates in complex processes of metabolic reactions involving enzymatic and non-enzymatic reactions with organic compounds catalyzed by ionizing radiations resulting in the formation of free radicals (Rajamani *et al*., 2010). Free radicals have surplus free-floating electrons rather than having harmonized pairs and therefore unstable, but are highly reactive, moves freely through blood stream and in order to attain stability attacks nearby molecules including proteins, carbohydrates and nucleic acids damaging them by stealing their electrons through a process called oxidation. Types of free radicals include the hydroxyl radical, the superoxide radical, the nitric oxide radical and the lipid peroxyl radical (Chaitanya *et al*., 2010). External sources like air pollutants, industrial chemicals, cigarette smoke, alcohol, oxidized poly unsaturated fats and cooked food (Bagchi and Puri, 1998) also contribute to the formation of free

radicals leading to irreparable damage to the several organs, causing malfunctions.

The role of free radicals has been implicated in the development of at least 50 diseases. A few of them include arthritis, inflammatory diseases, kidney diseases, cataracts, inflammatory bowel disease, colitis, lung dysfunction, pancreatitis, drug reactions, skin lesions and aging. Free radicals are also associated with liver damage due to alcohol consumption and the development of emphysema due to cigarette smoking.

Aging is the prime mechanism oriented with the free radical accumulation in the humans as suggested in the Free Radical Theory of Aging (Harman, 1956). A symptom of aging such as atherosclerosis is considered to be due to oxidation by free radicals. The primary site of free radical damage is the mitochondrial DNA. Damage to the mitochondrial DNA cannot be readily repaired and leads to the shutting down of mitochondria causing cell death and ageing (Speakman *et al*., 2004). Bombardment of free radicals with atoms of metals like mercury, lead, cadmium and even pesticides amplifies the production of free radicals several million times resulting in mitochondrial damage. Severe mitochondrial damage in the cells leads to apoptosis occurs due to a cascade initiated by Bcl-2 proteins on the surface of mitochondria. Destruction properties of free radicals will not limit only to the process of aging but also plethora of diseases via various metabolic activities.

Accumulation of free radicals forms cataracts in the human eye. Scavenging of free radicals takes place in the eye, which gets hampered due to age-related insufficient production of antioxidant scavenging systems leading to the formation of an opaque spot on the eye lens causing blindness (Jose *et al*., 1991).

Myocytes are the source of free radical accumulation in the heart. Free radicals damage proteins and calcium pumps on the sarcoplasmic reticulum, resulting in the accumulation of calcium. High levels of calcium cause erratical contraction of the myocytes causing arrhythmia (Marczin *et al*., 2003). Spread of arrhythmia to other cells disrupts heartbeat, causing severe complications.

Free radicals produced due to external sources especially radiation leads to cancer (Dreher and Junoda, 1996). Most of the radiation energy is taken by the cells, which is absorbed by the water causing one of its oxygen-hydrogen covalent bonds to split and forms free radical. This free radical reacts with another molecule in microseconds of its generation attacks and injures the macro molecules of the cell such as DNA, disrupting its strands and causing mutations in its bases (Curtis *et al*., 2006). However, free radicals that are produced during combustion may last little longer in the lungs binding to other air pollutants leading to lung cancer (Lee *et al*., 1999).

Role of antioxidants in promoting Human Health: Antioxidants are the molecules, capable of limiting the macro molecule oxidation of free radicals

by terminating the chain reactions, which are the main source of free radical formation in the cell. The critical role of antioxidants in ameliorating the free radicals have been elaborately studied, still it is not clear whether the production of free radicals is the consequence or the cause of a disease. Broadly antioxidants are classified into two types namely enzymatic and non-enzymatic. The non-enzymatic antioxidants are again classified into hydrophilic and hydrophobic. Hydrophilic antioxidants can dissolve into blood and cytosol and react with free radicals. Hydrophobic antioxidants protect the cell membrane from lipid peroxidation, a mechanism by which free radicals degrade the membrane lipids.

The role of antioxidants in scavenging the deleterious effects of free radicals is complex, which depend on the interactions of various metabolites and enzyme systems having synergistic and interdependent effects on one another (Chaudière and Ferrari-Iliou, 2006). The performance level of antioxidants also depends on the concentration, reactive potentiality with the specific free radical, interaction and function with other antioxidant family members (Vertuani*et al*., 2004).

Antioxidants may be defined as any substance that when present at lowconcentrations compared with those of an oxidizable substrate, significantly delays orprevents oxidation of that substrate in a chain reaction. Humans have evolved a highly complicated antioxidantprotection system, which involves a variety of endogenous and exogenous compoundsthat are

able to function interactively and synergistically to neutralize free radicals. These include antioxidant enzymes that catalyze free radical quenching reactions, metal bindingproteins that sequester free iron and copper ions that are capable of catalyzing oxidative reactions, diet-derived antioxidants, and other low molecular weight compounds such as α-lipoic acid.

Antioxidants have become a popular research topicbecause they cannot be generated by the human body and hence have to be consumed inthe diet. Many fruit and vegetables have been found to be rich sources of antioxidants.Since a large portion of the human diet is based on fruit and vegetables, it is important tounderstand the biological and biochemical interactions between these dietary antioxidantsand living systems.A major benefit from diets rich in fruit and vegetables may be increasedconsumption of antioxidant vitamins such as ascorbate (vitamin C) and tocopherol (vitamin E), vitamin like compound-glutathione and pigments such as phenolics-flavonoids and anthocyanin and carotenoids. These compounds along with dissolved sugars, acids, salts, amino acids andsome water-soluble pigments are situated in large central vacuoles of parenchyma cells,the main structural unit of edible portion of most fruit and vegetables. They act as major cellular redox buffers that can effectively quenchreactive oxygen species (ROS) by donating one or more electrons to ROS. Natural phytochemicals act synergistically to increase their antioxidant capacity such that thetotal antioxidant effect is greater than the sum of the individual antioxidant

activities, andthe isolation of one compound will not exactly reflect the overall reaction. Plants have a similar nonenzymatic ROS scavenging system including the ascorbate-glutathione cycle in chloroplasts, glutathione peroxidise cycle in peroxisomes, as well as tocopherol, flavonoids, alkaloids, and carotenoids.

Free radicals: Chemically, a free radical is any atom such as oxygen or nitrogen with at least oneunpaired electron present and is able to exist independently. Free radicals can easily be formed in three ways:

- by the homolytic cleavage of a covalent bond, generally incurring by high energy input;
- by the loss of asingle electron from a normal molecule;
- by addition of a single electron to a normal molecule

These free radicals that are highlyreactive molecules can be extremely damaging to the lipids, proteins and cellular DNA, which may lead to many biological complications, including carcinogenesis, mutagenesis, aging, and atherosclerosis. The oxygen-derived free radical is an important group formed during metabolism. One of these reactions found in biological pathways is the respiratory burstprocess, which result in free radical products termed ROS. Examples of ROS include superoxide, hydroxyl radicals, and non-oxygen free radical hypochlorites. Investigations have suggested that ROS are involved in mediating of certain typesof inflammatory tissue injury and the most likely sources of these oxidizing agentsare produced via phagocytic leukocytes.

Activation of phagocytes via interaction of certain pro-inflammatory mediators or bacterial components with specific membrane receptors of leucocytes triggers the assembly of the multicomponent flavoprotein NADPH oxidase which catalyzes the production of superoxide anion radicals. Superoxide will rapidly and spontaneously/enzymatically dismutase toproduce hydrogen peroxide and other free radicals. Besides being produced from superoxide, hydrogen peroxide can also begenerated by other oxidase enzymes, such as glycollate and monoamine oxidase, or by the peroxisomal pathway that is for β-oxidation of fatty acids. The production of hydrogen peroxide inhuman plasma was found to have involvement of an enzyme activity named xanthine oxidase. The level of xanthine oxidase has beenfound to increase as a result of tissue injury.

Sources of free radicals: Free radicals emanate from the environment, from other free radicals in chain reactionsand from many normal biological processes *in-vivo*.Free radical reactions are initiated continuously in cells and tissues in the body fromboth enzymatic and non-enzymatic reactions. Enzymatic reactions serving as sources offree radical reactions include those involved in phagocytosis, prostaglandin biosynthesisand in the Cytochrome P450 system. Free radicals also arise in the non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiation.

Sources of free radicals from the environment include tobacco smoke, ozone derivedfrom air pollution, automobile exhaust emissions, excessive radiation, pesticides, deepfriedfoods, hydrogenated oils and toxic metals which we inhale or digest. The destructive free radical nitrogen dioxide forexample, which is the result of a reaction between nitric oxide and oxygen, isformed in cigarette smoke and vehicle exhaust and has been implicated in respiratoryillnesses and irreversible lung damage.

Cancer is the uncontrolled virtually autonomous growth of abnormal cells that can arisein any organ or tissues of the body. A transformed neoplastic or cancerous cell is simplya once-normal cell which continues to grow and multiply without limitation. This maybe due to a multiplicity of endogenous and exogenous factors, including oxidativestress, which initiate a change in the cell's DNA, resulting in a tumour. Onlytransformed cells that escape detection by the immune system have the opportunity to become tumours. The immune system usually isolates and destroys the abnormal cellsbefore they proliferate enough to be noticeable as a tumour. Free radicals can compromise immune cell function reducing immune responses which can allow theabnormal cells to continue growing. The question ofwhy the risk of cancer increases with age is an interesting one. Harman, (1993) suggests that this increase is probably due in part to the increased level of endogenousfree radical reactions with advancing age and inadequate antioxidant defences resulting in an increased rate of mutations in proto-oncogenes (involved in

normal cell growth and development) and tumour-suppressing genes coupled with the progressive diminishing capacity of the immune system to eliminatetransformed cells.

The role of ROS in carcinogenesis

- Carcinogenesis is a complex process with the *in vivo* generation of ROS leading tooxidative DNA damage being a significant contributory factor. critical factor in mutagenesis is cell division. Cancer is rare in non structural alterations in DNA such as gene sequence amplification;
- translocations and base pair mutations the oxidised form of guanine has altered base-pairing properties;
- activation or inhibition of signal transduction pathways - over expression of agrowth factor receptor is commonly involved in the majority of squamous cell carcinomas of the lung;
- abnormal cell-to-cell communication that allows unrestricted cell proliferation;
- interference with genes that modulate cell growth preventing programmed cell death by apoptosis or necrosis;
- damage to proteins such as DNA repair enzymes compromising repair of a mutation once it has occurred.

The immune system keeps an ever-present vigil to protect us from invading organismsand remove damaged, aged or altered cells which have the potential to cause cancer.White blood cell membranes, like all cell

membranes, are composed of lipids, whichare highly susceptible to free radical attack. Numerous links have now beenestablished between free radical reactions and altered immune cell function (Niki *et al,*, 1991).

ROS can decrease the membrane fluidity of white blood cells, significantly reducingtheir function (Baker and Meydani, 1994). Loss of membrane fluidity has been directlyrelated to the decreased ability of lymphocytes to respond to challenges of the immune system (Bendich 1994; 1999) Free radicals can also damage the DNA of immune cellsresulting in mutations and reduced cell function (Fabiani *et al*, 2001). Ironically, freeradical damage forms the basis of some chemotherapy drugs and radiation used incancer treatment (Cottier *et al*, 1995). Well-documented side effects like hair loss,reduced immunity and gastro-intestinal disturbances result from the barrage of freeradicals that indiscriminately destroy healthy cells as well as malignant ones (Buckman, 1996).

Antioxidants and cancer: Epidemiological data provides strong evidence of a cancer prophylactic effect of highintakes of vegetables, fruits and whole grains containing high levels of antioxidantmicronutrients and phytochemicals (Diplock et al, 1998; Eastwood, 1999; Kaur and Kapoor, 2001; Meydani, 2001). Some naturally-occurring phytochemicals such as phenolic/polyphenolic compounds, e. g. epigallocatechingallate from green tea (Bushman, 1998; Nie *et al*., 2002), curcumin from turmeric (Nagabhushan, 1992; Sriganth and Premalatha 1999), genestein from soy and

red clover (Lian, 1999; Ren, 2001) and silymarin frommilk thistle (Jiang *et al*., 2000), may reduce cancer risk according to initial trials (Lamson and Brignall, 1999).

Ascidians are dominant organisms in many marine communities, having a wide geographic distribution. This ecological success is because of their ability to synthesize secondary metabolites, which possess an important defensive role against predation. They have increasingly become the target of natural products research. A natural product is a chemical compound or substance produced by a living organism - found in nature that usually has a pharmacological or biological activity for use in pharmaceutical drug discovery and drug designing. Research typically focuses on sessile organisms or slow moving animals because of their inherent need for chemical defences.

Many marine sedentary organisms produce components with unique structural pattern, for their chemical defence which do not occur in terrestrial plants. Due to physical and chemical conditions of the marine environment, almost every class of marine organism exhibits variety of molecules with unique structural features, which are not found in terrestrial natural products. Organisms with no apparent physical defence, like sessile organisms, are believed to have evolved chemical defences to protect themselves.

Marine organisms have been reported to be a rich source of biologically active compounds, especially ascidians which are most prominent sources of new compounds. The majority of metabolites reported

from ascidians are derived from amino acids and it is an important source in drug discovery. They are considered as a nuisance as they grow on all underwater marine structures and are usually thrown away. Such discards may have a wealth of natural products. Marine organisms, especially those that are a nuisance to the environment like biofoulers can be screened for antioxidant activity.

Many marine sedentary organisms produce components with unique structural pattern, for their chemical defence which do not occur in terrestrial plants. Sponges, bryozoans and tunicates are important source of new active principles for drug development.

The marine environment is an excellent source of novel chemicals, not found in terrestrial sources. According to Davidson, 1993; Faulkner, 2002 and Blunt *et al*., 2006 marine organisms such as ascidians, sponges and soft corals containing symbiotic microorganisms are a rich source of bioactive compounds. Dhorajiya *et al*., 2012 expressed that some of the compounds derived from marine organisms have antioxidant properties and anti-cancer activities, but they are largely unexplored.

Ismail *et al*., 2008 and Dellai *et al*., 2010 noted that since the few last decades, marine environment have been recognized to be a rich source of bioactive metabolites with varied biological and pharmacological activities. Chakraborty and Ghosh, 2010 suggested that bioactive peptides with novel

structures have also been shown in ascidians. Sac-like sea squirts inhabiting the sea floor produce complex anti-tumor compound which is hundreds to studies on antioxidant property of ascidians especially in *Phallusia nigra* and *Didemnum psammathodes* are lacking. As ascidians are available along the Tuticorin coast an attempt has been made to assess their *in vitro* antioxidant activity.

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Objectives

- To prepare the ethanolic extract of *Phallusia nigra* and *Didemnum psammathodes*
- To test the *in-vitro* antioxidant activity of ethanolic extract of *Phallusia nigra*
- To test the *in-vitro* antioxidant activity of ethanolic extract of *Didemnum psammathodes*
- To compare *in-vitro* antioxidant activity of ethanolic extract of *Phallusia nigra* and *Didemnum psammathodes.*

A review of literature is a comprehensive summary of previous research on a topic. The review of literature surveys scholarly articles, books, and other sources relevant to a particular area of research.

Palakkal and Ganesan 2005 tested antioxidant activity of the extract of *Macaranga peltata* and the correlation between the total phenolic content and antioxidant activity. Kumaran *et al*., 2007 observed a statistical correlation between the antioxidant properties and phenolic contents of methanolic extracts of stem barks, root bark, leaves and fruits from *Morus alba*. Sreelatha and Padma 2009, studied that the extracts of *Moringa oleifera* both mature and tender leaves have potent antioxidant activity against free radicals, prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage. According to Bhaskar and Balakrishnan, 2009 the extracts of the roots of *Carissa carandas* and *Pergularia daemia* possess antioxidant properties and could serve as free radical inhibitors or scavengers.

Sharma and Kumar, 2011 observed *in vitro* antioxidant activity of petroleum ether, ethanolic, and aqueous extracts of fruits of *Rubus ellipticus* using DPPH radical scavenging and reducing power assay. Beta hydroxy acid was used as a standard antioxidant for DPPH radical scavenging activity extracts of *Rubus ellipticus* fruits possess significant free radical scavenging

and reducing power properties at concentration-dependent manner. Mahdi-Pour *et al*., 2012 observed the antioxidant activity of methanolic extracts of various parts of *Lantana camara*. According to Carbonera *et al*., 2014 supplementation of tilapia diet with ethanolic extract of acerola fruit residue resulted, in an improvement of the antioxidant capacity of the fillets. Nahid *et al*., 2017, evaluated the antioxidant, antimicrobial and phytochemical constituents of the methanol extract of *Artemisia indica*. The powerful antioxidant activity is attributed to the greater amount of total phenol and flavonoid compound in the ethanolic leaf extract of *Memecylon umbellatum* as stated by Anbukkarasi *et al.,* 2017.

Trad *et al*., 2018 observed the butanolic extract of *Ephedra alte* had high phenolic contents and exhibited high antioxidant activity both *in-vitro* and *in-vivo*. Quispe *et al*., 2018 investigated antioxidant activity of the ethanolic extracts of peel, flowers, gel and root of *Aloe vera* as the capturing of the DPPH• and ABTS•+ radicals, while the iron-reducing antioxidant power (FRAP) was measured by spectroscopic methods. Keerthana and Visweswaran 2018 observed that the drug Seeraga choornam has promising therapeutic antioxidant activity when compared with the standard drug. This research work can help for medical practitioners to use this poly herbal compound for the treatment of cancer.

Yarnpakdee*et al.,* 2019 studied the extract of *Cladophoraglomerata* could be a source of alternative natural antioxidant in lipid based muscle

food. Sharma *et al.,* 2019 determined *in-vitro* antiradical activity and *in-vivo* metabolism of polyphenol. Tekin and Küçükbay, 2020 the extracts of flowers of *Punica granatum* L. had high content of flavonoids and other phenolics with antioxidant activity. Arshan *et al*., 2020 observed the phytochemical analysis of extracts *Annona squamosa* linn leaves had glycosides, saponins, tannins, flavonoids, phenols, etc. In-vitro antioxidant activities clearly suggest that methanol extract has higher antioxidant activity than the other extract due to a higher presence of phenolic and flavonodal constituents in the methanol extract.

Hamidinia *et al.,* 2008 stated that ethanolic extract of *Eudistoma viride* showed a promising antioxidant potential against free radical induced oxidative damage. Zhang *et al*., 2010 observed antioxidant activity of five polysaccharides extracted from five algae including one brown alga *Laminaria japonica*, one red alga *Porphyra haitanensis* and three green algae *Ulva pertusa*, *Enteromorpha linza* and *Bryopsis plumose.* Revathi *et al*., 2015 observed the maximum antioxidant activity in the methanol extract of *Hypnea valentiae* and antioxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. Priya *et al*., 2016 stated that the ethanolic extract of colonial ascidian, *Eudistoma viride* by DPPH method reveals a promising antioxidant potential against free radical induced oxidative damage. Elya *et al.,* 2018 according to them the methanol extract of the ascidian *Didemnum* sp. exhibited antioxidant

activity. Praba*et al.,* 2020 stated that the antioxidant activity of two colonial ascidians such as *Eudistoma amplum* and *Polyclinum nudum*, were tested by DPPH scavenging test with concentrations ranging from 5 to 150 µg/ml based on the reduction of the maximum absorption of the DPPH radicals in the presence of an antioxidant compound.

Ekanayake *et al*., 2004 stated that the flesh and skin of *Eptatretus burgeri* (hag fish) showed higher DPPH radical scavenging activities when compared with commercial antioxidants.

ANIMAL MATERIAL

Samples of simple ascidian *Phallusia nigra* Savigny, 1816 were collected from the under surface of the barges of Tuticorin harbor. The specimens of colonial ascidian *Didemnum psammathodes* were collected from the intertidal rocky shore area of Thoothukudi north break water Tamilnadu. The samples were washed with sea water to remove sand, mud and overgrowing organisms at the site collection, and then transported to laboratory. Identification up to the species level was carried out based on the key to identification of Indian ascidians by Meenakshi, 1997.

