ASSESSMENT OF WATER QUALITY PARAMETERS ALONG THE SELECTED COASTAL AREAS OF THOOTHUKUDI

A Short – term project submitted to
ST.MARY'S COLLEGE (AUTONOMOUS)

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in partial fulfilment of the requirements for the degree of
BACHELOR OF SCIENCE IN BOTANY

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CERTIFICATE

It is certified that this short term project work entitled "**Assessment of water quality parameters along the selected coastal areas of Thoothukudi**" submitted to **St. Mary's College (Autonomous)** affiliated to **Manonmaniam Sundaranar University** in partial fulfilment of the requirements for the degree of Bachelor of Science in Botany, and is a record of work done in the Department of Botany, St. Mary's College (Autonomous), Thoothukudi during the year 2019-2020 by the following students.

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Abbreviation

mg/L –milligram per litre

C - Celsius

DO –Dissolved oxygen

BOD –Biochemical Oxygen Demand

COD – Chemical Oxygen Demand

TTPS - Thoothukudi Thermal Power Station

INTRODUCTION

The ocean covers approximately 70% of the earth's total surface area. In the total water content of the earth, 97% is present in the oceans. Oceans are rich source of biodiversity in which population may exceed in trillions (Meiaraj and Jeyapriya. 2019).. Oceans are the main regulatory agent of the earth's climate. Coastal region plays a key role in the country's economy due to it availability of resources, productive habits and wealthy biodiversity. Water is an indispensible component of human resource.

India has about 7,500 kms of coastline. The Tamil Nadu coastline is about 10% of the length of the total coastal length of the Arabian Sea, Bengal Bay and the Indian Ocean (Kumar *et al.*, 2006). Seawater covers approximately 71 per cent of the earth's surface, an area of about 361 million square kilometres (139 million square miles) comprising the major ocean areas. In the deepest parts the bottom lies more than 10, 000 m from the surface, and the average depth is about 3700 m. Although marine organisms are unevenly distributed, they occur throughout this vast extent of water and have been brought up from the deepest places. Close to land the sea is mostly shallow, the bottom shelving gradually from the shore to a depth of about 200 m. This coastal ledge of shallow sea bottom is the continental shelf. About 8 per cent of the total sea area lies above it. Its seaward margin is termed the continental edge, beyond which the water becomes much deeper. The steeper gradient beyond the continental edge is termed the continental slope. Seawater is evidently an excellent medium for an abundance and variety of life (Tait and Dipper, 1998).

Seawater is an extremely complex solution, its composition being determined by an equilibrium between rates of addition and loss of solutes, evaporation and the addition of fresh water. The original source of seawater is uncertain, but was probably by

condensation of water vapour and solutes released into the atmosphere from hot rocks and volcanic action at an early stage of the Earth's history. Probably all natural elements are present in solution in the sea, and all the constituents needed for the formation of protoplasm are present in forms and concentrations suitable for direct utilization by plants. The transparency of the water and its high content of bicarbonates and other forms of carbon dioxide provide an environment in the upper layers of the sea in which plants can form organic materials by photosynthesis (Tait and Dipper, 1998).

It is uncertain to what extent the composition of seawater may have changed during geological time, but it is not thought to have varied very widely over the period that life has existed. At present, the principal cations are sodium, magnesium, calcium, potassium and strontium and the chief anions are chloride, sulphate, bromide and bicarbonate. These make up over 99.9 per cent of the dissolved material, forming approximately a 3.5 per cent solution. The amount of inorganic material dissolved in seawater expressed as weight in grams per kilogram of seawater is termed the salinity and usually amounts to about 35 g/kg, i.e. S = 35 parts per thousand (generally written 35‰). The salinity of most ocean water is within the range 34–36‰. High salinities are associated with low rainfall and rapid evaporation, especially where the circulation of the water is relatively poor (Tait and Dipper, 1998).