SYSTEMATIC POSITION

Phallusia nigra belongs to

Phylum : Chordata

Subphylum : Urochordata

Class : Ascidiacea

Order : Enterogona

Suborder : Phlebobranchia

Family : Ascidiidae

Genus : *Phallusia*

Species : *nigra*

Didemnum psammathodes belongs to

Phylum : Chordata

Subphylum : Urochordata

Subclass : Tunicata

Class : Ascidiaceae

Order : Enterogona

Suborder : Aplousobranchia

Family : Didemnidae

Genus : *Didemnum*

Species : *psammathodes*

EXTERNAL APPEARANCE

Plate – 1 Individuals are oval or elongated, laterally compressed with the free edges thick and rounded. The size varies from 1.5 cm to 9.5 cm. Attachment is by the posterior end or by one third of the posterior left side. In a few specimens the posterior basal part had a long flat creeping process for attachment. The anterior end narrows to a terminal branchial siphon. Atrial siphon is one third from the anterior end on the dorsal surface directed anteriorly. There are 8-10 branchial and 6-8 atrial lobes with ocelli in between them. The lobes are rounded without any tentacular fringe. The whole anterior part of the body is curved dorsally which is characteristic of the

species so that the two apertures are quite close together. Test is firm, smooth, shiny and jet black in colour.

Plate -2 shows the colony of *Didemnum psammathodes*. It is thin and soft.In the test spicules are few, but abundant ovoid faecal pellets are present. Live and preserved colonies are grey in colour.

PREPARATION OF POWDER

The specimens were dried under shade. The dried animals were homogenized to get a coarse powder. The dried powder of the tunicate *Phallusia nigra*and *Didemnum psammathodes* was used.

PREPARATION OF EXTRACT

Soxhlet extraction is a method used for the extraction of valuable bioactive compounds from various natural sources.

It is a simple and convenient method for infinitely repeated cycle of extraction with a fresh solvent until complete exhaustion of the solute in the raw material. During extraction with soxhlet, the process of distillation is implicated. It consists of heating a solution up to boiling and then condensed send back to the original flask. 50 g of the powder was introduced in a thimble. This thimble is then deposited in a distillation flask, filled with ethanol solvent. After reaching to a submersion level, a siphon absorbs the solvent in the thimble-holder and then release it back into the distillation flask. This solution contains the extracted solutes. This process is done continuously until the extraction is completed. (Azmir, 2013). The separation

of the extract from the solvent is made by natural evaporation method. It is taken in a conical flask and the mouth of the flask was covered by poratedaluminium foil. After the complete evaporation of ethanol, the extract was used for carrying out the experiment. The crude extracts were kept at -20^0 C until further processing.

***DPPH (2,2-diphenyl- 1-picrylhydrazyl) radical scavenging*DPPH Assay**

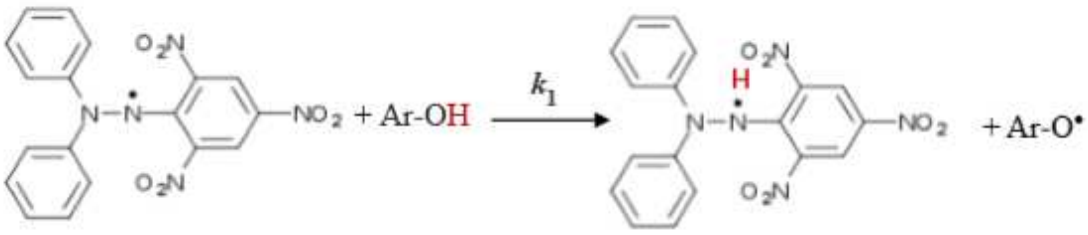
The study of free radical in the antioxidant component is assayed by DPPH radical scavenging activity (Blois, 1958). This method is based on the reduction of DPPH in methanol solution in presence of a hydrogen donating antioxidant resulting in the formation of the non-radical form DPPH-H. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Koleva, 2002). DPPH in methanol (0.1 mM) was prepared. To 3 ml of ethanolic extract of *Phallusia nigra* and *Didemnum psammathodes* at different concentrations (20, 40, 60, 80, 100 μg/ml), 1 ml of DPPH in methanol was added. After vigorous shaking the mixture was kept undisturbed at room temperature for 30 min. The absorbance was measured at 517 nm in a UVVis spectrophotometer. Ascorbic acid was used as the standard. A reagent blank was also run simultaneously.

While DPPH can accept an electron or hydrogen radical to become a stable, diamagnetic molecule, it can be oxidized only with difficulty and then irreversibly. DPPH shows a strong absorption band at 517 nm due to its odd

electron and solution appears a deep violet colour, the absorption vanishes as the electron pairs off. The resulting decolorization is stoichiometric with respect to the number of electrons taken up. The alcoholic solutions of 0.5 mMare densely colored and at this concentration, the Lambert-Beer law is obeyed over the useful range of absorption (Blois 1958).

DPPH assay is considered a valid accurate, easy and economic method to evaluate radical scavenging activity of antioxidants, since the radical compound is stable and need not be generated.

The DPPH method is the most frequently used assay for the evaluation of the free radical-scavenging capacity of plant extracts. The reaction mechanism involves the H transfer from a phenolic compound to the DPPH radical. Interaction of the DPPH radical (purple-coloured) with a phenolic compound, which is able to neutralize its free radical character, leads to the formation of yellow colorless hydrazine and the resulting effect can be quantified spectrophotometrically at 515 nm.



The H-transfer from a phenolic compound (AR-OH) to DPPH

The capabilityof scavenging DPPH radical was calculated using the following equation.

$$\text{Percentage of Inhibition} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \text{x } 100$$

CALCULATION OF IC_{50}

For each concentration of sample solution, the IC_{50} values reflecting the concentration that produced 50% inhibition of DPPH radicals were calculated by dividing (Blank Absorbance – sample absorbance) with blank absorbance, then times 100%. The equation y=a+bx was determined from linear regression, where, x was the concentration (µg/mL) and y was the percentage inhibition (%). Antioxidant activity was expressed as the IC_{50}, previously defined. The IC_{50} was obtained from the x value after setting y=50. From the equation y=a+bx, the value of $IC_{50}$was calculatedby dividing (50-a) with b.

Plate - 1. External appearance of *Phallusia nigra* Savigny, 1816

Plate - 2: *Didemnum psammathodes*

Screening of the *in-vitro*antioxidant activity of the ethanolic extracts of ascidians *Phallusia nigra* and *Didemnum psammathodes* was assessed by DPPH radical scavenging activity.

The results of DPPH radical scavenging activity of different concentrations of ethanolic extracts of *Phallusia nigra* and *Didemnum psammathodes* and standard ascorbic acid is given in Table 1 & 2. The radical scavenging effect was found to increase with increasing concentrations.

The antioxidant activity of ethanolic extract of *Phallusia nigra* and *Didemnum psammathodes* were assessed based on their ability to scavenge the DPPH free radicals. Ethanolic extract of *Phallusia nigra* exhibited significant scavenging activity with 50.65%, 57.37%, 63.44%, 69.9% and 71.63% at 20, 40, 60, 80 and 100 µg/ml concentration respectively. Percentage of scavenging activity of *Didemnum psammathodes* was 22.62%, 29.34%, 29.34%, 50% and 66.72% at 20, 40, 60, 80 and 100 µg/ml concentration respectively.

The IC_{50} values indicated the concentrations required to inhibit 50 % of the DPPH free radicals. The IC_{50} values of *Phallusia nigra* and *Didemnum psammathodes*were 13.71 and 34.16 µg/ml respectively.

Table:1 Effect of ethanolic extract of *Phallusia nigra* on in-vitro anti oxidant activity

Concentration (µg/ml)	Absorbance	Percentage of Scavenging Activity	IC_{50}
20	0.301	50.65	13.71
40	0.26	57.37	
60	0.223	63.44	
80	0.186	69.5	
100	0.173	71.63	
Ascorbic acid	0.044	92.78	
Blank	0.61		

Table:2 Effect of ethanolic extract of *Didemnum p*sammathodes on in-vitro anti oxidant activity

Concentration (µg/ml)	Absorbance	Percentage of Scavenging Activity	IC_{50}
20	0.472	22.62	34.16
40	0.431	29.34	
60	0.38	37.7	
80	0.305	50	
100	0.203	66.72	
Ascorbic acid	0.044	92.78	
Blank	0.61		

Figure 1: Effect of ethanolic extract of *Phallusia nigra* on percentage of Scavenging Activity

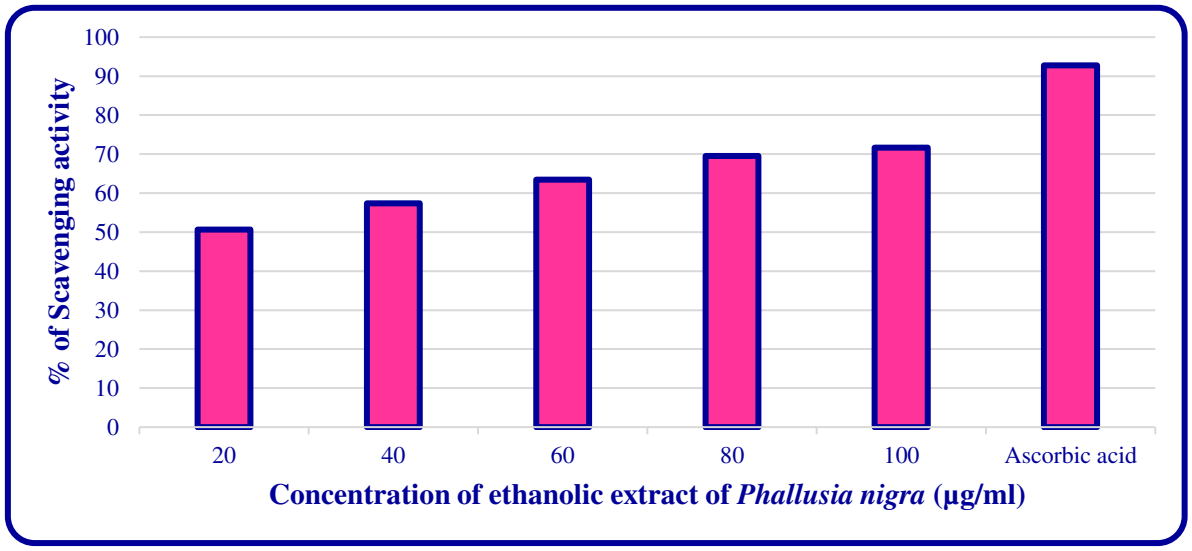


Figure 2: Effect of ethanolic extract of *Didemnum psammathodes* on percentage of Scavenging Activity

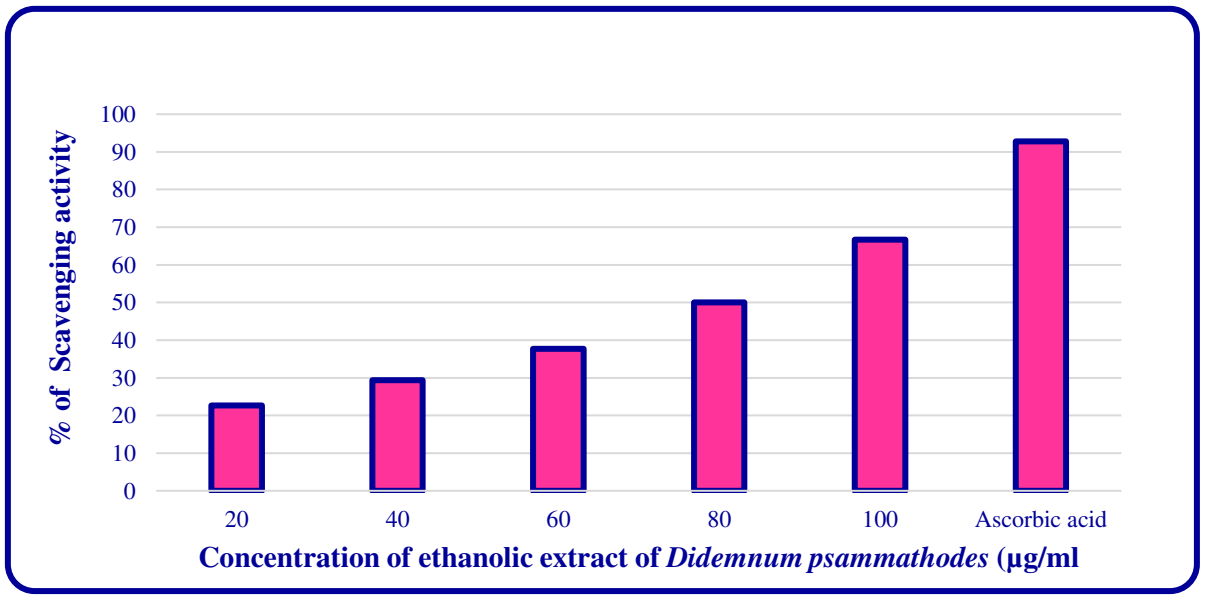


Figure 3: Effect of ethanolic extract of *Phallusia nigra* on percentage of Scavenging Activity

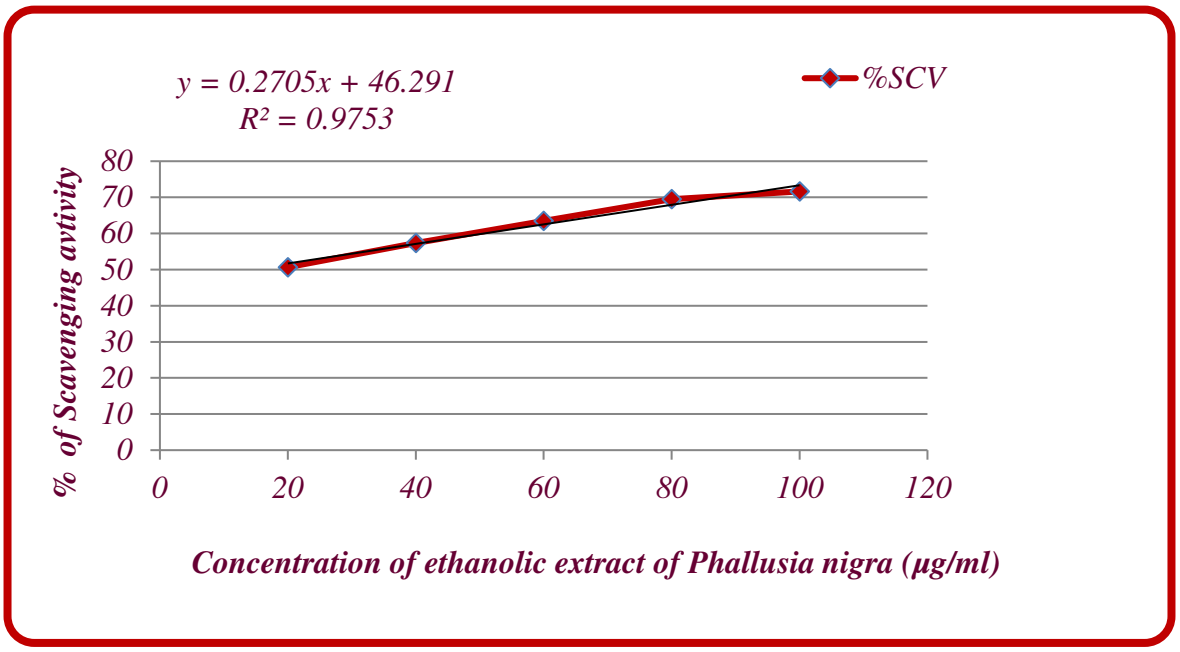
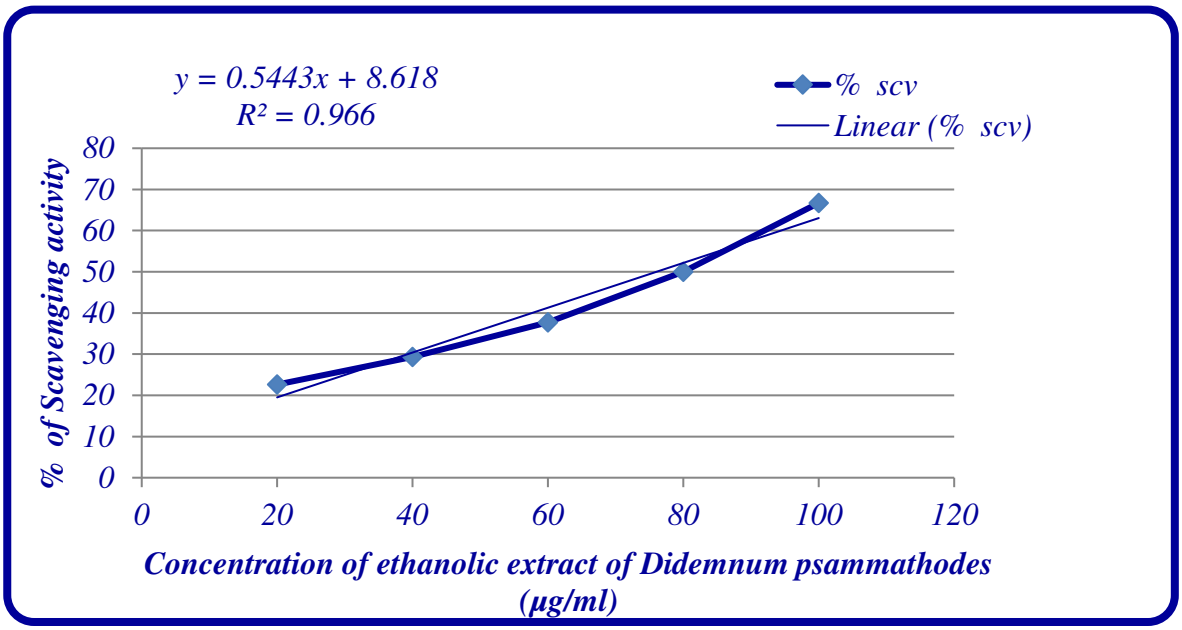


Figure 4: Effect of ethanolic extract of *Didemnum psammathodes* on percentage of Scavenging Activity



The secondary metabolites of animal origins are gaining immense consideration recently due to their wide range of biological activities. Thus intake of the functional foods for their benefits has markedly increased with the awareness of their safety and nil side-effects. In this study an *in-vitro* antioxidant assay has been performed to evaluate the free radical scavenging properties of extracts of *Phallusia nigra* and *Didemnum psammathodes*. The *in-vitro* antioxidant activity was dependent on the concentration of the extract.

According to Priya *et al*., 2016 the strongest antioxidant activity of ethanolic extract of *Eudistoma viride* may be due to the presence of flavonoids and phenolic compounds.High radical scavenging was observed in *Eudistoma viride*. The findings of the present study support the view of ascidians are promising sources of potential antioxidants and may be efficient in preventing agents in some other diseases. Roselin *et al*., 2018 suggested that the effectors include non enzymatic antioxidants like vitamin C, E, glutathione, thiol compounds, carotenoids and flavonoids.The present study clearly indicates that the different extracts of *Didemnum psammathodes* show strong antioxidant activity.

According to Esmaeili *et al*., 2015 samples with high level of phenolic content also contain flavonoids in great amount. The rich-flavonoid plants

could be a good antioxidant source that would help increase the overall antioxidant capacity of an organism and guard it against lipid peroxidation. Arshan *et al*., 2020 reported that the increased radical scavenging activity of the leaf extract could be due to the high content of phenolic compounds which were the main antioxidant components and their total contents were directly proportional to their antioxidant activity.

A preliminary chemical screening of the ethanolic extract of *Phallusia nigra* showed the presence of alkaloids, terpenoids, flavonoids, glycosides, phenolic compounds and tannins as stated by Priya *et al.,*2018. The reports of the current study are coincides with considerable DPPH scavenging activity which was better than the reports of earlier studies (Thakur and Singh 1965). Therefore, in this study, the presence of theflavonoids and phenols in all the tested extracts of *Phallusia nigra* might have contributed to the antioxidant activity.

Kumaran and Bragadeeswaran 2017 reported that the ethyl acetate extracts of ascidian *E. viride* and *D. psammathodes* showed antioxidant activity. Fractionation of the *Didemnum sp.* extract showed thatthe ethyl acetate fraction had the highest antioxidant activity. Further, fractionation of the ethyl acetate fraction by accelerated column chromatographyshowed that fraction VI had the highest antioxidant activity. The most active fraction contained alkaloids, steroids/triterpenoids, saponins, and glycosides. Antioxidants are the substances which inhibit oxidation, which has the ability

to remove the potentially damaging oxidizing agents in a living organism. Many phytochemicals present in the plants are able to reduce or prevent the oxidative damage to the human cells which can cause even cancer in humans. DPPH radical was used for evaluation of free radical scavenging and the total phenolic content was determined by the folin-Ciocalteaureagen. Polyphenols are responsible for the antioxidant activity, the obtained amount of total polyphenols in the extract indicated the extract to possess a high antioxidant activity as stated by Ganesan and Palakkal, 2019.

According to Sofna and Banjarnahor 2014 cardiovascular protective effect of flavonoids resembles in their antioxidant activity. Flavonoids as a strong antioxidant have also been used in certain study related to diabetes mellitus. The ability of flavonoids to interfere with cancer treatment has been tested in a series of flavonoids compound.The studies evaluating the antioxidant properties prompted by flavonoids have currently expanded to a wider range of therapeutic applications. Underlying the antioxidant properties of flavonoids have been concisely described.

According to literature survey, the antioxidant activity may be due to the presence of flavonoids. The simple ascidian *Phallusia nigra* and colonial ascidian *Didemnum psammathodes* contain flavonoids, which could be the reason for this significant antioxidant activity. The activity observed is in a very good correlation with the composition, where the most active extracts are those rich in polyphenol and flavonoids.

Conclusion

From the results, it was concluded that the ethanolic extracts of *Phallusia nigra* and *Didemnum psammathodes* have appreciable antioxidant activity. The radical scavenging effect was found to increase with increasing concentrations.

* Antioxidant activity can be determined using other assays also.
* A further study on isolation, purification, structure determination and subsequent recognition of the novel mechanism of action of the clinically effective agent is suggested.
* Chemotherapeutic and spectroscopic techniques can lead to the development of new drugs.
* As the extract of *Phallusia nigra* and *Didemnum psammathodes* showed antioxidant activity, other species of ascidians can also be tried.

Antioxidants are the molecules, capable of limiting the macro molecule oxidation of free radicals by terminating the chain reactions, which are the main source of free radical formation in the cell.

The role of free radicals has been implicated in the development of at least 50 diseases. A few of them include arthritis, inflammatory diseases, kidney diseases, cataracts, inflammatory bowel disease, colitis, lung dysfunction pancreatitis drug reactions, skin lesions and aging.

The marine environment is an excellent source of novel chemicals, not found in terrestrial sources. In the present study, *in vitro* antioxidant activity of ethanolic extract of *Phallusia nigra*and *Didemnum psammathodes* was assessed. Samples of ascidians were collected and identification up to the species level was carried out based on the key to identification of Indian ascidians by Meenakshi, 1997.

The specimen was dried under shade and was homogenized to get a coarse powder. Soxhlet extraction is a method used for the extraction of valuable bioactive compounds from various natural sources. The study of free radical in the antioxidant component is assayed by DPPH radical scavenging activity (Blois, 1958). Percentage of inhibition and IC_{50}were calculated.

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AN ANALYSIS OF THE NUTRITVE VALUE OF MARINE BIVALVE *DONAX FABA* (GMELIN,1791) FROM THOOTHUKUDI COAST

A project submitted to

ST.MARY'S COLLEGE (Autonomous), THOOTHUKUDI

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

in partial fulfillment for the award of the degree of

Bachelor of Science in Zoology

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CERTIFICATE

This is to certify that the project entitled **An analysis of the nutritive value of marine bivalve *Donax faba* (Gmelin, 1791) from Thoothukudi Coast** is submitted to **St.Mary's College (Autonomous), Thoothukudi** in partial fulfillment for the award of the degree of **Bachelor of Science in Zoology** and it is a record of the work done during the year 2020-2021 by the following students.

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CONTENTS

INTRODUCTION

1. INTRODUCTION

Indian coast is blessed with number of ecosystems such as coral reefs, mangroves, lagoons, estuaries, sandy beaches and rocky shores. Among these, the coral reef and rocky shores are having rich assemblage of molluscan fauna. The molluscs are abundant in rocky shores, as it provides a better substratum and act as a feeding and breeding ground, ensuring a favorable environment for their survival. The molluscan fishery comprises an important component of the Indian sea food Industry. Although many species of the phylum molluscan are indigenous to the continent, the most important classes of commercial interest are the bivalves (oysters, clams, scallops and mussels), gastropods (conchs, whelks, abalones and periwinkles) and cephalopods (Gopalsamy Idayachandiran *et al*., 2014)

The edible species of marine bivalve molluscs are tasteful and it will get more importance next to fish and prawn. Marine molluscs are economically important species and it is easy to cultivate in coastal region. The marine molluscs are having leading components of bivalve fishery in aquaculture coastal area (Jones and Alagarswami, 1973) and it forms an important source of nutrition for coastal folks (Verlecar *et al.,*2006). In general, bivalves are found in abundance in seashores and estuaries and form the food of many coastal people in India. The knowledge on biochemical composition of any edible organisms is extremely important since the nutritive value is reflected in its biochemical contents

(Nagabhushanam and Mane, 1978). The demand for protein rich food is increase, especially in developing countries, stimulating the exploration of unexploited or non-traditional resources.

Molluscs are important for marine ecology and human diet, since it is an important source of nutrients. The variations in the total protein, carbohydrate, lipid, and fatty acid content of the mature and immature green mussel, *Perna viridis* from the Ennore estuary of Madras coast. Consumption of marine molluscs provides an inexpensive source of protein with a high biological value, essential minerals and vitamins. Molluscs are also a good source of minerals such as calcium, potassium, zinc, iron, phosphorus and copper. As the world population is growing, the per capita consumption of seafood is also increasing rapidly. Because of health consciousness, the modern day man is interested in taking seafood more in view of its nutritional superiority than all other sources of food accessible to him.

From India, a total of 371 species of molluscs belonging to 220 families and 591 genera have been documented and of these 1900 are gastropods, 1100 bivalves, 2210 cephalopods, 41 polyplacophorans and 20 scaphopods (Appukuttan, 1996). India has long coast line of 8129 km with rich marine fishery resources consisting of chiefly fishes, crustaceans and molluscs. There is a high demand for animal protein which can be utilized either for human consumption or as fish meal

or manure. Due to lack of awareness, molluscs are not popular food in India. The knowledge on biochemical composition of any edible organisms was extremely important since the nutritive value reflected in its biochemical contents (Nagabhushanam and Mane, 1978).

The molluscs are considered as a low fat and high protein content food. The quality of protein is usually assessing by its amino acid composition. The amino acid composition in turn is helpful in assessing the nutritive value of organism (King *et al*., 1990). Utilization of molluscs as source of food especially for coastal community has increased rapidly. The molluscs are one of the most delicious and protein rich food among the sea foods. Moreover, they serve as cheap food, raw material for cottage industries (Srilatha *et al.,* 2013). The bioactive components of marine mollusks provide a variety of metabolites, and some of which can be used for drug development (Chellaram *et al.,* 2010). The shellfish proteins are rich in essential amino acids, which are required for the growth, reproduction and synthesis of vitamins.

Shellfish also contain significant amounts of "good" fats called omega-3 fatty acid and also provide high quality protein with all the dietary essential amino acids for maintenance and growth of the human body (Fernandez-Eeiriz *et al*., 1996). Factors such as water temperature, nutrient availability and reproductive cycle of molluscs can influence the biochemical composition (Orban, 2002).

The knowledge on biochemical composition of any edible organisms is extremely important since the nutritive value is reflected in its biochemical contents (Nagabhushanam and Mane, 1978). A new species should be recommended for human consumption only after assessing the nutritive value of the species with regards to its nutritional qualities (Ajaya Bhaskar, 2002) The demand for protein rich food is increasing, especially in developing countries, stimulating the exploration of unexploited or non-traditional resources. Molluscs are important for marine ecology and human diet, since it is an important source of nutrients. Consumption of marine bivalves provides an inexpensive source of protein with a high biological value, essential minerals and vitamins (Astorga-Estpana, Rodrugues-rodriguez and Romero 2007). Molluscs are also a good source of minerals such as calcium, potassium, zinc, iron, phosphorus and copper.

Hence, the present study has been made on attempt to estimate the proximate composition of economically important marine bivalve through estimating their major biochemical composition such as total protein, carbohydrate and lipid content, and mineral content in the whole-body tissue.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Molluscs are one of the earliest recorded group of living organism. Their presence on planet earth since the paleozoic era 540 million years ago has been proved beyond doubt. Abundance in size and diversity and their dual roles as predators and prey make molluscs an indispensable component of tropical marine ecosystems.

Navarro and Villaneuva (2000) estimated the lipid and fatty acid composition of stages of cephalopods an approach to their lipid requirements. Navarro *et al.,* (2000) reported that molluscan muscle contains little saturated fat and significant amount of vitamin C. Okuzumi and Fujii (2000) studied the nutritional and functional properties of squid and cuttle fish. Thilagavathi and Christy Ponni (2000) evaluated the nutritional value of marine bivalve *Donax variabilis* from Porayar coastal area, Nagapattniam District. Ajaya Bhaskar (2002) showed the nutritional composition of molluscan sea food. Astorga–Espana *et al.,* (2007) compared the mineral and trace element concentrations in two molluscs form the Strait of Magellan (chile).

Babu *et al.,* (2010) reported *Bursa spinosa*-a mesogastropod fit for human consumption. Babu *et al.,* (2010) analyzed the nutrient content of same commercially important molluscs of Bangladesh. Chelladurai and Moorthy (2010)

should the biochemical composition of some commercially important marine gastropods from Tuticorin coast. The proximate study and nutrient content was analyzed in six commercially important molluscs *Pila globosa, Bellamya bengalensis, Melania tuberculata, Lamellidens marginalis, Anisus convexiusculus* and *Helix* species. These species were assessed for their proximate and mineral compositions designed to establish their nutritive values on the weight basis.

Periyasamy *et al.,*(2011) showed the nutritional value of gastropod *Babylonia spirata* from Thazhanguda, southeast coast of India. Periyasamy (2012) studied the biochemical, antimicrobial and anticoagulant potential of *Acanthochiton amahensis* from Tranquebar, southeast coast of India. Srilatha *et al.,* (2013) investigated the proximate, amino acid, fatty acid and mineral composition of clam, *Meretrix casta* from Cuddalore and Parangipettai coast, southeast of India. Periyasamy *et al.,* (2014) reported the biochemical composition of marine bivalve *Donax incarnatus* from Cuddalore southeast coast of India. Gopalsamy Idayachandiran *et al.,* (2014) studied the nutritional value of marine bivalve *Donax cuneatus* from Cuddalore coastal waters.

There remains no considerable study on bivalve with regard to their nutritive value for human consumption although clams are being consumed in some parts of India and other country, very limited studies have been made on bivalves. In view

of that, the present work was aimed to study the nutritive value of marine bivalve *Donax faba.*

OBJECTIVES

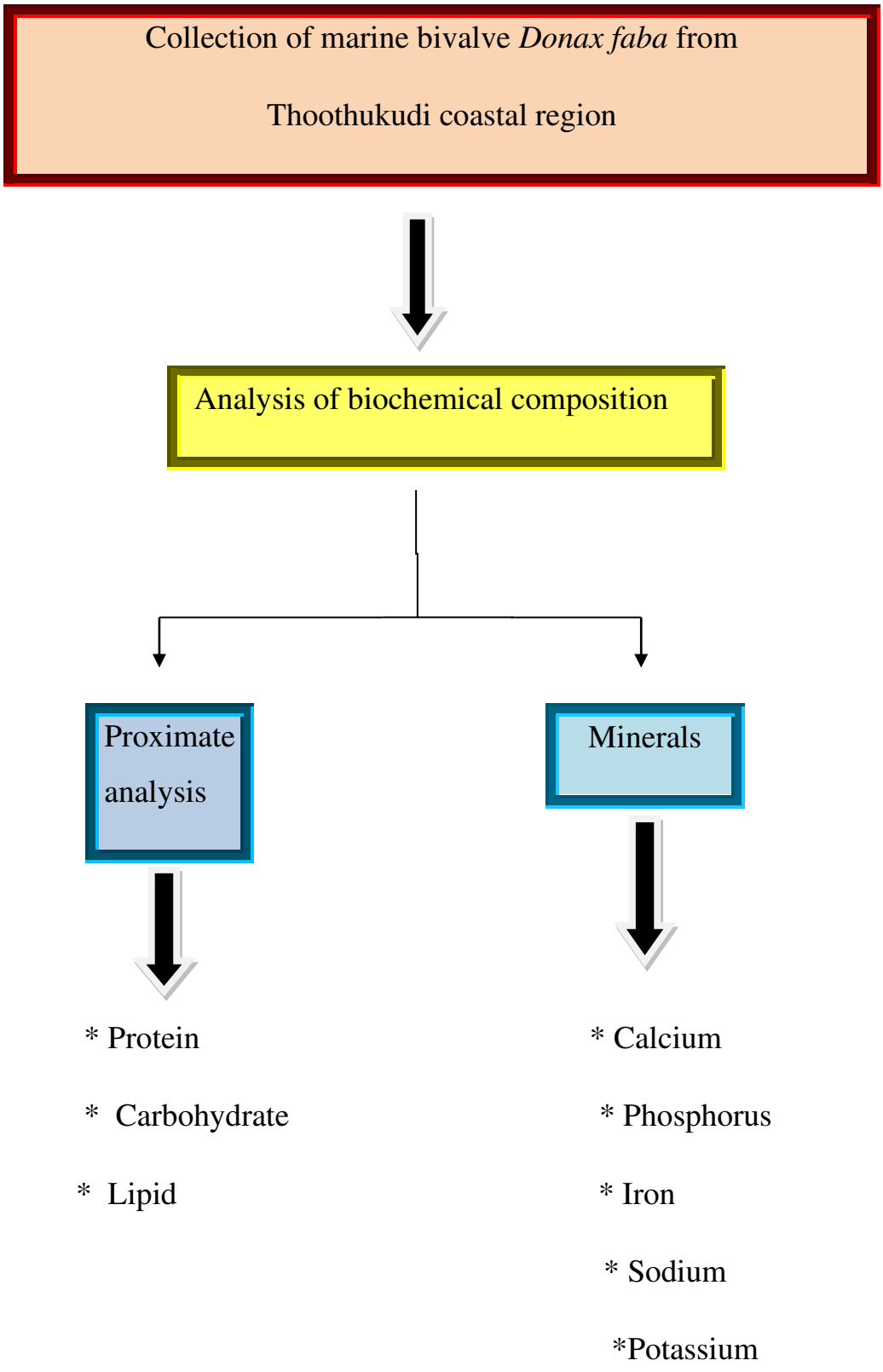
3. OBJECTIVES

The marine molluscs have been given more importance, because they have both ecological and economical importance to mankind. Marine molluscs are commercially valuable species and easy to cultivate in coastal areas. Consumption of marine molluscs provides an inexpensive source of protein with high biological value, essential minerals and vitamins. Though bivalve muscles are being consumed in coastal people and islands, in India. The present study has been investigated with the following objectives.

- To estimate the proximate composition viz., protein, carbohydrate and lipid of *Donax faba.*
- To analyse the mineral contents in the marine bivalve *Donax faba.*

EXPERIMENTAL DESIGN

4.EXPERIMENTAL DESIGN



MATERIALS AND METHODS

5. MATERIALS AND METHODS

5.1 SYSTEMATIC POSITION OF EXPERIMENTAL ANIMAL

***Donax faba* (Gmelin, 1791)**

Phylum	:	Mollusca
Class	:	Bivalvia
Order	:	Cardiida
Super family	:	Tellinoidea
Family	:	Donacidae
Genus	:	*Donax*
Species	:	*Faba*

5.2 STUDY AREA

Specimens of *Donax faba* used in the present study were collected from Gulf of Mannar Coastal region. Gulf of Mannar is situated on the South-east Coast of India. This area is remarkable for its richness and variety of fauna and the inshore sea bottom forms an ideal habitat for the growth of the shell fishes which sustain a good fishery. The Indian part of Gulf of Mannar covers approximately an area of 10,500 km^2 along lat 8°35′ - 9°25′ N and long 78°08′ - 79°30′E (Figure1).

It is a part of the southward extension of Bay of Bengal, it meets in the Indian ocean. This geographical area runs from Pamban island including

Rameshwaram to Cape Comarin along the South east coast of India to a distance of above 170 nautical miles. This coast contains a rich biological diversity of flora and fauna largely due to diversified microhabitats such as mangroves, corals, seaweed beds, sea grasses, sandy, rocky and muddy shore etc. The faunal diversity is also well pronounced with reference to different molluscan groups.

5.2.1 COLLECTION OF EXPERIMENTAL ANIMAL

Specimens of *Donax faba* were collected during low tides from the sea in their natural habitat that is intertidal zone and from reefs by divers, brought to the laboratory and maintained under laboratory conditions for further observations.

The wedge clam, *Donax faba* is a filter feeding organism belonging to the family Donacidae.The length of the shell is 17.35-25.1 mm, height is 11.35-18.21 mm and width is 5.8-9.66 mm. The colour of the shell is freckled white with red rays. Interior of the shell is glossy white, with orange or purple irregular radiating bands. The shape of the shell is triangularly ovate, compressed, inequilateral shell. It has posteriorly placed pointed umbo. Anterior margin is rounded and posterior margin is short and truncated. Ligament is brown colored. In the left valve the cardinal area consists of triangular anterior tooth and a laminate posterior tooth. In the right valve cardinal complex consists of a bifid tooth, laterals very distinct, provided with upper space to accommodate the laterals of the left valve. Adductor

muscle scars are well developed. Anterior adductor scar more or less triangular, posterior adductor scar rounded with elongated anterior ends. *Donax faba* is an important and profitable resource on the southern Indian coast, where it is exploited for food and ornaments. Along south west coast of India, wedge clams *Donax faba* is an important bivalve with a good fishery potential.

5.3 ANALYSIS OF BIOCHEMICAL COMPOSITION

5.3.1 Estimation of protein

The protein content of the tissue was estimated following the method of Lowry *et al*., (1951). A known weight of tissue was homogenized with 5ml of 10% Trichloroacetic acid (TCA) and 8ml of distilled water using a tissue homogenizer and centrifuged at 3000 rpm for 10min. The supernatant was decanted. The residue was dissolved in 5ml of 0.1N sodium hydroxide and kept in a water bath at 60-70°C for 10min. From this, 0.5ml of solution was pipetted out into a clean test tube. To this 4.5ml of carbonate copper solution (50ml of 2% sodium carbonate + 0.5ml of 0.5% copper sulphate + 0.5ml of 2% sodium potassium tartarate) was added. They were thoroughly mixed well by lateral shaking. After this, the test tubes containing the solution were kept undisturbed at room temperature for 15min. To this, 0.5ml of Folin-ciocalteau phenol reagent was added. Test tubes were shaken well for uniform mixing and kept in room temperature for another 30min. The resultant blue colour was read at 640 nm against a reagent blank in a

UV-VIS Spectrophotometer-118. The standard was obtained by using bovine serum albumin and percentage of protein was calculated by using the following formula.

$$\text{Percentage of protein} = \frac{\text{Standard value x OD value}}{\text{Weight of the tissue}} \text{ x } 100$$

5.3.2 Estimation of carbohydrate

Carbohydrate was estimated following the slightly modified method of (Seifter *et al*., 1950) using anthrone reagent. A known weight of the tissue was homogenized with 2ml of 10% Trichloroacetic acid (TCA) and 8ml of distilled water. The homogenate was centrifuged at 3000rpm for 10min. The supernatant was collected and measured. One ml of the supernatant was taken in a clean test tube. To this 4ml of anthrone reagent was added and mixed well. The test tube containing the mixture was kept at room temperature. The developed colour was read at 620nm against a standard reagent blank in a UV-VIS Spectrophotometer-118 model. Standard curve was obtained by using glucose and the percentage of carbohydrate was calculated by using the following formula.

$$\text{Percentage of carbohydrate} = \frac{\text{Standard value x 0D value}}{\text{Weight of the tissue}} \text{ x } 100$$

5.3.3 Estimation of lipid

Lipid was estimated by following the method of Bradgon (1951). A known weight of the tissue homogenized well with 5ml of chloroform solution and the solution was centrifuged at 3000rpm for 15min. The supernatant was evaporated to dryness by keeping it in an oven. 3ml of 2% potassium dichromate was added which was followed by 3ml of distilled water. The developed colour was read at 640nm against a reagent blank in a UV-VIS Spectrophotometer-118 model. The standard curve was obtained by using cholesterol and the percentage of lipid was calculated by using the formula.

$$\text{Percentage of lipid} = \frac{\text{Standard value x 0D value}}{\text{Weight of the tissue}} \text{ x } 100$$

5.3.4 Estimation of minerals

The samples were oven dried at 60ºC for 24 hours and used for the estimation of mineral content.

Sample preparation and derivatization

500mg of dried sample was digested by microwave sample preparation system (Anton paar Multiwave 3000) using an acid mixture containing nitric acid and perchloric acid (3:1V/V). The residues were dissolved in 2N hydrochloric acid and filtered through Whatman No.1 filter paper and the volume was made up to 25ml with de-ionized water in a standard flask. The clear solution was used to

measure the concentration of different minerals. Minerals such as calcium, sodium and potassium were analyzed using digital flame photometer (Model CL 22D) pre-calibrated with respective standards. Phosphorus and iron determinations were performed by optical emission – spectrophotometer (Perkin Elmer Model Optima 2100 DV). The trace minerals were quantified on the basis of peak areas and comparison with a calibration curve obtained with the corresponding standards.