The major constituents of seawater, and some of the minor constituents, remain virtually constant in proportion (conservative constituents), certain minor constituents fluctuate in amount due to selective absorption by organisms (non-conservative constituents). The latter include nitrate, phosphate, silicate, iron and manganese. Nitrogen in combined form is present in seawater as nitrate, nitrite, ammonium ions and traces of nitrogen-containing organic compounds. Nitrate ions predominate, but in the uppermost 100 m and also close to the bottom there are sometimes appreciable amounts

of ammonium and nitrite formed by biological activity. Phosphorus is present almost entirely as orthophosphate ions H_2PO_4 and HPO_4^{2-} with traces of organic phosphorus. The viscosity of seawater decreases considerably with rise of temperature and increases slightly with increase of salinity. Viscosity influences both sinking and swimming speeds, and in many organisms must also have effects on feeding rates and respiration (Tait and Dipper, 1998).

The rate of photosynthesis increases with rising temperature up to a maximum, but then diminishes sharply with further rise of temperature. Different species are suited to different ranges of temperature and photosynthesis is probably performed as efficiently in cold water by the phytoplankton of high latitudes as it is in warmer water by the phytoplankton native to the tropics. Seasonal variations of production rate in temperate latitudes are related to changes of both temperature and illumination. Apart from its direct effect on rate of photosynthesis, temperature also influences production indirectly through its effects on movement and mixing of the water and hence on the supply of nutrients to the euphotic levels (Tait and Dipper, 1998). Living processes involve energy exchanges. Energy for life is drawn primarily from solar radiation, transformed into the chemical energy of organic compounds by the photosynthetic processes of plants; thence transferred through the ecosystem by movements of materials within and between organisms, mainly through the agencies of feeding, growth, reproduction and decomposition. An ecosystem is therefore essentially a working, changing and evolving sequence of operations, powered by solar energy (Tait and Dipper, 1998).

The word 'plankton' is taken from a Greek verb meaning to wander and is used to refer to those pelagic forms which are carried about by the movements of the water rather than by their own ability to swim. These organisms are called planktonts. The

plants of the plankton are the phytoplankton, the animals the zooplankton (Tait and Dipper, 1998). Marine phytoplankton dominate primary production across 70% of earth's surface, play a pivotal role in channeling energy and matter up the food chain, and control ocean carbon sequestration. The diversity of phytoplankton species in open waters has intrigued ecologists for at least half a century, but the global pattern of this diversity and its underlying drivers have been unclear. This is a critical gap in our understanding of the oceans since the richness of phytoplankton species, a key element of their diversity, may enhance resource use efficiency, and thus primary production, as often seen in terrestrial systems (Righetti *et al.*, 2019).

Phytoplankton is an essential component in marine life and plays fundamental role in the biodiversity, bio-productivity and biogeochemical cycle of marine ecosystem. Besides being a primary producer, phytoplankton serves the energy needs of planktivorous organisms and is helpful in predicting the fishery potential of the region (Falkowski *et al.*, 1998). Distribution of phytoplankton species shows spatiotemporal variations due to hydrographical factors and serves as indicators of water quality of the environment (Liu *et al.*, 2004). The study of phytoplankton community structure in response to environmental variables is considered very useful for evaluating ecosystem changes of both the long-term and short-term scales (Biswas *et al.*, 2010).

The phytoplankton is responsible for most of the primary production in the sea. There would be virtually no life in the ocean without the photosynthesis carried out by these microscopic plants. On land the energy-fixing plants dominate the landscape in the form of grasses, shrubs and trees. In contrast the phytoplankton is only visible to us as a cloudiness or discolouration of the water when reproduction is rapid and a 'bloom' occurs. Some planktonts can only float passively, unable to swim at all. Others are quite active swimmers but are so small that swimming does not move them far compared to

the distance they are carried by the water. The swimming movements serve chiefly to keep them afloat, alter their level, obtain food, avoid capture, find a mate or set up water currents for respiration (Tait and Dipper, 1998).