RESULTS

6. RESULTS

The proximate composition (%) such as protein, carbohydrate and lipid contents of *Donax faba* were estimated and the results of proximate composition revealed that protein was found to be dominant (18%) followed by carbohydrate (16%), lipid (12%) in the tissue of marine bivalve *Donax faba* (Figure 2). The analysis of the nutrients from the flesh of marine bivalve *Donax faba* results that bivalves are moderate sources of protein. Other than that molluscs can be regarded as a source of carbohydrate also.

The species *Donax faba* recorded the highest amount of minerals such as calcium, phosphorus, iron, sodium and potassium (mg/100g). The analysis of the minerals in the flesh of *Donax faba* results that bivalves are good sources of minerals. The highest amount of calcium was recorded from the flesh of *Donax faba*(302.42 mg/g) followed by phosphorus (135.46 mg/g), iron (95.74 mg/g), sodium (42.79 mg/g) and potassium (30.86 mg/g) (Figure 3). There is large quantity of calcium in bivalve flesh.

Figure 1. Map showing the study area Gulf of Mannar

Thoothukudi coastal region

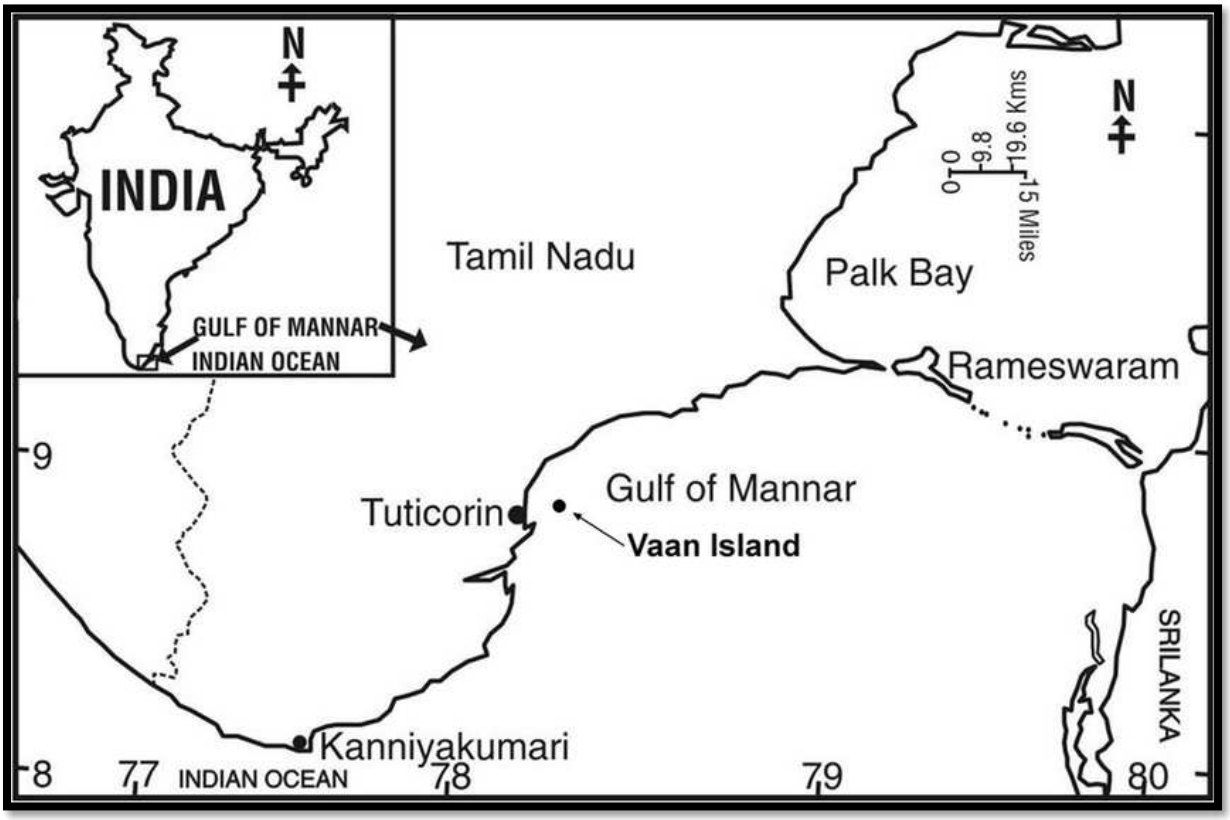


Plate 1 showing the marine bivalve *Donax faba*

Figure 2: Showing the proximate composition of marine bivalve *Donax faba*

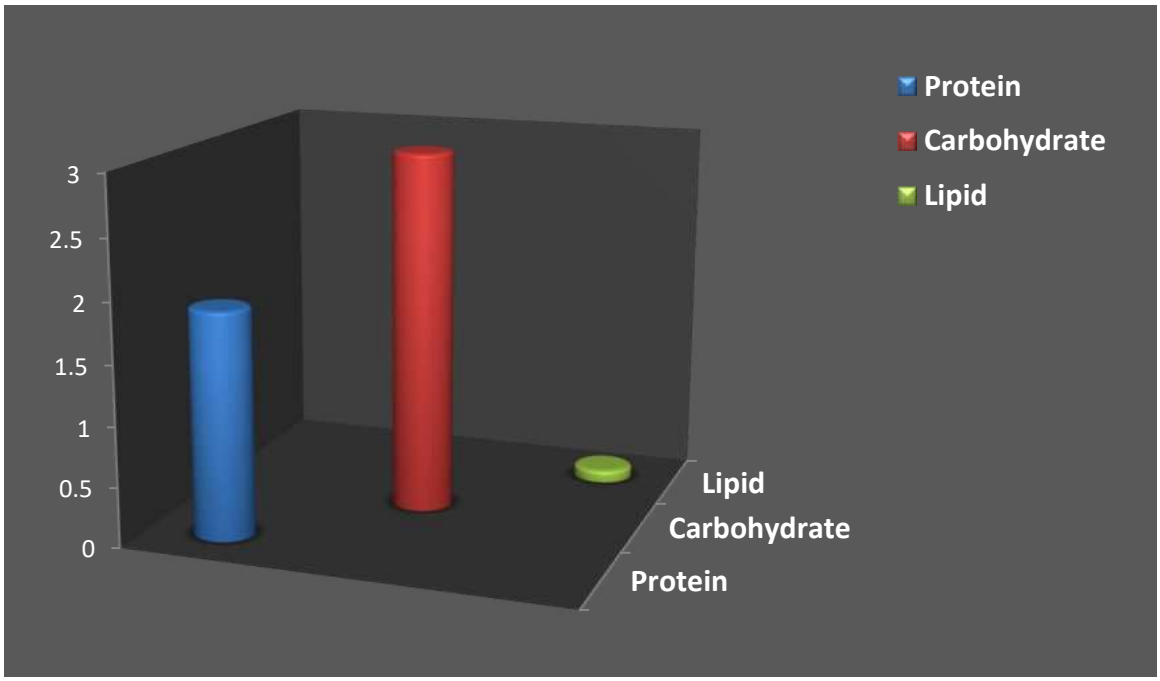
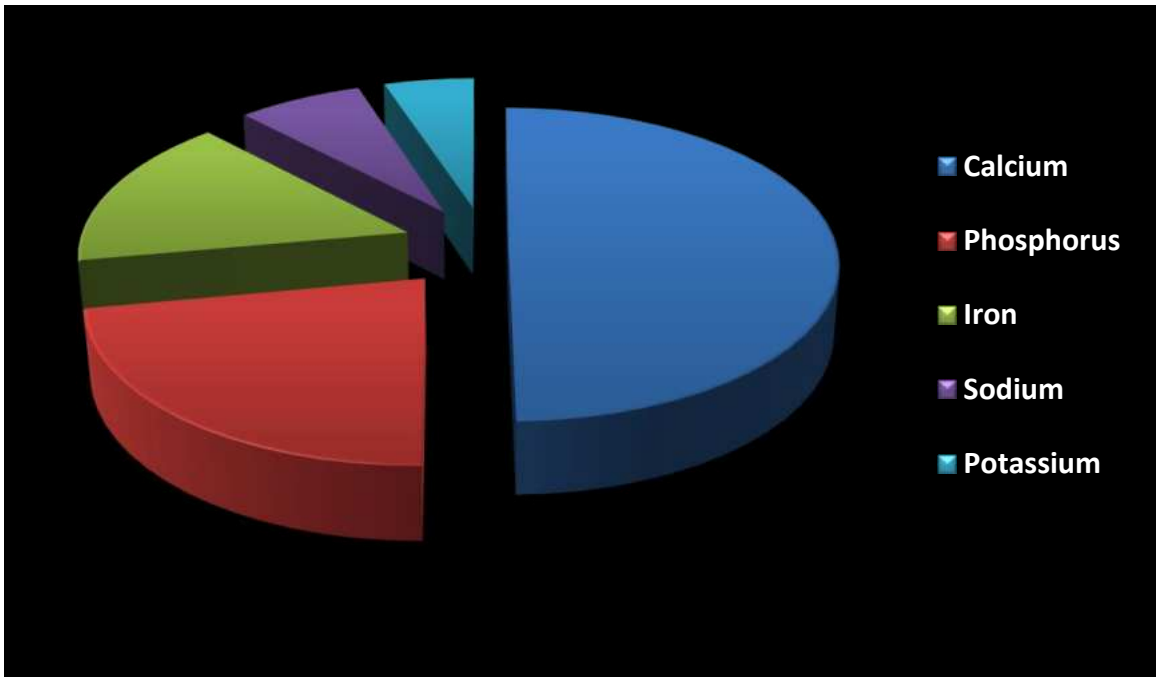


Figure 3: Showing the mineral profile of marine bivalve *Donax faba*



DISCUSSION

7. DISCUSSION

Biochemical studies are much important from the nutritional point of view. The biochemical constituents in animals are known to vary with season, size of the animal, stage of maturity temperature and availability of food etc. Biochemical components such as protein, carbohydrates and lipids are essential for body growth and maintenance. The results of the present study revealed that the protein content in *Donax faba* was about 18%. The molluscs are reported to contain high amount of protein ranged between 40-78% (Ananadakumar, 1986). The protein content was about 44.8 and 46.1% in males and females of *Rapana rapiformis* respectively. Arularasan (2009) has studied the protein content on male and female species of an edible sea snail of *Strombus canarium* that has protein content in the range from 47.86 to 70.18% taken from Gulf of Mannar. Shanmugam *et al.,* (2001) have mentioned the protein level of *Bursa spinosa* which varied from 18.71 to 29.81% at Parangipettai coast. 23.51% of protein was observed by Periyasamy *et al.,* (2014) on marine bivalve *Donax incarnatus* at Cuddalore coast.

The protein content in *D. incarnatus* body tissue was 26.93%. Babu *et al.,* (2010) assessed the percentage of protein which was ranged from 19.25% to 27.9% in the mesogastropod, *Bursa spinosa*. The protein content varied from 29.81% to 18.71%as reported by Shanmugam *et al.,* (2001). Arularasan *et al.,* (2009)

analyzed the percentage of protein which ranged from 47.86% to 70.18% in males and from 49.64% to 72.21%in females of *Strombus canarium*.

Carbohydrates are a group of organic compounds including sugars, starches and fiber, which is a major source of energy for animals. The percentage of carbohydrate in the body tissue of *L. quadricentus* is 10.13% and 4.69% in *N. pyramidalis* and the carbohydrate content in *Pythia plicata* ranged from 0.84% to 3.04%. The lipids are highly efficient source of energy, in that they contain more than twice the energy of carbohydrate and proteins. The lipid content of tissue were 1.34 and 2.11% in *Rapana rapiformis*, 0.85%-2.12% in male and 0.95%-2.96% in female, the highest level of lipid content 10.38% in *Babylonia zeylanica* and 1.97% in *Pleuroploca trapezium* were observed. This clearly indicated that the gastropods are potential source for the proximate composition and as human consumption. The determined research showed that marine bivalve *D. faba* tissue is a valued food due to high quality protein.

In the present study, the carbohydrate content was found to be 16% in *Donax faba*. In similar study, the percentage of carbohydrate in the body tissue of *D. cuneatus* was about 8.3% (Rajkumar, 1995). The carbohydrates reserve may be utilized under unfavorable conditions generally in molluscs and the greater variation found in the tissue indicates that the level of mobilized carbohydrates reserves may fluctuate widely and rapidly in response to fluctuation in condition.

Lipids are highly efficient as sources of energy and they contain more than twice the energy of carbohydrate and proteins. In the present study, the lipid content was found to be 12% in the marine bivalve *Donax faba.*

Lipid content of *D. cuneatus* was reported as 1.24% (Okuzumi and Fujii 2000). However, the reported lipid content in *Rapana rapiformis* ranged between 0.85-2.12% in male and 0.95-2.96% in female (Rajkumar, 1995). In *Babylonia zeylanica* and *Pleuroploca trapezium* species 10.38% and 1.97% of highest lipid content were noticed by Nirmal (1995). Shanmugam (2007) has reported that *Donax cuneatus* to contain good quantity of saturated, mono and polyunsaturated fatty acids in the range of 35.28%, 12.71%, 11.72% respectively.

Minerals also constitute important components of hormones, enzymes and enzyme activators in human nutrition. In the present study the levels of nutritionally significant minerals were investigated in *Donax faba*. Among the macro minerals, calcium (302.42 mg/g) was found to be higher and other minerals such as phosphorus, iron, sodium and potassium were also detected in significant level in *Donax faba*.

Very similar to the present study Coombs (1974) reported more than 40% of soluble copper and zinc in the oyster *Ostrea edulis*. Devadas (1994) reported that sea foods in general are excellent sources of I, CA, P, Na, Fe, Zn and oysters are

good sources of Fe and Cu. The nutritional values of snails got less emphasis in India and are not well studied. This is perhaps the important work on bivalves nutrient content and showed significant result for their use in both agro-industry and human consumption.

In general sea food is one of the most nutritionally balanced foods which helps to control weight and goes a long way towards preventing heart diseases. The nutritional values of bivalve are not brought to the limelight so far, so consumption of these nutrient rich molluscs has not attracted attention. The results of the present study provide ample information about the nutritional values such as protein carbohydrate, lipid and minerals composition which suggested consuming the bivalves *Donax faba.*

SUMMARY

8. SUMMARY

- The proximate compositions (%) such as protein, carbohydrate and lipid contents of *Donax faba* were estimated and the results of proximate composition reveled that protein was found to be dominant (18%) followed by carbohydrate (16%) lipid (12%) in the tissue of marine bivalve *Donax faba.*

- The analysis of the nutrients from the flesh of marine bivalve *Donax faba* results that bivalves are moderate sources of protein.

- The species *Donax faba* recorded the highest amount of minerals such as calcium, phosphorus, iron, sodium and potassium (mg/100g).

- The analysis of the minerals in the flesh of *Donax faba* results that bivalves are good sources of minerals.

- The highest amount of calcium was recorded from the flesh of *Donax faba* (302.42 mg/g) followed by phosphorus (135.46 mg/g), iron (95.74 mg/g), sodium (42.79 mg/g) and potassium (30.86 mg/g). There is large quantity of calcium in bivalve flesh.

CONCLUSION AND SUGGESTIONS

9. CONCLUSION AND SUGGESTIONS

Molluscs are widely used for various purposes like human consumption, poultry feed, fish feed, lime fisheries etc. Some other species are also collected and used as feed to ducks and fish ponds highly nutritious flesh and shells of molluscs should be used more vastly throughout the country, as those are abundant here. The present work reveals that other available molluscs could be used effectively for the same purpose. Popularization of bivalve as human food can also supplement the protein requirement of the poor inhabitants of the coastal area. In the present study, results of proximate composition revealed that protein was found to be dominant (18%), followed by carbohydrate (16%) and lipid (12%) in the tissue of marine bivalve *Donax faba.* The mineral profile ranged as calcium (302.42 mg/g), phosphorus (135.46 mg/g), iron (95.74 mg/g), sodium (42.79 mg/g) and potassium (30.86 mg/g) respectively.

Sea food is one of the most nutritionally balanced foods which helps to control weight and goes a long way towards preventing heart diseases. The nutritional values of bivalve are not brought to the limelight so far, so consumption of these nutrient rich molluscs has not attracted attention. The result of the present study confirmed that the marine bivalve *Donax faba* contains higher nutritional values.

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A COMPARATIVE STUDY ON THE PHYSICO-CHEMICAL PARAMETERS OF SOIL SAMPLES FROM THOOTHUKUDI DISTRICT

A project submitted to

ST.MARY'S COLLEGE (Autonomous), THOOTHUKUDI

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

in partial fulfillment for the award of the degree of

Bachelor of Science in Zoology

by

C.JENIFER	18AUZO14
J.MARIA ANISHA	18AUZO22
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St.Mary's College (Autonomous), Thoothukudi

(Re-accredited with 'A+' Grade by NACC)

April 2021

CERTIFICATE

This is to certify that the project entitled **A Comparative Study on the Physico-Chemical Parameters of Soil Samples from Thoothukudi District** is submitted to **St. Mary's College (Autonomous), Thoothukudi** in partial fulfillment for the award of the degree of **Bachelor of Science in Zoology** and it is a record of the work done during the year 2020-2021 by the following students

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CONTENTS

INTRODUCTION

1. INTRODUCTION

Soil is a complex and dynamic ecosystem whose functionality is related to the link that exist between chemical, physical and biological parameters and resident microbial communities. Soil is a vital component, medium of unconsolidated nutrients and materials from the life layer of plants. Soil developed as a result of pedogenic processes through weathering of rocks, consisting of inorganic constituents possessing definite chemical, physical, mineralogical and biological properties, having variability from depth to surface of the earth, and provides a medium for plant growth (Thakre *et al.*, 2012). Soil physico chemical properties influence the behavior of soil and hence, knowledge of soil property is important (Sumithra *et al*., 2013). Soil testing is the only way to determine the available nutrient status in soil. Soil properties that are sensitive to changes can be used as indicators to improve soil quality.

Analysis of soil is carried out for the studies of various parameters like total organic carbon, available nitrogen (N), Phosphorus (P_20_5) and Potassium (K_2O), pH, electrical conductivity, soil texture, bulk density, chloride, fluoride and moisture content. The fertility of the soil depends on the concentration of N, P, K, organic and inorganic materials, conductivity. The physicochemical properties

such as moisture content, nitrogen, phosphorus and organic matter are required for the growth of plants.

Soil quality can be defined as the balance between high active and high microbial biodiversity (Li *et al*., 2013). The management of soil quality plays an important role in protecting, the environment through preserving biodiversity and good agricultural practices (Lemaire *et al*., 2014). Many different microorganisms live in the environment, including bacteria, archaea, fungi, yeast, protozoa and microalgae. Soil microorganisms play a central role in decomposing organic matter, determining the release of mineral nutrients and in nutrient cycling and have direct and indirect effect on both crop growth and quality (Handelsman *et al*.,1998; Kozdroj 2013; Nannipieri *et al*., 2003). The structural composition of the soil microbiome can be studied in detail using next generation sequencing (NGS) (Grzadziel and Galazka, 2018)

In addition to the composition and activity of soil microbiomes, the quality of the soil is also defined by its physical, chemical and biochemical parameters (Furtak and Gajda, 2018). Among the parameters used to assess the quality of soil, one can distinguish pore size distribution (PSD), organic matter content, pH value, the total content and nitrogen, microbial biomass carbon and nitrogen contents, enzymatic activity and glomalin contents (Gajda and Furtak, 2018).

The physical and chemical parameters of the soil influence the microbial community and its activity. It is considered that a certain minimum number of species is necessary for the functioning of ecosystems, although the relationship between the microbiological diversity and the function of the soil system is not well known. A total of 80-90% of soil processes are reactions which microorganisms mediate (Nannipieri *et al*., 2003; Grzadziel and Galazka, 2018). Soil quality is the result of interaction between physical, chemical and biological properties (Li *et al*., 2013).

The quality and health of soil determine agricultural sustainability and environmental quality which jointly determines plant, animal and human health. The status of available micronutrients in soils and their relationship with various physico-chemical properties have been attempted by several investigator (Kumar and Babel, 2011; Methur and Sudan, 2011 and Ganorkar and Chinchmalatpure, 2013). The quality of the soil depends both on its physical properties (color, texture, moisture contents, pH, organic matter content etc.) and chemical properties (cation exchange capacity, organic matter contents, phosphate, phosphorus, nitrate, nitrogen, nitrite, etc).

The physical and chemical properties largely determine the suitability of a soil for its planned use and management requirements to keep it most productive to

a limited extent, the fertility of the soil determines its possible uses (Jaiswal, 2004). Soil physico-chemical properties play a vital role in determining the heavy metals pollution in the environment. When a soil is examined, its color indicates its condition. The surface soil colour varies from almost white to shades of brown and grey to black. Light color indicates low organic matter content while clave color indicates a high organic matter content (Jaiswal, 2004). Agriculturalists have always been interested in studying soil moisture. More recently, the potential impact of soil moisture content on run-off has been understood by a wider audience. The temporary storage of rainfall in the soil and aquifer layers of a catchment can be significant in the overall catchment water balance. Soil moisture variations occur predominantly in the first metre below the surface (Nathalie Cools and Bruno De Vos, 2013).

pH is generally acknowledged to be the principal factor governing concentration of sociable and plant available metals (Brallier *et al*., 1996). Metal solubility tends to increase at lower pH and decreases at higher pH values (Garia-Mirangana, 1984). It was found that cation exchange capacity increases as the clay content in the soil increases in the soil. It was also found that the cation exchange depends on the negative charge on the surfaces of soil colloids and the relative charges on the metal species in solution and on the soil surface (Evans, 1989).

The cause of the electrical conductance is the existence of particles with electric charge which, from a microscopic point of view, are loosely bound to specific positions within materials and thus, are capable of conveying electric charge. The materials featured by these particles are able to conduct electricity and thus are known as conductors. Liquid water is a conductor because under natural conditions, it contains dissolved ions which are movable charged particles. Soil, the other material that concern us here the most, is a composite conductor in which water, solid particles, and air are present in variable quantities and arrangements, and in which the electric charge carriers are water dissolved and solid particle loosely adsorbed ions (Fernando Visconti and José Miguel de Paz, 2016).

Soil alkalinity or salinity is a condition that results from the accumulation of soluble salts in soil. Most of the alkaline soils are found in the desert environments throughout the world. Although saline soils do occur in humid regions in areas affected by sea water, the most extensive occurrences are in arid regions, where they usually are found in low-lying areas where evaporation concentrates the salts received from more elevated locations in surface water, ground water, or irrigation water.

Calcium and magnesium are essential plant nutrients. They are called “secondary” nutrients because plants require them in smaller quantities than

nitrogen, phosphorus. The presence of higher levels of nitrite and nitrate, nitrogen in the soil is a clear indication of humus rich nitrogenous substance, which could be favorable to plant growth but may have implication for the ground with quality. Chlorine is important for the fertility of soils and being a nutrient for crops. However, anthropogenic activities often lead to excessive accumulation of the anion of chlorine, (Cl^-) in soils, either directly by applying animal wastes that are usually rich in Cl^- or via atmospheric depositions from industrial and municipal sources. Micronutrients (Cu and Fe) largely exist in the soil as positively charged metal cations bound as minerals or adsorbed to the surfaces of colloids or soil particles (Godson *et al*., 2004).

A comprehensive study on the physical and chemical properties of the soil is important for explaining the relationship between basic soil processes and in monitoring changes in the environment. So, the present study has been carried out with a view to investigate the physico chemical properties of different soil samples from Thoothukudi district.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The soil forms the intermediate zone between the atmosphere and the rock cover of the earth, the lithosphere. The soil may be defined as the uppermost weathered layer of the earth's crust in which mixed organisms and products of their death and decay materials were found. It may also be defined as the part of the earth's crust in which plants are anchored. It also forms interface between water bodies (hydrosphere) and the lithosphere and thus forming a part of biosphere. Soil sampling is perhaps the most vital step for any soil analysis.

Chik (2011) studied the chemical effects on soil compaction characterizations through electrical conductivity. Thakre *et al*., (2012) revealed the physico chemical characterization of red and black soil of Wardha Region. Sani *et al*., (2012) observed the physico chemical parameters of soil in some selected dump sites in Zaria and its environs. Onojake and Osuji (2012) assessed the physico chemical properties of hydrocarbon contaminated soil. Sumithra *et al*., (2013) reported a case study on physico-chemical characteristics of soil around industrial and agricultural area of Verraguntla, Kadapa District, Andhra Pradesh.

Rajesh Ganorkar *et al*., (2013) showed the physico - chemical assessment of soil in Rajura Bazar in Amravati District of Maharashtra. Kiran Chaudhari (2013) studied the physico chemical parameters of soil samples. Wagh *et al*., (2013)

observed the physico-chemical analysis of soil from eastern part of Pune City. Anita Joshi Raj *et al*., (2013) reported the comparative analysis of some important physico-chemical parameters of surface soil and underground water samples of fluorotic areas of Agastheeswaram union, South India.

Bednarek *et al*., (2014) observed the physico-chemical properties of surface layer after the flood in the middle Vistula river valley. Adesina *et al*., (2014) found in the effect of crude oil population in soil and maize and cowpea growth. Nazir Ruqia *et al*., (2015) showed the accumulation of heavy metals Ni, Cu, Cd, Cr, Pb, Zn, Fe in the soil, water and plants and analysed the physico-chemical parameters of soil and water collected from Tanda dam Kohat.

Beena and Jaya (2016) evaluated soil contamination in the surrounding of Kerala Minerals and Metals Limited (KMML) industrial area in Kollam District, Kerala. Sowinski *et al*., (2016) revealed the distribution of heavy metals in soil in a post glacial river valley - A geochemical landscape approach. Marshal Soni (2016) described the soil samples for its physico-chemical properties from Abohar city. Madan Lowry *et al*., (2016) reported the physico-chemical and biological properties of hydrocarbon contaminated soil of Mathura region. Rajesh *et al*., (2017) assessed the soil nutrients and physico chemical parameters in the region of Hiwarkhed village of Amravati district.

Arshi Iram and Khan (2018) analyzed the soil quality using physico-chemical parameters with special emphasis on fluoride from selected sites of Sawai Madhopur Tehsil, Rajasthan. Zhang *et al*., (2018) found the changes in soil physico-chemical properties and soil bacterial community in mulberry intercropping system. Karolina Furtak *et al*., (2019) analysed the soil properties, bacterial community composition and metabolic diversity in fluvisols of a floodplain area.

From the above review it is pronounced that knowledge on the physico chemical parameters are lacking for the soil samples of Thoothukudi district. Considering the importance of the physico chemical parameters, the present work on soil analysis is planned and carried out covering all the above countenances.

OBJECTIVES

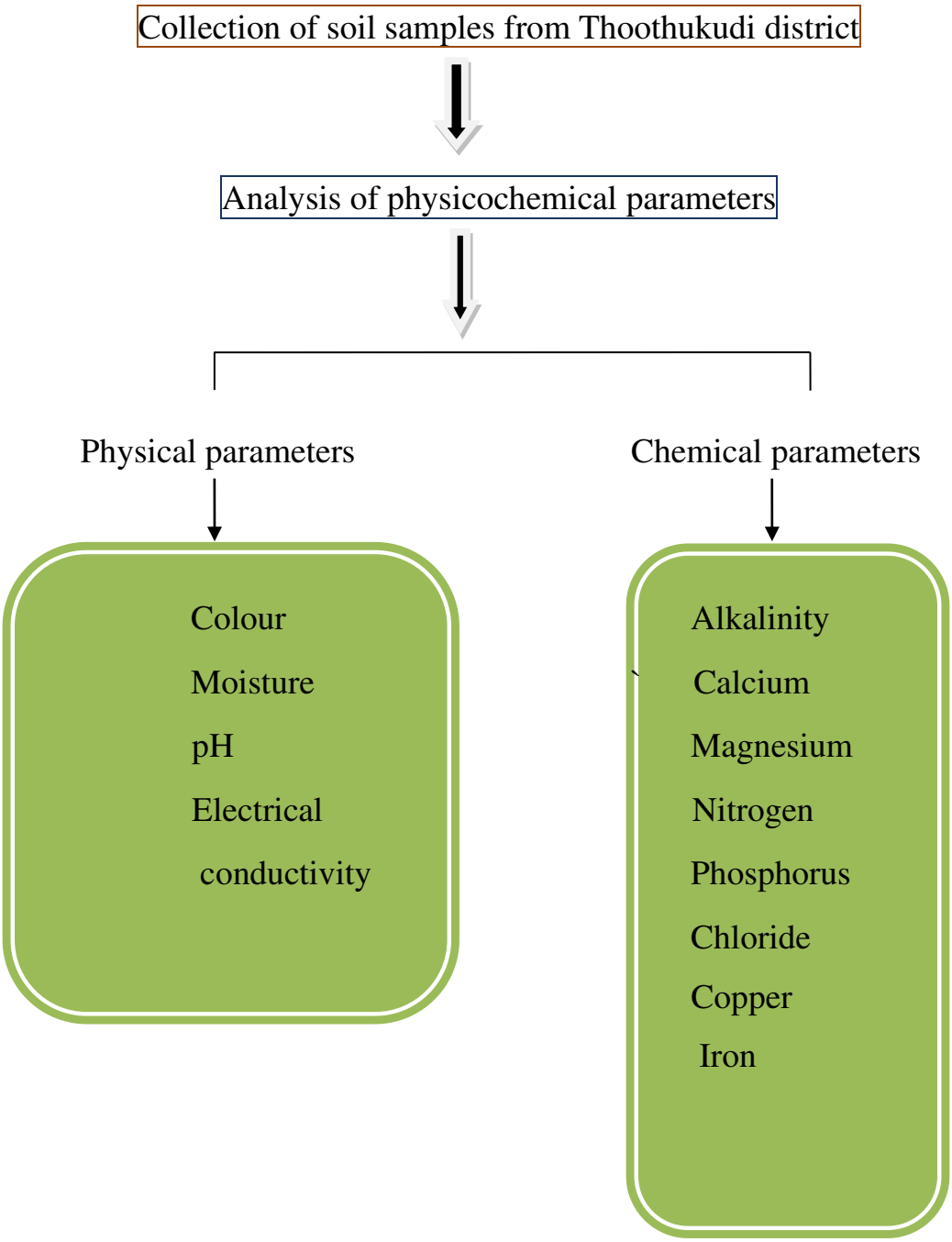
3. OBJECTIVES

The economies for most developing countries primarily depend on agriculture. Studying the physicochemical properties of soil is important for sustainable management of the agricultural resources and economies growth. Lack of agricultural inputs, traditional farming methods, over grazing and continuous cultivation practice, coupled with environmental factors aggravates the degradation of soil physico chemical properties. Therefore, the main objectives of the present study are

- To assess the physicochemical properties of three different soil samples from Thoothukudi district.
- To investigate the physicochemical parameters like colour, moisture, pH, electrical conductivity, alkalinity, calcium, magnesium, nitrogen, phosphorus, chloride, copper and iron.

EXPRIMENTAL DESIGN

4. EXPRIMENTAL DESIGN



MATERIALS AND METHODS

5. MATERIALS AND METHODS

5.1 Collection of soil samples

Soil samples was collected from three different areas Narippaiyur, Bangla street, Vembar regions of Thoothukudi district (8.7642° N, 78.1348° E). The sample was taken from 0-10 cm depth from the surface area and collected inside sterile sample bottles and transported to the laboratory for analysis.

5.2 Analysis of Soil Sample

The collected samples were subjected to analysis of physico – chemical parameters.

5.3 Physico - chemical parameters

5.3.1 Colour

Colour is an easily observable characteristic of soils and is integral to the taxonomic classifications of soils. The colour of three different samples were independently measured in the laboratory undercontrolled lighting conditions by direct visual examination.

5.3.2 Moisture

1g of soil sample was weight into dry crucible. The crucible was then placed

in an air circulated oven at 105°C and dried to constant weight (for 6 hours). The sample was cooled in a dessicator and re-weighed. The percentage air dried moisture from the loss in weight was then determined as follows:

$$\% \text{ moisture content} = \frac{\text{Loss in weight}}{\text{Initial weight}} \times 100$$

5.3.3 pH

The pH of the soil sample was measured with the help of pH paper. Dip a strip of wide range pH paper into the soil sample. Compare the colour developed with the pH chart given with the paper. This gives the approximate pH. Based on this, the pH value was calculated.

5.3.4 Electrical Conductivity

25 g of air dried soil sample was placed into 250 ml beaker. Distilled water was added slowly drop by drop uniformly over the entire soil surface until the soil appears to have been wetted. A stainless steel spatula was used to form a homogeneous soil saturated paste. The beaker was then covered with the petri-dish. 50 ml distilled water was added and shaken for 1 hour. 40 ml of the diluted extract was placed into 100 ml beaker and the conductivity meter was inserted and the electrical conductivity of the soil was recorded in µmho/cm.

5.3.5 Alkalinity

The soil sample (50ml) was taken in a conical flask and 2 -3 drops of phenolphthalein indicator was added and titrated against 0.02 N Sulphuric acid to the colourless endpoint. Noted the reading as 'P'. Then to the same solution 2-3 drops of methyl orange indicator was added and continued the titration until the yellow colour turned into orange (end point). Noted the reading as't' which was the volume of titrate used for both the tirations. Total alkalinity in soil sample was calculated and expressed the values as mg/l.

$$\text{Phenolphthalein alkalinity (mg/l)} = \frac{\text{ml of titrant x N x 50 x 1000}}{\text{Sample volume (ml)}}$$

$$\text{Total alkalinity (mg/l)} = \frac{\text{ml of titrant x N x 50 x 1000}}{\text{Sample volume (ml)}}$$

5.3.6 Calcium and Magnesium (APHA, 1998)

EDTA Titrimetric method was adopted. 5ml of soil sample was taken in a conical flask and 5ml of ammonium buffer was added. It was diluted with 100ml distilled water and a pinch of eriochrome black –T was added. This solution was warmed to 60^{o}C and was titrated against the EDTA from the burette. The end point was from the salmon red to blue. This titre value was taken as A and is the measure of total amount of calcium and magnesium.

Another 5ml of sample was taken in a conical flask and 5ml of sodium hydroxide was added followed by a pinch of murexide as an indicator. This solution was diluted with 100ml distilled water and it was titrated against EDTA until the colour changes from pink to deep violet. This value was taken as B and is a measure of amount of calcium.

By subtracting the titre value for calcium from the titre value of the total amount of Ca and Mg gives the value for magnesium alone and it is calculated and expressed in mg/l.

$$\text{Amount of calcium in the sample} = \frac{F \times B \times 1000}{\text{Vol of the sample}}$$

Factor value for Ca = 2 mg

Factor value for M g =1.12mg

$$\text{Amount of Mg in the sample} = \frac{F \times (A-B) \times 1000}{\text{Vol of the sample}}$$

5.3.7 Nitrogen

Take 10 gm of air-dry soil in a 300 ml flask and add 20 gm of catalyst mixture and 35 ml of sulphuric acid. Heat the contents from bottom of the flask for about 2 hours. Cool the contents, add about 100 ml of distilled water, wait for about 5 minutes, and deliver the supernatant into 1 liter distillation flask A of

Kjeldhal distillation assembly. Wash the residue with a little of distilled water several times and transfer the supernatant each time to the same distillation flask. Add 100 ml of sodium hydroxide solution and a few granules of zinc. Take 25 ml of boric acid cum indicator solution in a 500 ml Erlenmeyer flask B and place it below distillation assembly so that the lower open ends of the condenser is dipped in solution. Heat the distillation flask on a hot plate and collect about 150 ml of distillate in flask B. Remove the flask with distillate and titrate the distillate (which has turned blue due to dissolution of ammonia) against 0.1N hydrochloric acid. Turning of blue colour to light brown-pink indicates the end point. Run distilled water blank in the same manner.

$$\text{Kjeldhal Nitrogen (mg/g)} = T1 - T2 \times N \times 14 / W$$

5.3.8 Phosphorus

10ml of freshly prepared mixed reagent was added to 50ml of soil samples. The contents of the flask were thoroughly mixed, and allowed to stand for about 15minutes at the room temperature. The optical density was measured at 882 nm. By comparing the absorbance of the sample with that of standard solution the total phosphorus was calculated and expressed in mg/g.

5.3.9 Copper

In a 200-ml Erlenmeyer flask place 10 g of air dried and sieved (mesh 2 mm) soil sample, with 30 ml water for analysis and 10 ml nitric acid 65 % and heat for 1 hour. After cooling slowly and under swinging add 23 ml ammonia 25 %. The pH - value should be 9 - 10. Filter off the insoluble parts and wash the filter 3 times with water for analysis. Collect the filtrate and the washings in a 200-ml volumetric flask, fill up to volume with water for analysis and mix well.

Copper content in mg/kg Cu = analysis value in mg/l Cu x 20

5.3.10 Chloride

100ml of soil sample was taken in a conical flask. The sample was filtered first if it contains a lot of suspended matter. 1ml of potassium chromate indicator solution was added and run in silver nitrite solution taken in a burette. Swirled the flask during the addition of silver nitrate. The titration was continued until the precipitate was formed and the solution turned a pink- orange colour. The chloride ion in water sample was calculated and expressed in mg/l.

Chloride ion = (A - 0.2) X N X 354.5

Where

A - standard silver nitrate used

0.2 – Blank value attributed to potassium chromate

N – Normality of silver nitrate determined by standardisation against sodium chloride

354.5 is a constant

5.3.11 Iron

100ml of soil sample was taken in a conical flask, 4ml dilute $HNO_3$was added, heated to boiling. A few drops of N/8 $KMnO_4$ was added until a slight permanent pink colour develops. Filtered if necessary, washed, cooled and then 4ml of ammonium thiocynate solution was added. Matched immediately with the standard. Reading of the concentration from the standard with which it matches and expressed in mg/l.

RESULTS

6. RESULTS

The basic physical and chemical soil properties are given in table 1; figures 2,3 and 4. The analyzed physical and chemical properties show the wide variation range, which can be seen in the result. The colour of the soil varied among three stations. Station I showed the white colour, black colour was observed in station II and brown colour was observed in station III. The moisture content of soil in station I was found to be 9.374, in station II was 9.978 and station III was 9.834. The pH of the soil samples was found to be 7.61 in station I, 7.58 in station II and 7.68 in station III. Electrical conductivity of soil samples were found to be 0.19 µmho/cm in station I, 0.14 µmho/cm in station II and 0.17 µmho/cm in station III.

The alkalinity level of soil samples of three stations were 360 mg/g in station I, 220mg/g in station II and 420 mg/g in station III. The percentage of calcium content was 34.03% in station I, 23.62%in station II and 28.82 in station III. The magnesium level was recorded as 52.64% in station I, 60.53% in station II and 43.84% in station III. Most important factor which decides the soil productivity is N:P:K ratio. Phosphorus considered as micro nutrient, is utilized by plant in the form of $H_2PO_4^-$ & HPO_4^{-2} species. Appropriate concentration of phosphorus (P) is necessary for maintaining a balance between the other plant nutrients and ensuring the normal growth of the crop. The nitrogen level was observed as 529mg/g in station I, 459mg/g in station II and 572mg/g in station III.

The phosphorus content was recorded as 55.32mg/g in station I, 35.99mg/g in station II and 24.08mg/g in station III. Chloride is mentioned as hydrological and chemically insert substance. The chloride content ranged as 106.35mg/g in station I, 141.8mg/g in station II and 123.98 mg/g in station III. The amount of copper was found to be 5.6ppm in station I, 4.9ppm in station II and 3.1ppm in station III. The amount of iron was ranged as 19.6ppm in station I, 14.3ppm in station II and 12.5ppm in station III.