The word 'pollution' is now widely used as a convenience term for virtually any substance released into the environment by human activities which has a deleterious effect on marine organisms and ecosystems or is a nuisance to mankind. Effects of these materials include smothering and poisoning of organisms, interference with physiology and behaviour, and increase or decrease in biological productivity with consequent effects on organisms in other parts of the food web (Tait and Dipper, 1998). Pollution is the introduction by man, directly or indirectly, of substances or energy into the marine environment resulting in deleterious effects of such nature as to endanger human health, harm to living resources ecosystems and hinder marine activities quality of seawater. The marine environment mainly contaminated by waste disposal. The wastes of society can be placed on land or in the water. It also penetrates directly to the marine environments (Padmanaban and Kumar, 2012).

Environmental pollution of the coast, inshore water and deep ocean is one of the important topical issues in the context of human health and global warming. Coastal environment plays a main role in nation's wealth by virtue of the resources, productive habitats and rich biodiversity. The contamination of seawater, including trace metal concentration affects marine organisms and then people consuming them causing some carcinogenic and non-carcinogenic impacts in their body (Meiaraj and Jeyapriya. 2019). Pollution can be measured in terms of water quality or the effects of pollutants on marine biota. Water quality is the general descriptor of water properties such as its physical, chemical and biological characteristics. Water quality can be affected by the release of effluents from either point or non-point sources. Monitoring and assessing the quality of

surface waters are critical for managing and improving its quality (Malarvannan and Balamurugan, 2018).

In recent years there has been much concern over the extent to which the oceans may be adversely affected by their use as a dumping ground for an ever-increasing quantity and variety of human and industrial wastes. The oceans are so great in volume that overall accumulation of persistent pollutants can only occur very slowly. Organisms have a great capacity to respond to gradual environmental changes by adaptation, acclimatization and evolution. Except where the pollution load is heavy, its effects on marine life are likely mainly to influence the fringes of a species' distribution, where the population is already under environmental stress. In such areas any additional burden may increase mortality (Tait and Dipper, 1998).

Despite some scaremongering of impending world catastrophe through oceanic pollution causing the widespread demise of marine life, a reasonable view of the present situation seems to be that these dangers are remote compared with far more imminent threats to human survival arising from our inability to live peaceably together. The oceans do have a capacity to absorb wastes and in some cases may be a much safer place for disposal of certain wastes than storage on land, with the attendant hazards to human populations of contamination of food or freshwater supplies (Tait and Dipper, 1998).

The coastal areas of Tuticorin are assuming greater importance owing to increasing human population, urbanization and accelerated industrial activities. These anthropogenic activities have put tremendous pressure on the fragile coastal environments. In general, the near shore regions are of great concern now. Coastal pollution in Tuticorin has seriously affected the exploitable living resources, recreational and commercial uses of coastal areas and the overall integrity of the marine and coastal ecosystems. Hence protection of the coastal and marine regions from continuing

pollution becomes the most essential in coastal resources management. Effective planning for controlling and combating coastal pollution requires knowledge about the magnitude of the pollution, the entry, transport and the state of pollutants in the marine environment and their effects on marine ecosystems (Padmanaban and Kumar, 2012).

It was thought that human being, living only on one-third of the portion of globe, cannot pollute this vast amount of water, as the marine ecosystems are capable of serving as sink for all the pollution caused by us. However, in reality this is not true. We have come to realize that our waste, even in small quantities, has huge effect on ocean communities and species. Future generations will judge this period to have been an Age of Waste. The earth's resources of energy and useful materials are being rapidly consumed and dispersed, often for trivial or destructive purposes, with remarkably little forethought.

REVIEW OF LITERATURE

The territorial waters of the coastal one highly productive ecosystems in marine waters. However behind its potential, development usually also mainly concentrated in coastal areas, so that often creates a negative impact on the potential of these resources, human activity in exploiting natural resources on it, such as industry, agriculture, fisheries, mining, navigation, and tourism are often multiple overlap, so it is not uncommon benefit or value to the ecosystem will be down. These activities often produce waste pollutants harmful to the marine waters life of or destroy fishes habitat in coastal areas (Spanton and Saputra, 2017).