Figure 1. Map showing the study area Thoothukudi District

Table 1: Measurement of Physico chemical parameters in station I, II and III

PARARMETERS	STATION I	STATION II	STATION III
Colour	White	Black	Brown
Moisture	9.374	9.978	9.834
pH	7.61	7.58	7.68
Electrical Conductivity (µmho/cm)	0.19	0.14	0.17
Alkalinity (mg/g)	360	220	420
Calcium (%)	34.03	23.62	28.82
Magnesium (%)	52.94	60.53	43.84
Nitrogen (mg/g)	529	459	572
Phosphorus (mg/g)	55.32	35.99	24.08
Chloride (mg/g)	106.35	141.8	123.98
Copper (ppm)	5.6	4.9	3.1
Iron (ppm)	19.6	14.3	12.5

Figure 1: Physicochemical parameters in station I

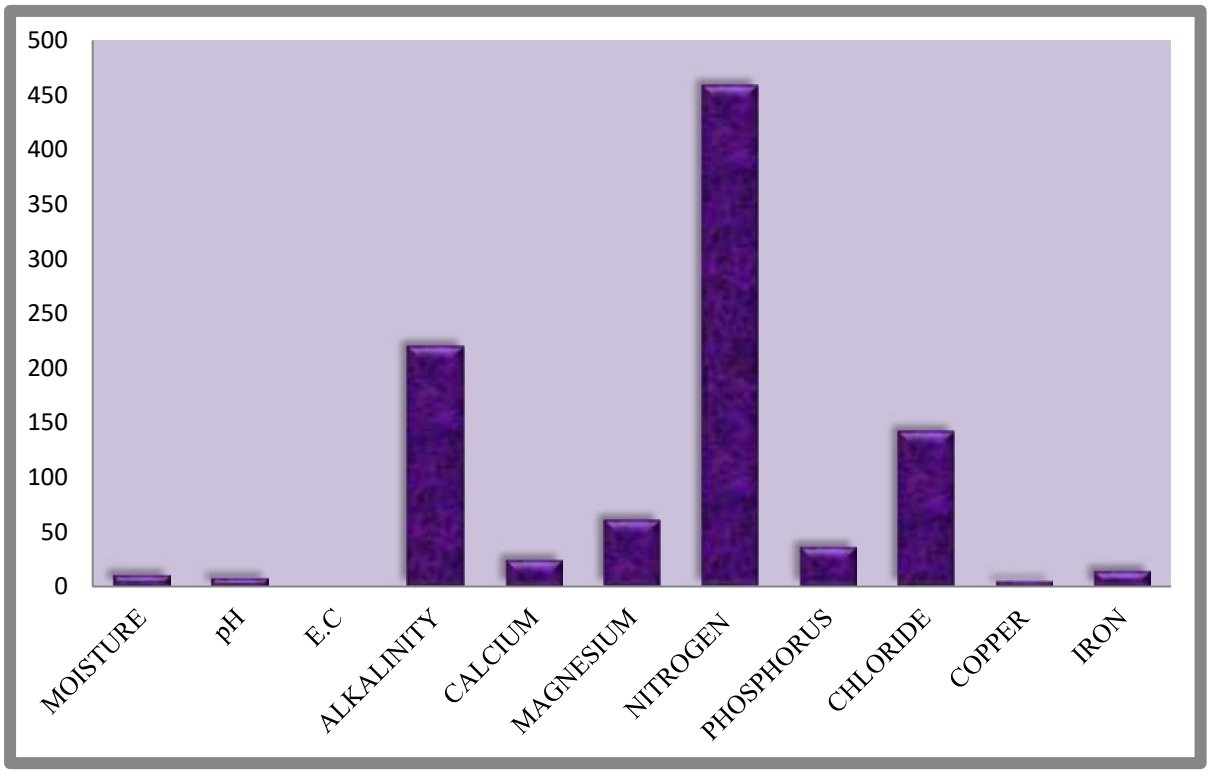


Figure 2: Physicochemical parameters in station II

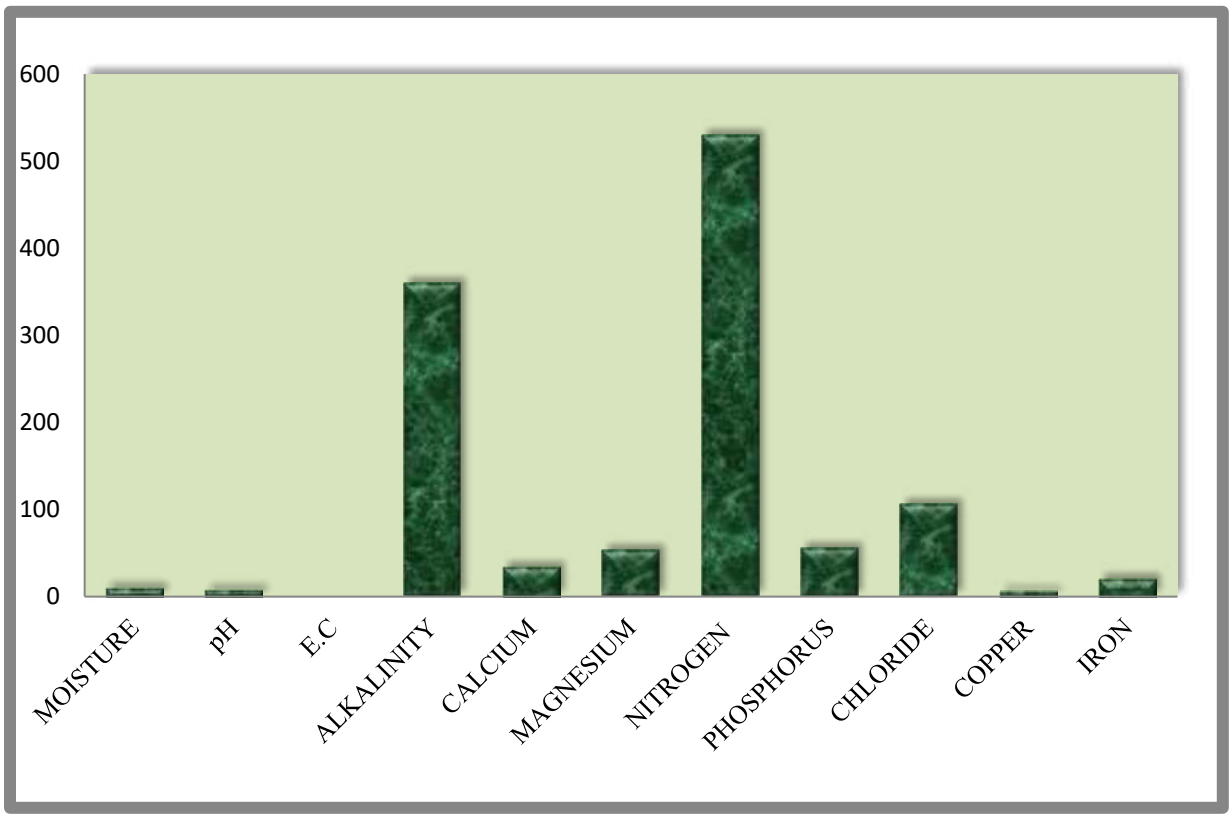
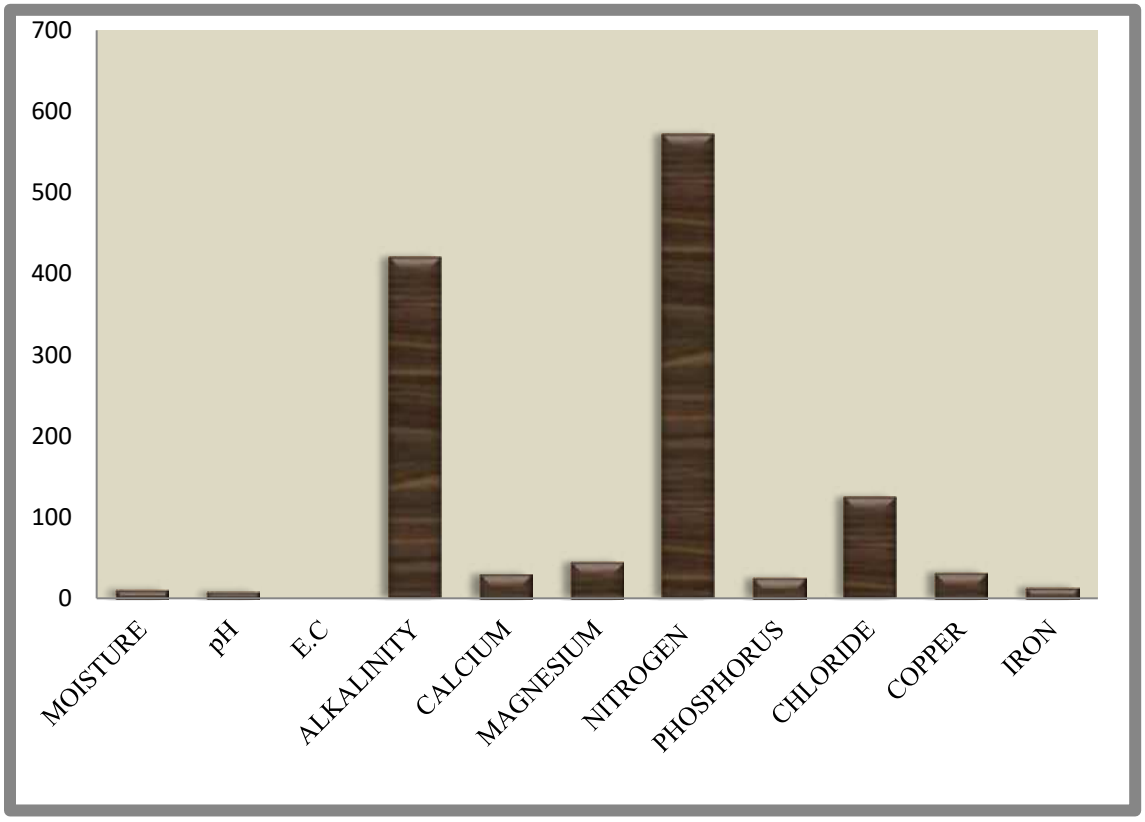


Figure 3: Physicochemical parameters in station III



DISCUSSION

7. DISCUSSION

Soil structure is influenced by different properties of soil constituents and by its environment (Cammeraat and Imeson, 1998). Natural environment is clean, but due to multifarious activities of man, it gets polluted resulting in environmental pollution. The physico-chemical study of soil is based on various parameters like colour, pH, moisture, electrical conductivity, alkalinity, calcium, magnesium, nitrogen, phosphorous, chloride, copper and iron. Three different soil samples were obtained and analyzed for its physico-chemical properties.

The most significant property of soil is its pH level. Its effects on all other parameters of soil. Therefore, pH is considered while analyzing any kind of soil. If the pH is less than 6 then it is said to be an acidic soil, the pH range from 6-8.5 it's normal soil and greater than 8.5 then it is said to be alkaline soil (Marshal Sonic, 2016). In the present study the pH of station I is 7.61, 7.58 in station II and 7.68 in station III.

pH can affect the availability of nutrients and activity of many essential micro-organisms, and most of the sample found alkalinity is not good for microbes. Several researchers showed that the texture of soil remain a major

constraint to crop production. In this context, Nyabyenda (2005) reported that the production of grain legumes had been low due to declining soil fertility as a result of soil impoverishment in organic matter content and corresponding texture.

Electrical conductivity is also a very important property of the soil, it is used to check the quality of the soil. It is a measure of ions present in solution. Electrical conductivity is a very quick, simple and inexpensive method to check health of soils. In the present study, electrical conductivity was found to be 0.19 µmho/cm in station I, 0.14 µmho/cm in station II and 0.17 µmho/cm in station III.

Alkalinity is a measure of saline or salt effected soil, the pH of these soil is greater than 7. In the present study, the alkalinity level of soil samples of three stations were 360 mg/l in station I, 220mg/l in station II and 420 mg/l in station III. Calcium values ranged as 34.03 in station I, 23.62% in station II and 28.82 % in station III. Calcium is essential in the proper functioning of plant cell walls and membranes. Sufficient calcium must also be present in actively growing plant parts, especially storage organs such as fruits and roots. Properly limed soils with constant and adequate moisture will normally supply sufficient calcium to plants. High humidity and poor soil drainage hinder calcium movement into these plant parts and should be avoided.

Magnesium values ranged as 52.64 % in station I, 60.53 % in station II and 43.84% in station III. Magnesium acts together with phosphorus to drive plant metabolism and is part of chlorophyll, a vital substance for photosynthesis. Low magnesium levels in many soils will normally not cause problems provided the exchangeable cations are in good balance. If Mg levels are low and lime is required, dolomitic lime (rich in Mg) will be recommended. If Mg is low and lime is not required, Epsom salt (magnesium sulphate) may be incorporated at a rate of 5-10 lbs/1000 square feet.

Phosphorus is the most important element present in every living cells. It is one of the most important micronutrient essential for plants growth. Phosphorous most often limits nutrients remains present in plant nuclei and act as an energy storage. In the present study, phosphorus of station I 55.32 mg/g, 35.99 mg/g in station II and 24.08 mg/g in station III. Phosphorus helps the transformation of solar energy into chemical energy. Among other important functions, phosphate provides plants with a means of using the energy harnessed by photosynthesis to drive its metabolism. A deficiency of this nutrient can lead to impaired vegetative growth, weak root systems, and fruit and seed of poor quality and low yield (Raman and Sathiyanarayanan, 2009).

In present study variability in soil physicochemical parameters may contribute to the variation in nutrient storage and availability water retention and transport and binding and stability of soil aggregates. Normally, black loamy soil has good N: P: K ratio. Soil texture directly or indirectly influences soil function such as soil erosion, water availability (Adhikari, 2009). The sandy soil can quickly be recharged but its holding capacity is not good. As texture becomes heavier, the wilting point increases because fine soils with narrow pore spacing hold water more tightly than soils with wide pore spacing (Thakre, 2012). The bulk density depends on compaction, consolidation of the soil but it is negatively correlated to the organic content. According to Micheni (2004) the soil organic matter plays an important role in maintaining soil quality.

In the study area soil organic matter content varies from very less to more than sufficient and it's directly influenced by soil texture and moisture content. Chloride is an undesirable content but it's unavoidable, because it is an essential micronutrient for optimal growth. Both potassium and chloride play the main role to neutralize the charges, and as the most important inorganic osmotic active substances in plants cells and tissues. The association of potassium and chloride is related to the opening and closing of stomata (Oberg and Sanden, 2005), (Talbott and Zeiger 1996), (FIXEN and Adv Agron, 1993). In the most of site soil samples potassium content was in average range. Potassium is known to affect cell division,

cell permeability formation of carbohydrates, translocations of sugar, varies enzymes actions and resistance of some plants to certain diseases (Miller and Turk 2002). Soils are basically categorized on behalf of soil fertility and presence of micro nutrient. In presence finding site soil is less nutrient so farmers use more fertilizer and phosphate fertilizer.

Copper in soils is strongly immobilized by the composition of the soil absorption complex (10, 46) (i.e., organic matter, Fe-, Mn-oxyhydroxides, and nature of the humic substances). Soluble humic and fulvic acids may increase the solubility and mobility of the elements; once in a neutral to alkaline reaction environment, they form stable complexes with the carboxyl, hydroxyl, and amino groups of these compounds. In the present study, copper contents were found to be 5.6pm in station I, 4.9ppm in station II and 3.1 in station III.

Iron values ranged in 19.6 ppm in station I, 14.3ppm in station II and 12.5ppm in station III. The iron is essential to plants, but required in very small amounts. In most properly limed soils they are available in sufficient quantities. The values of iron lie between 0.1% to 1.24 %.

Chemical-intensive practices in agricultural fields increasing fluoride contamination and other pollution problems of a magnitude that exceeds normal limits. Plants take up fluoride through fine hair rootlets from the soil. The most

prominent factors that dictate the amount of F in most soils are the quantity of clay minerals, the soil pH and the concentrations of Ca and P in soil (Begum *et al., 2008*). Same results found in the study of (Larsen, and Widdowson, 1971; Chhabra *et al.,*1980 ; Omueti Jai and Jones, 1980) high adsorption of fluoride by soil mineral components is at about pH 6 to 8 (Andrews *et al*., 2004; Clark *et al.,* 1983; Clark *et al.,* 1976; Desaai *et al*., 1988; Grewal *et al*., 1992; Hand book of agriculture, 2001; Hassink, 1992; Iram and Khal 2016; Jackson Mi 1958; Jezierska Madziar and Pinskwar 2003; Omueti Jai and Jones 1977; Praveen *et al.,* 1993; Stevens *et al.,* 2000; Walkley and Black 1934; Wenzel and Blum 1992). From, the present study it was confirmed that three different stations showed wide variations in the physico chemical parameters.

SUMMARY

8. SUMMARY

- The basic physical and chemical soil properties are given in table 1, figures 2, 3 and 4.
- The analyzed properties show the wide variation range, which can be seen in the result.
- Station I showed the white colour, black colour was observed in station II and brown colour was observed in station III.
- The moisture content of soil in station I was found to be 9.374, in station II was 9.978 and station III was 9.834.
- The pH of all samples was found to be ranged in 7.61 in station I, 7.58 in station II and 7.68 in station III.
- Electrical conductivity of soil samples range in 0.19 in station I, 0.14 in station II and 0.17 in station III.
- The alkalinity level of soil samples of three stations was 360 mg/g in station I, 220mg/g in station II and 420 mg/g in station III.
- The percentage of calcium content was 34.03% in station I, 23.62%in station II and 28.82in station III.

- The magnesium level was recorded as 52.64% in station I, 60.53% in station II and 43.84% in station III.
- The nitrogen level was observed as 529mg/g in station I, 459mg/g in station II and 572mg/g in station III.
- The phosphorus content was recorded 55.32 mg/g in station I, 35.99 mg/g in station II and 24.08 mg/g in station III. Phosphorus considered as micro nutrient, is utilized by plant in the form of $H_2PO_4^-$ & HPO_4^{-2} species.
- The Chloride content ranged as 106.35mg/g in station I, 141.8mg/g in station II and 123.98 mg/g in station III.
- The amount of copper was found to be 5.6ppm in station I, 4.9ppm in station II and 3.1ppm in station III.
- The amount of iron was ranged as 19.6ppm in station I, 14.3ppm in station II and 12.5ppm in station III.

CONCLUSION AND SUGGESTIONS

9. CONCLUSION AND SUGGESTIONS

In the present study physicochemical parameters of three stations were analyzed. The results of the present study revealed the values of physicochemical parameters of soil. The analyzed physical and chemical properties showed the wide variations in the result. It is important to agricultural chemists for plants growth and soil management. The results of the present study will help to identify the type and degree of soil related problems and to suggest appropriate reclamation measure and also to find out suitability for growing crops. It will also help to study the soil genesis. High moisture content signifies that the water available to the plant in the soils is also high. On the basis of this study farmers can get an approximate idea about the amount of which fertilizers and nutrients are needed to soil for increasing the percentage yield of crops. High organic matter content indicates human-rich soil. This implies that all the soils studied were highly fertile. However, when the waste soils are used as manure there is fear of heavy metals accumulation.

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Insilico Characterization of *Allium cepa* lectin-

A potent antiviral protein

A Project submitted to

ST.MARY'S COLLEGE (Autonomous), THOOTHUKUDI

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

In partial fulfilment for the award of the degree of

Bachelor of Science in Zoology

By

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April 2021

CERTIFICATE

This is to certify that the project entitled "Insilico Characterization of Allium cepa lectin - A potent antiviral protein" is submitted to St. Mary's College (Autonomous), Thoothukudi in partial fulfillment for the award of the degree of **Bachelor of Science in Zoology** and it is a record of the work done during the year 2020-2021 by the following students

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CONTENTS

1. Introduction

Lectins are carbohydrate binding proteins found in all the organisms-microbes, plants and animals. Lectins exhibit a wide range of biological activity from haemagglutination to antiviral activity. Lectins do not cause any antigenic stimulation within the immune system but they have the basic capacity to bind analogously to an antibody(Komth et al., 2006). Lectins bind to glycoproteins present on the cell surface of animal cells and viruses. In the early 1990's Peumans and Van Damme defined lectins as = a class of proteins of nonimmune origin that posses at least one non-catalytic domain that specifically and reversibly bind to mono-or oligosaccharides‖. Their carbohydrate binding ability is very specific (Rini, 1995). They may bind to a soluble carbohydrate or to a carbohydrate moiety that is part of glycoprotein or glycolipid which are usually multivalent possessing more than one sugar binding site. The lectins cause lymphocyte mitogenesis and cause haemagglutintion, aggregagate immunoglobulins, trigger complement pathways, inhibit fungal and possess insecticidal activity. Some lectins are documented to have antiviral activity.

Antiviral lectins have been reported from bacteria, plants and animals. Antiviral activity of lectins is attributed to its interaction with the glycoprotein of viral envelope. Such antiviral lectins have been identified in bacteria, plants and marine algae. A study on antiviral lectins will help them to be harnessed for therapeutic use against viral diseases like the Covid19.

2. Objectives

In the light of above background ,the objectives set for this study were;

- Sequence retreival and In silico analysis of *Allium cepa* lectin.
- Molecular modeling of a *Allium cepa* lectin

3. Review of literature

Lectins were first described in *1888 by Stillmark*, who observed that crude extracts of castor beans (Ricinus communis) contained a toxic substance named ricin that agglutinated human and some blood cells. However the modern age of lectinology started nearly 100 years later. Lectins were initially found and described in plants, but subsequent years multiple lectins were isolated from microorganism and from animals indicating that they were extensively distributed. (Sharon and Lis, 2004). They encompass different members that are diverse in their sequences, structures, binding site architecture, carbohydrate affinities and specificites as well as their larger biological roles and potential applications (Kumar *et. al.* 2012)

The lectins bind to a soluble carbohydrate or to a carbohydrate moiety that is part of glycoprotein or glycolipid which are usually multivalent possessing more than one sugar binding site. The use of carbohydrate-binding activity rather than haemagglutination activity is considered as the functional criterion of lectins. Goldstein et al., (1986) defined lectins as the carbohydrate-binding proteins (or glycoproteins) of non-immune origin that agglutinate cells and/or precipitate glycoconjugates. Later with the observation that some lectins contain a second type of binding site that interacts with non-carbohydrate ligands, lectins were redefined as carbohydrate-binding proteins other than antibodies or enzymes. Because some plant enzymes (like Type2 ribosome

inactivating proteins [RIP] and the class I chitinases) being fusion proteins are built of carbohydratebinding domain tandemly arrayed with a catalytic domain, these lectins cannot be excluded. On the other hand, observation that some proteins that are related to lectins, but lack carbohydrate-binding domain insisted on including functionality as a criterion. Now the only prerequisite for a protein to be named a lectin is the presence of at least one non-catalytic domain that binds reversibly to a specific carbohydrate (Peumans and Van Damme, 1995).

Structure of lectins

Lectins are mainly made-up of carbohydrate binding proteins or glycoproteins of nonimmune origin which binds the cells or precipitates, glyco-conjugates or sometimes, both (Goldstein *et al.,*1980). The carbohydrate moieties specify the lectins associated reaction. Generally lectins are classified into four groups, based on their affinity to bind with 1. Glucose/mannose 2. Galactose and N-acetyl-D-galactosamine 3. L-fucose 4. Sialic acids

Biological role:

The biological role of lectins is speculative. Lectins may be involved in sugar transport or carbohydrate storage. The microbial lectins may play an important role in adhesion to the surfaces colonized by the microorganisms and hence important host pathogen interactions. Lectins agglutinate erythrocytes because of which lectins are used as probes to investigate cell surface structures and functions. They are used as drug

carriers, reagents to study cell surface receptorsof bacteria, protozoa, and higher organisms.Lectins have demonstrated potential for use in histologic studies to stain certain tissue types (Boerwinkel *et al.,* 2014), or diagnostics in biosensors as well as helping to understand and modulate cellular processes including host defense from infectious agents.

.**Antiviral activity of lectins**

Lectins bind to monomeric and oligosaccharide substrates with high specificity (Fig. 3.1). The lectins that are evolutionarily present in all the organisms from bacteria to animals they play multifaceted roles in biological systems including self-recognition, protein folding, and cell movement and adherence. The viruses recognize and enter the host cells through the host-cell surface proteins by utilizing its glycosylated envelope proteins that have affinity for host cell-surface proteins. The lectins exhibit antiviral activity as they bind to the carbohydrate moieties present on the viral envelope through their carbohydrate binding domain and stop their entry into the host cell. The evolutionary development of viral glycosylation as a mechanism to both enhance viral uptake and evade host organism defenses has resulted in a co-evolution of lectins specific to non-self carbohydrate structures. The continuing challenges in enabling broad spectrum viral suppression support the study of lectins as viral entry inhibitors to provide prophylactic and potentially therapeutic agents against viral infections

Antiviral lectins generally contain internal repeats within their primary sequences each comprising a carbohydrate recognition domain (CRD). The duplication of binding domains provides a mechanism for increased avidity for branched carbohydrate structures. The sequence identity of lectins ranges from ~10 to 100% across genera with the greatest variability localized to the loop regions that contribute to the CRD for some classes. Sequence homology is found predominantly in the core structural components of the lectin. Disulfide bonds are present in some antiviral lectinstructures, but are not a prerequisite.

Antiviral lectins interact predominantly with high-mannoseglycan structures added as post-translational modifications to theenvelope proteins of viruses (Bokesch *et al*., 2003). The envelope proteins share sequence homology across enveloped viruses, adopt similar tertiary and quaternary structure, and perform equivalent functions (Fig. 3.2). Lectins inhibit the viral entry by interacting with the glycosylated moieties of the Envelope protein complex preventing the conformational rearrangements required for viral fusion Antiviral lectins have been isolated from various sources and have been listed in Table 3.1 (Bacterial lectins), Table 3. 2 (Plant lectins) and Table 3.3 (Animal lectins)

Table 3.1. Antiviral lectins from Bacteria

LECTIN	SOURCE	ACTIVITY
Actinohivin	*Longsiporum albid* K97 – 0003	HIV
Cyanovirin	*Nostoc ellisosporum*	HIV , HIV – 2, SIV , HCV , HSV – 1, Influenza A and B , Ebola &MARV
Microvirin	*Microcystis aeruginosa* PCC7806	HIV
MVL	*Microcystis viridis* NIES – 102	HIV & HCV
Scytovirin	*Scytonema varium*	HIV , Ebola , HCV & MARV
OAA	*Oscillatoria agardhii* NIES – 204	HIV
PFA	*Pseudomonas fluorescens*	HIV
MBHA	*Myxococcus xanthus*	HIV
BOA	*Burkholideria oklahomensis* EO147	HIV

Table 3.2. Antiviral lectins from Plants

LECTIN	SOURCE	ACTIVITY
Jacalin	*Artocarpus integrifolia*	HIV
BanLec	*Musa acuminate*	HIV , HCV , H1N1, & H3N2
GRFT	*Griffithsia sp*	HIV – 1, HIV – 2 ,HCV , HSV – 2 , SARs – CoV various avian CoV subtypes , BCoV , IBV , MHV, PCoV , HCoV
SCL	*Scilla campanulata*	HIV
NPL	*Narcissus pseudonarcissus*	HIV , & SIV HSV , Rabies , & Rubella
GNA	*Galanthus nivalis*	HIV – 1 , HIV – 2, SIV , & FIV
HHA	*Hippeastrum hybrid*	HIV – 1, HIV-2, SIV & FIV
PCL	*Polygonatum cyrtonema Hau*	HIV
ConA	*Canavalia ensiformis*	HIV & HSV

UDA	*Urtica dioica*	HIV , CMV , RSV , H1N1, & SARS – CoV
MHL	*Myrianthus holstii*	HIV – 1
NICTABA	*Nicotiana tabacum*	HIV – 1/2 , HSV , Influenza A / B & RSV

Table 3.3. Antiviral lectins from Animals

LECTIN	SOURCE	ACTIVITY
CVL	*Chaetopterus variopedatus*	HIV – 1
SVL – 1	*Serpula vermicularis*	HIV – 1
SVL – 2	*Serpula vermicularis*	HIV – 1
CGL	*Crenomytilus grayanus*	HIV – 1
DTL	*Didemnum ternatanum*	HIV – 1
DTL – A	*Didemnum ternatanum*	HIV – 1
GSL	*Savaglia savaglia*	HIV – 1

Sequence retrieval:

All nucleotide and amino acid sequences used in this study were retrieved from the NCBI database . Homologous sequences from other species were obtained with the BLAST (Basic Local Alignment Search Tool) search algorithm on the NCBI

Sequence analysis:

Physicochemical parameters were analyzed by the ProtParam (http://web.expasy.org/protparam) (Gasteiger et al., 2005). The identification of the functional domains of the protein, its classification and ontology were performed using the Prodom server (http://prodom.prabi.fr/prodom/) (Servant et al., 2002), which is a database of families of protein domains of homologous segments.

Domain prediction of *Allium cepa* lectin

The secondary structure of the deduced amino acid was predicted using PSI-PRED (http://bioinf.cs.ucl.ac.uk/psipred/) online server.

Homology modeling of Allium cepa

Homology model of Allium cepa lectin was constructed by comparative modeling in SWISS-MODEL (http://swissmodel.expasy.org). SWISS-MODEL is a fully automated protein structure homology modeling server, accessible via ExPASy web server. The model was then energy minimized and refined using modrefiner refinement tool (http://zhanglab.ccmb.med.umich.edu/ModRefiner/). The model was validated by

Verify 3D analysis from SAVES server (http://services.mbi.ucla.edu/SAVES/) which determines the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigning a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar, etc) and comparing the results to good structures (Liiethy et al., 1992). In addition, PROSA (Wiederstein, M & Sippl, M. J. 2007) which calculates an overall quality score for a specific input structure and PROCHECK (Laskowski, R.A et al., 1993) which checks the stereo chemical quality of a protein structure by analyzing residue-by-residue geometry and overall structure geometry by Ramachandran plot analysis were used.

Physiochemical properties of the protein sequence

Analysis of various features of gene and protein molecules helps to predict the type of structure and cellular localization of a given protein or an entire protein family is an indispensable complement to homology-based analysis. Protein sequence analysis classifies the protein into families, predicting domains and important sites. In addition, identification of certain structural features of proteins, such as signal peptides, transmembrane segments, or coiled-coil domains, may provide some functional clues. The physiochemical property of the *Allium cepa* lectin protein was analyzed by the ProtParam server. Table 5.1 shows the molecular weight, the theoretical isoelectric point (pI) and the Grand mean of hydropathicity (GRAVY) of the protein sequences evaluated.

Table 5.1. Physiochemical analysis of *Allium cepa* lectin

Allium cepa Protein sequence

```
        10         20         30         40         50         60
MARNVLVNNE GLYAGQSLVE EQYTFIMQDD CNLVLYEYST PIWASNTGVT GKNGCRAVMQ

        70         80         90        100        110        120
VDGNFVVYDV KGRAVWASNS RRGNGNYILV LQKDRNVVTY GSDIWSTGAY MKKEGGAVVM

       130        140
AMNGNVDGGS VIGPVTVNQN VTAAAA
```

Number of amino acids: 146

Molecular weight: 15712.69

Theoretical pI: **5.29**

Amino acid composition

CSV format

Amino acid composition:

Ala (A)	13	8.9%
Arg (R)	6	4.1%
Asn (N)	15	10.3%
Asp (D)	7	4.8%
Cys (C)	2	1.4%
Gln (Q)	6	4.1%
Glu (E)	5	3.4%
Gly (G)	17	11.6%
His (H)	0	0.0%
Ile (I)	5	3.4%
Leu (L)	7	4.8%
Lys (K)	5	3.4%
Met (M)	6	4.1%
Phe (F)	2	1.4%
Pro (P)	2	1.4%
Ser (S)	8	5.5%
Thr (T)	8	5.5%
Trp (W)	3	2.1%
Tyr (Y)	8	5.5%
Val (V)	21	14.4%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%
(B)	0	0.0%
(Z)	0	0.0%
(X)	0	0.0%

Total number of negatively charged residues (Asp + Glu): 12
Total number of positively charged residues (Arg + Lys): 11

Estimated half-life:

The N-terminal of the sequence considered is M (Met).

The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro).
>20 hours (yeast, in vivo).
>10 hours (Escherichia coli, in vivo).

Instability index:

The instability index (II) is computed to be 16.48
This classifies the protein as stable.

Aliphatic index: 82.67

Grand average of hydropathicity (gravy): -0.099

The relative volume of a protein occupied by its aliphatic side chains is termed as Aliphatic index (AI). Aliphatic index plays role in protein thermal stability. With a high Aliphatic

index, proteins are more thermally stable. Aliphatic amino acids also are hydrophobic in nature. The aliphatic index of the *Allium cepa* lectin was 82.67

Multiple Sequence Alignment and phyogenetic analysis of *Allium cepa* lectin

Multiple sequence alignment of different Allium lectins was performed using ClustalW of MEGA 6 tool. The phylogenetic neighbor- joining tree was constructed with 31 Allium lectins including the *Allium cepa* using MEGA 6 tool (Fig 5.1). The complement of the probability (1 - α) is computed in MEGA 6 and it is called as confidence probability (CP). The reliability of a branch length is high when the CP is high, thus the branch length is considered to be statistically significant. MEGA 6 inferred the evolutionary tree by a Neighbor-Joining (NJ) algorithm by using a matrix of pairwise distances. Bootstrap confidence levels are shown as percentages on nodes and confidence values are shown in branches. The constructed phylogenetic tree showed that isolated AAL belongs to the MMBL super family and sub clustered with other onion lectins.

Functional domain identification

Functional domains determine the active sites of a protein. Functional domain was identified using PRODOM server. Three functional domains were identified by the PRODOM server: PD330654, PDC8M6T0 and PD585253 in the lectin sequences analysed (Fig.5.2). The PD330654 functional domain is associated with mannose binding, actin monomer binding and pollen recognition functions, being an integral

component of the membrane which indicates its relevance for protein activity. No information was found on the activities of domain PD585253 in the server.

Conserved domain and Pfam analysis

CDD, the Conserved Domain Database, is part of NCBI's Entrez query and retrieval system. The protein sequence of lectin was analysed for the presence of conserved domain using the NCBI Conserved Domain Database Search. The results revealed the presence of mannose binding site and dimerization interface. Analysis using Prosite, Interpro scan revealed a possible mannose binding site and dimerization interface. (IPR001480) and (IPR036426) (Fig.5.3).

Secondary structure prediction and validation of lectin

Secondary structure of the sequence was predicted using PSIPRED online server.There are 146 amino acids with one α-helix , thirteen β-sheets and the rest of the regions are defined as loops (Fig.5.4).

Molecular modeling of *Allium cepa* lectin protein

BLASTp search with the lectin sequence against the PDB database showed 73% significant similarity with template d1kj1d for structure prediction (Table 5.2). Therefore, lectin model was generated based on homology method of protein prediction using Phyre server. The predicted 3-D model, binding pocket and the spatial arrangement

of the active site residues in protein conformation inferred from the 3-D model of *phaZ* are presented in Fig.5.5 and 5.6.

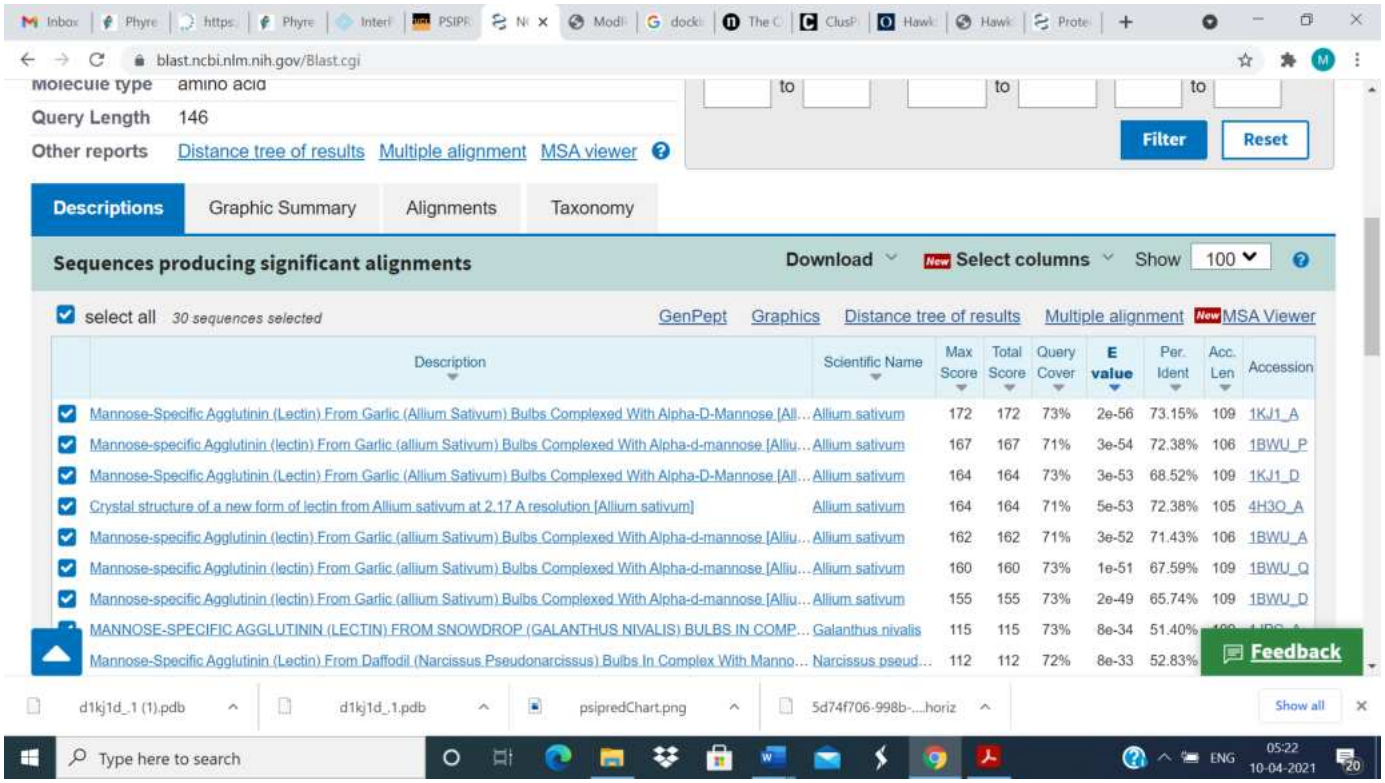

Table 5.2: BLASTp analysis of *Allium cepa* lectin

The crucial step in structure prediction is evaluation of protein structure quality. The quality assessment of *phaZ* protein structure was done using Structure Analysis and Verification server (SAVEs).This server integrates quality evaluation, PDB structure validation tools based on the goodness of query structure depicted as a PROCHECK, ProSA (Z-scores), Verify 3D profile and ERRAT.

Energy minimization was performed using ModRefiner tool for the predicted structure of lectin and then the resulting energy minimised PDB structure was used as input for construction of the Ramachandran plot. The Ramachandran plot presented the

empirical distribution of data points visualising the structural validation. Ramachandran plot obtained from PROCHECK analysis revealed that *phaZ* model is stereochemically good.

Ramachandran plot for *phaZ* revealed that the phi-psi torsion angles for 90.7% of residues of *phaZ* are in the most favourable region (A, B, and L), 8.3% in additionally allowed region (a, b, l, p), 0.5% in generously allowed regions (~a, ~b,~l,~p) and less than 1 % of amino acid in the disallowed regions.The backbone conformation of amino acid residues were scattered in allowed regions thus proving the reliability of the predicted structures. The Ramachandran plot obtained for modelled structure of *phaZ* was shown in Fig.5.7.

The quality of the predicted model was validated using ProSA (Z scores). The ProSA Z-score represents the measure of model quality by estimating the degree of nativeness observed in a model and describes the likelihood of the model in comparable quality to experimentally determined structures.It predicts Z-score and residue energy plot of the protein structure. Z-score locates the position of the modelled structure in context of all the known protein structures (X-ray analysis and NMR spectroscopy) available in the PDB (represented as black dot in. Z-score for *phaZ* was found to be -7.69 which is largely negative and positioned in the range of protein structures available in PDB indicating that the quality of model is good (Fig. 5.8, 5.9).

6. Discussion

The sequence determines the structure and the structure determines the function of proteins. The curren works focuses on sequence analysis, structure determination and functional characterization of *Allium cepa.* The full length cDNA of *Allium cepa* lectin gene was amplified and submitted to NCBI Gen Bank (Gen Bank ID KM096569, 481 bp, mRNA *Allium cepa* mannose-binding lectin (MBL) mRNA, partial cds. (Kasthuri et al. 2014). Allium cepa gene sequence was retrieved and insilico analysis was performed to study the phyologenetic relationship, physiochemical properties, conserved domains and molecular model. Insilico physiochemical analysis was done and the isoelectric point (pI) was predicted to be 5.29. The isoelectric point (pI) is important for solubility, subcellular localization and interaction of a protein. At pHs different from the isoelectric point, the protein solubility increases due to the appearance of positive or negative charges on the protein chains, which favors the charge-moment interaction of the water dipole. At a time when a protein solution is in its isoelectric state, that is, when the protein manifests zero net charge in an aqueous system, protein-protein interactions increase, so less water interacts with protein molecules, which that their molecules approach, aggregate, and precipitate. The knowledge of the pI of a protein is of great importance, since, based on it, one can predict the net charge of the protein at a certain pH, which will be very important for the practice of experimental studiesas in the process of protein purification and maintenance of the same in a solution that favors its maintenance in the soluble form.