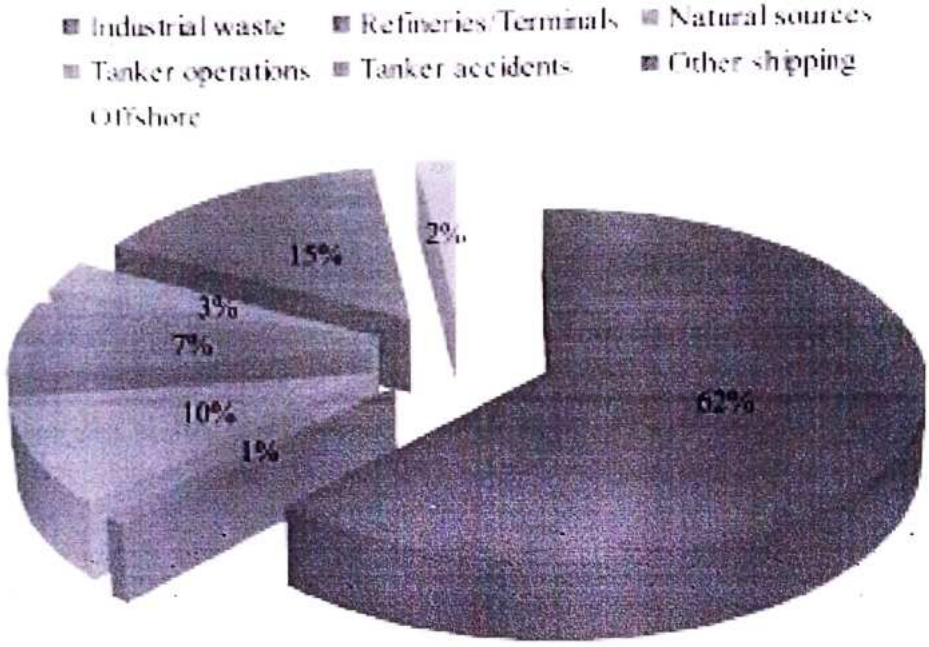


Fig. 1 — Statistics on various sources of marine oil release

Marine water bodies of Indian coast are studied by Baskar *et al.* (2013. The rainfall and fresh water in flow from land in turn moderately reduced the salinity, Ganesh *et al.* (2018), Ganesh *et al.* (2017). Seasonal and the spatial variations of physic-chemical parameters was correlated with population of living organisms by Nisha *et al.* (2018).

Studies pertaining to source, distribution and utilization of inorganic compounds were reported by Anjali *et al.* (2018).

Several workers all over the world have investigated physico-chemical parameters of various marine water ecosystems. Seasonal variation of physico-chemical parameters were studied at four different stations in Pondicherry mangroves, southeast coast of India by Kumar and Khan (2009). Prasanna *et al.* (2010) studied the temporal and spatial distribution of salinity, DO, BOD, turbidity, pH, hardness and dissolved nutrients of surface water collected from different points of Dhamra estuary during February-2007 to January-2008. Sekar *et al.* (2009) carried out analysis of seawater samples collected from four different sampling points in Thoothukudi coastal for the study of physicochemical characteristics. The physical and chemical parameters such as temperature, pH, salinity, nitrite, ammonia, silicates, dissolved oxygen and inorganic phosphate were studied using various analytical techniques. The studies revealed that the physical and chemical composition of all the samples collected from the sites mainly depends on seasonal variations and discharge from domestic and industries.

Marine phytoplankton communities which contribute to primary productivity are largely dependent on nutrient availability, light penetration and mixing within the water column in coastal areas, whereas in the open sea they are highly dependent on the depth of the mixed layer and light penetration. However, nutrient availability is frequently referred to as a key factor regulating phytoplankton biomass (Roelke *et al.,* 1999). Inorganic substances such as nitrogen, phosphorous and silicon are considered to be more important than other nutrients, because they play a key role in phytoplankton abundance, growth and metabolism (Grant and Gross, 1966). Seasonal pattern include changes in phytoplankton diversity, composition, biovolume and importantly, the

magnitude of primary production (Sawant and Madhupratap, 1996), but the photosynthetic response to limiting factors might be regulated by changes in species composition and diversity (Duarte *et al.*, 2006). Substantial work has been carried out relating to species composition and seasonal variation of phytoplankton in different coastal ecosystem of India (Menon *et al.*, 2000; Sahu *et al.*, 2012; Sivasankar and Padmavathi, 2012).