The isoelectric point of the sequences was 5.29, which is different between 4.65 (*A. ramosum*) and 8.96 (*A. altaicum*), showing that this lectin exhibits great variation of chemical properties between species analyzed. The mean hydrophobicity indicates solubility, with positive values for hydrophobic and negative for hydrophilic proteins. Sequences ranged in size from 138 (*A. ursinum*) to 159 (*A. pskemense*) amino acids. The mean hydrophobicity for the sequence analysed was -0.099. The mean hydrophobicity ranged from -0.295 (*A. tuberosum*) to -0.028 (*A. altaicum*), indicating that this protein is hydrophilic in character for all species as reported earlier (Jimenez *et al.,* 2019). The molecular weight varied from 14.83 kDa (*A. ursinum*) to 16.82kDa (*A. pskemense*). Molecular weight is employed in chromatography and electrophoresis, for example, to isolate proteins. However, it is not possible to generalize about it in relation to protein functions. The instability index value for the lectin protein was 16.48 indicating that the protein as a stable protein.

The active domain prediction suggested the presence of mannose binding domain. The secondary structure prediction of AAL revealed that the protein possess predominantly β-sheets and possess 3 mannose binding motifs of repeat sequences (QXDXNXVXY) which plays a major role in Alpha D-Mannose recognition. The presence of mannose binding domains relates to the antiviral activity of the protein

6. Conclusion and Suggestions

Conslusion

Insilico analysis of Allium cepa lectin gene was done. The phyologenetic analysis predicts the protein to be a mannose binding lectin closely related to other lectins isolated from the *Allium* species.

Suggestion for future:

In future the *Allium cepa* lectin

Alliun cepa lectin can be docked with the surface proteins of viruses and find its efficacy for antiviral activity.

The *Allium cepa* lectin can be overexpressed in bacterial system and its invitro and invivo antiviral activity can be assessed.

7. Summary

To summarize the work, the following bioinformatic analyses were done.

- Sequence retrieval of *Allium cepa* lectin from NCBI database
- Multiple sequence alignment and phylogenetic tree construction of the lectin gene
- Insilico physiochemical analysis of the *Allium cepa* lectin.
- Secondary structure prediction of the *Allium cepa* lectin protein.
- Functional domain prediction.
- Molecular modeling of the protein.

8. References

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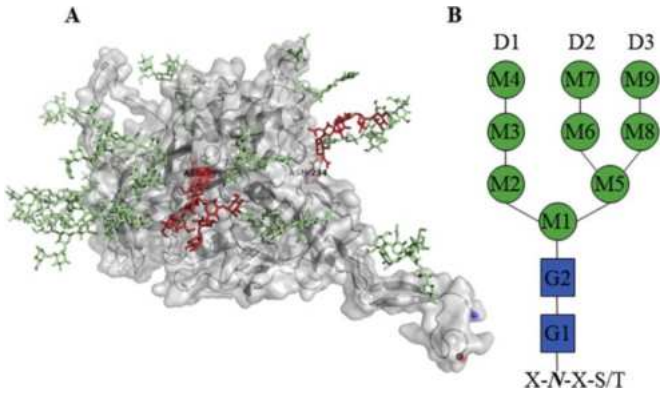

Fig. 3.1. A) The crystal structure of glycosylated gp120 monomer from HIV-1 B) Schematic of high-mannose (Adopted from Mitchell et al. 2017)

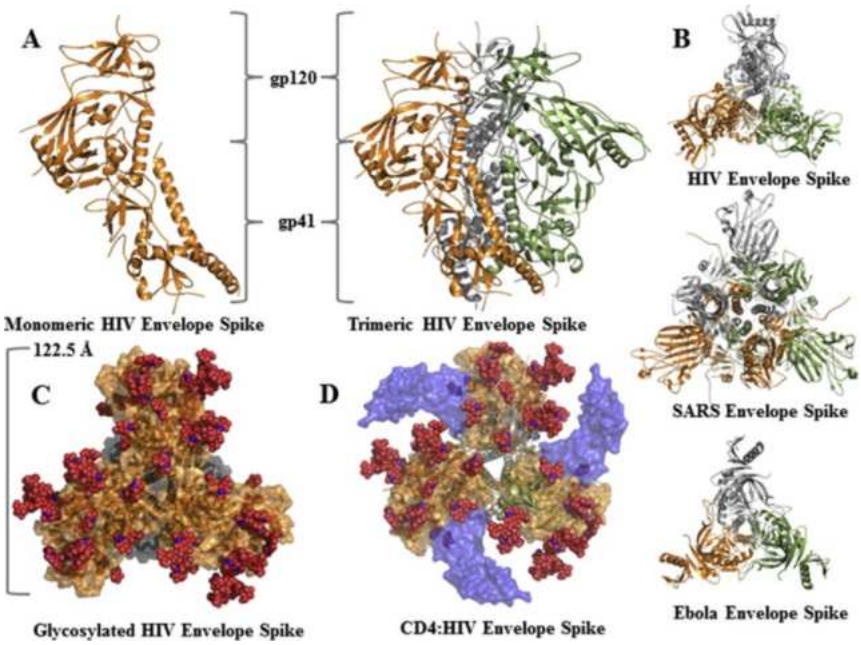

Fig 3.2. Viral Envelope Protein Complexes (Adopted from Mitchell et al. 2017)

Fig 5.1: Phylogenetic neighbor-joining tree of 31 Allium Lectin gene sequences showing the relationship between AAL and other Allium lectins. The isolated AAL gene is highlighted in brown box. Bootstrap confidence levels are shown as percentages on nodes and confidence values are shown in branches**.**

Fig.5.2. Functional domain analysis using PRODORM server

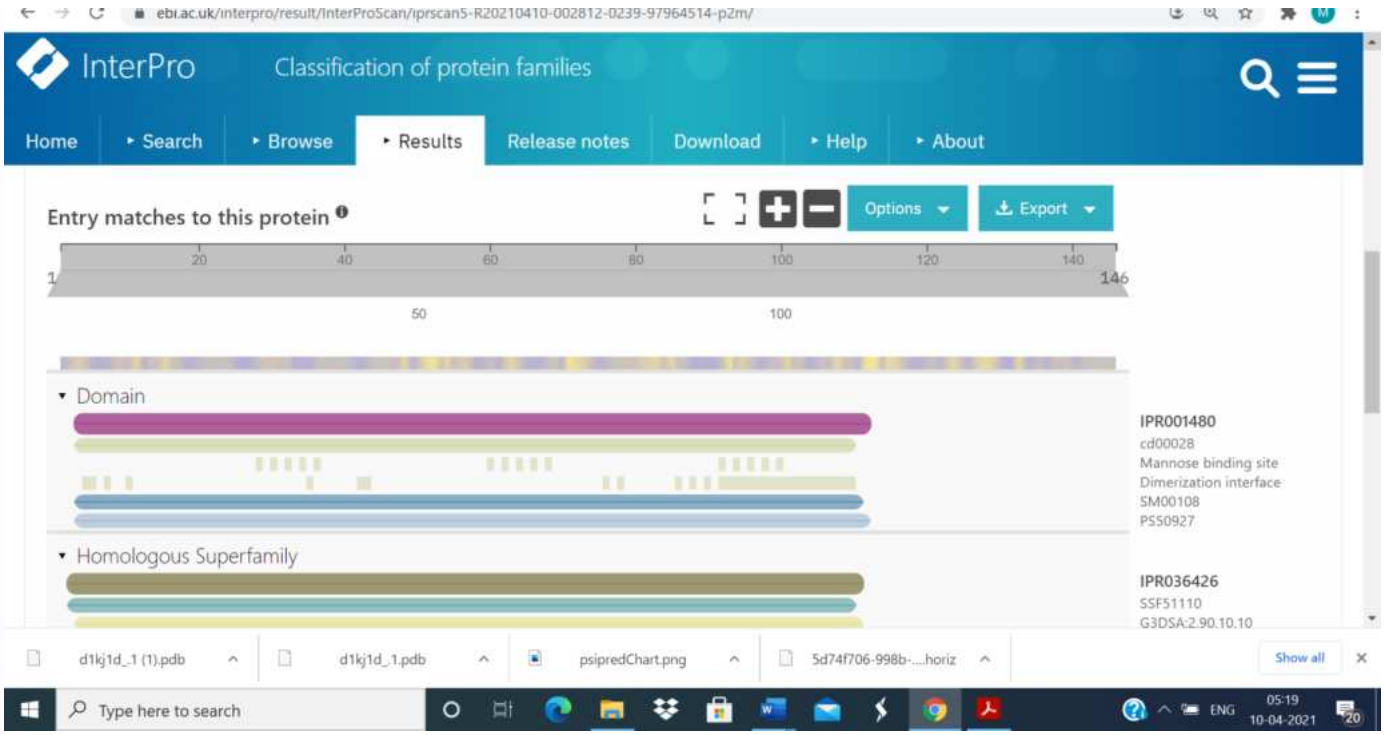

Fig. 5.3. Domain prediction using InterPro

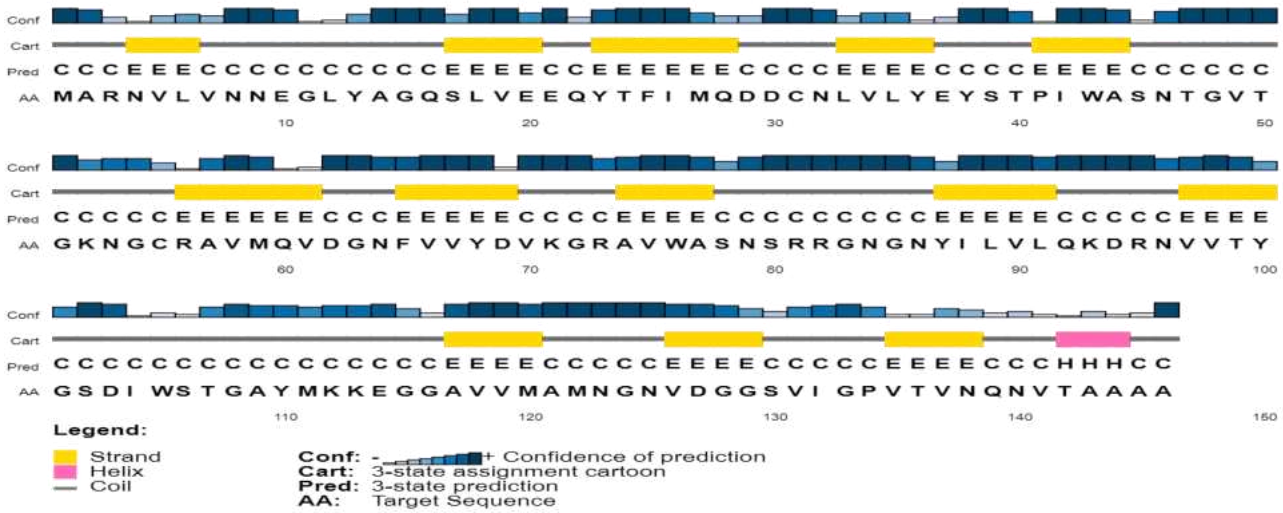

Fig. 5.4. Secondary structure prediction using PSIPRED

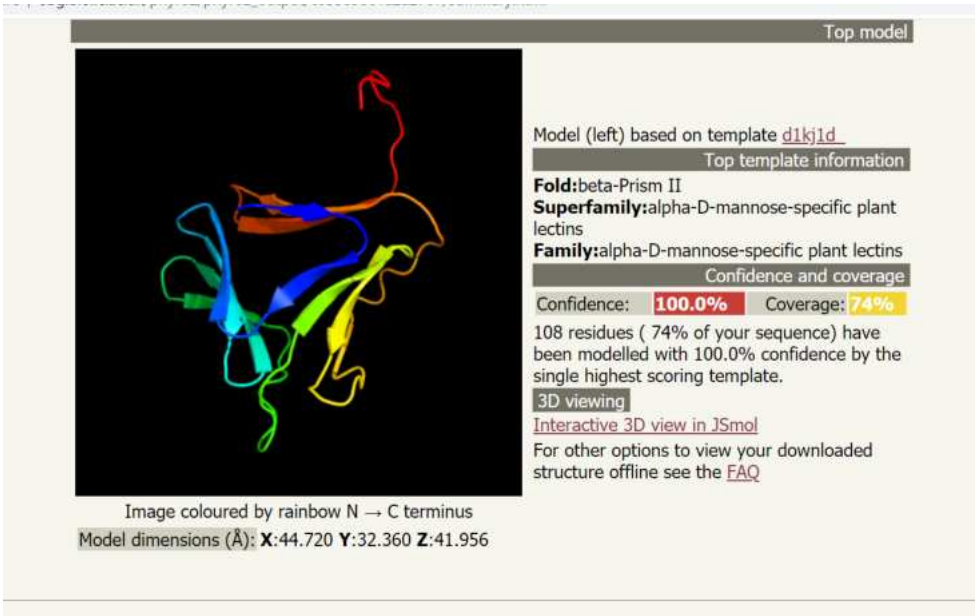

Fig. 5.5. 3D structure of *Allium cepa* lectin model with mannose binding sites predicted using SWISS-MODEL using the template of PDB ID: d1kj1d

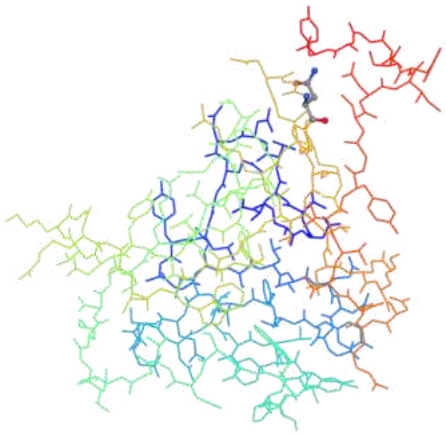

Fig. 5.6. 3D structure of *Allium cepa* lectin model with mannose binding sites predicted using SWISS-MODEL using the template of PDB ID: d1kj1d

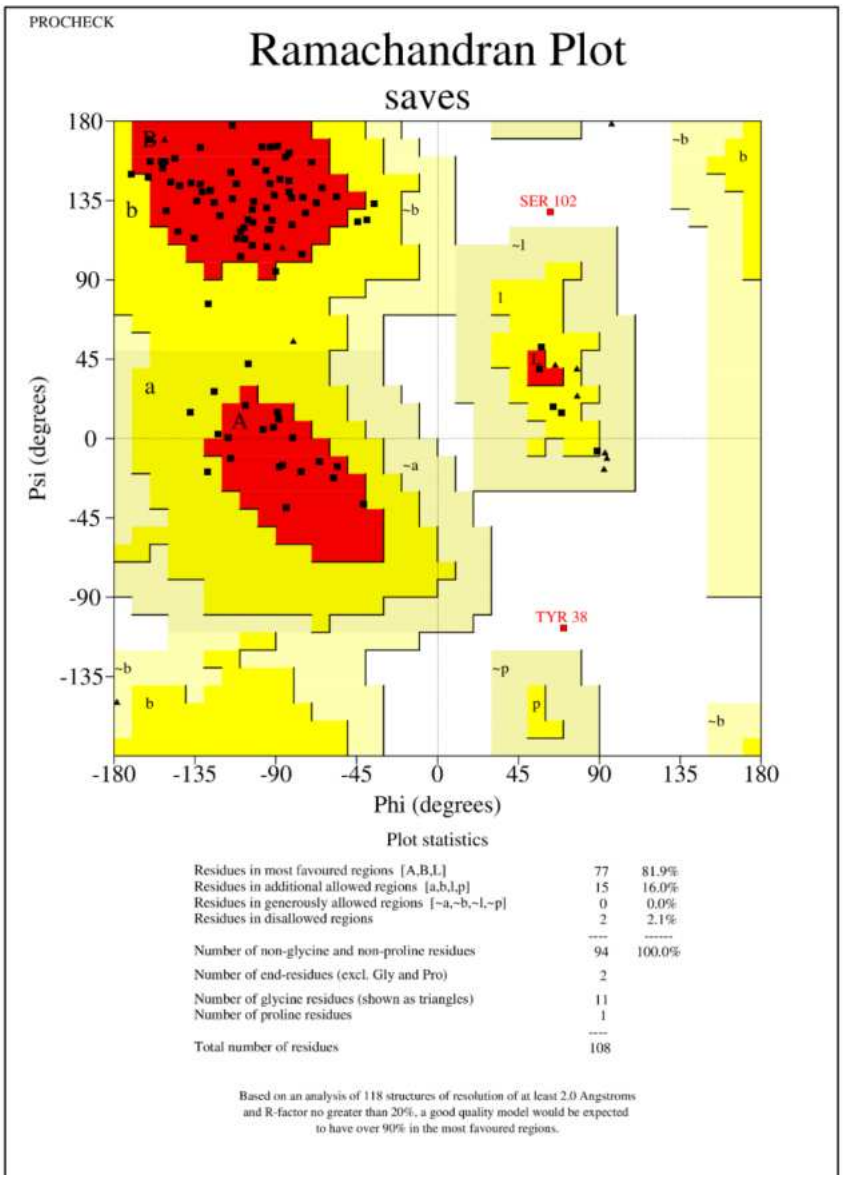

Fig.5.7. Ramachandran plot analysis of AAL model analyzed using PROCHECK. The most favorable conformation of Φ – Ψ values are colored red, additional allowed,

generously allowed and disallowed regions are indicated as yellow, light yellow and white fields, respectively.

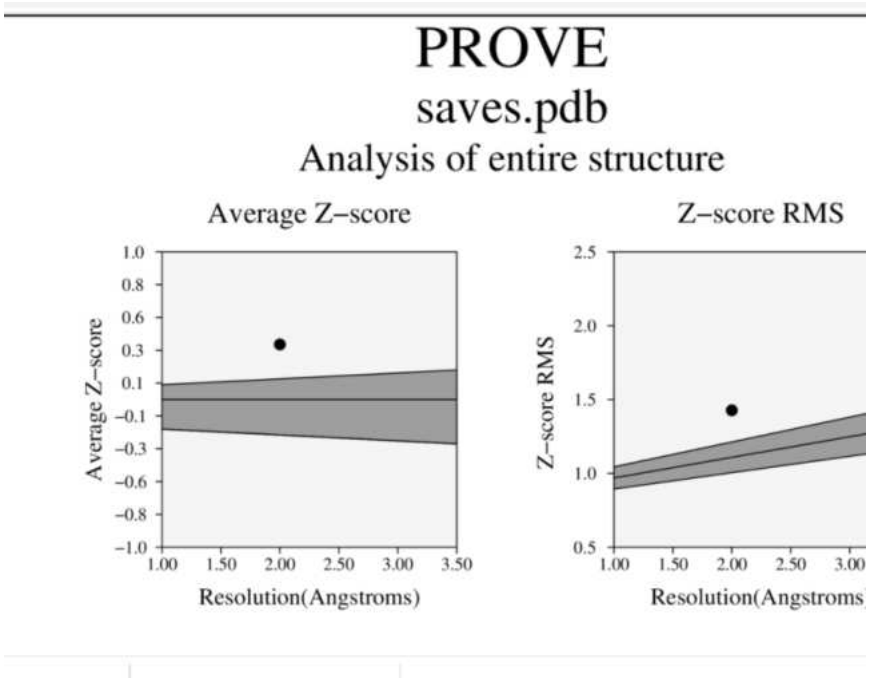

Fig. 5.8 Structure analysis

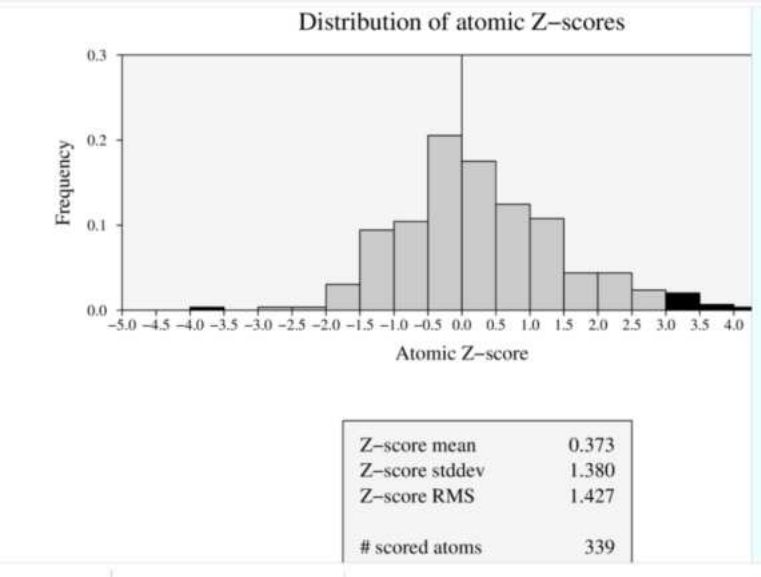

Fig. 5.9 Z-score analysis