Plankton is one of the important components of any aquatic ecosystem. This is obvious from abundant occurrence of planktonivorous animals in the marine ecosystems. Among plankton, phytoplanktons are the primary source of food in the marine pelagic environment, initiating the food-chain which may culminate even in large mammals (Waniek and Holliday, 2006). More than 95% of the primary production in the oceanic waters is contributed by only phytoplankon (Lewis, 1974). However, the shallow neritic zones of the coastal areas are comparably more productive due to the combined production of unicellular algae, macro-algae, symbiotic algae of coral reefs and the seagrasses. Among all, the drifting micro-algal (phytoplankton) population plays a major in determining the productivity of the coastal and marine environment.

Phytoplankton species composition, richness, population density, and primary productivity will vary from coast to coast and sea to sea depending upon the varying hydro biological features. It is worth mentioning that Reynolds (1993) has stated that changes in species composition and dominance of phytoplankton can be mediated by a variety of mechanisms including ambient temperature, light penetration, nutrient supply, and removal by zooplankton etc. However, such information on phytoplankton of the Palk Bay is very much limited. Krishnamoorthy and Subramanian (1999) reported that the west coast current and conglomeration of open ocean influenced the highest species

diversity of meroplankton in the Palk Bay and Gulf of Mannar. Sridhar *et al.* (2006) reported the seasonal behavior of distribution of phytoplankton in the Palk Bay region.

Seawater samples were collected by Malarvannan and Balamurugan (2018) from three different sampling points in Tuticorin coastal to study physico-chemical characteristics and parameters such as temperature, pH, salinity, nitrite, ammonia, silicates, dissolved oxygen and inorganic phosphate were studied using various analytical techniques. The studies reveal that the physical and chemical composition of all the samples collected from the sites mainly depends on discharge from the sources of pollutants.

The position of hydrographical parameters of estuarine and marine coastal waters within 1 km of Thoothukudi, Tamil Nadu, India. This study would be supportive in the ecological monitoring of the ecosystem (Nayagam *et al.*, 2019). The physicochemical qualities of sea water are very important for the health biodiversity in total Gulf of Mannar. The results pronounced variation in most of the water quality parameters with geographical location (Muthuraman *et al.*, 2019). Phytoplankton community structure and its relationship with selected physicochemical variables in the inshore waters of Tuticorin were analyzed (Asha *et al.*, 2018).

Studies in Kadalur, coastal village of Tamil Nadu, located in the neighborhood of the estuarine region Palar river to assess the quantitative distribution of Zoo plankton from the three stations 1 (Palar river), station 2 (Palar esturary) and station 3 (Kadalur sea coast) (Prabhahar *et al.*, 2011). The hydrographic variables and composition of phytoplankton communities in the Arabian Sea waters off Kasaragod, Kerala, Southern India was studied by Rai and Rajashekhar (2014). The analysis of physico-chemical and plankton reveals that there is a pronounced variation in most of the water quality parameters with variation in season and geographical location (Salvi *et al.*, 2014).

Study was conducted by Pitchaikani and Lipton (2016) to understand the role of available inorganic nutrients in controlling the abundance and structure of phytoplankton populations in traditional fishing grounds of Tiruchendur coastal waters. Trejos *et al.* (2014) investigated how the structure and adaptive capacity of phytoplankton communities will respond to changing environmental conditions. Jeyageetha and Kumar (2015) studied the of physico-chemical parameters of sea water in Tuticorin coastal area and assessing their quality.

Objectives of present study

- Estimate the baseline characteristics of the marine water in Thondhakadi coast along the salt pans located nearby Thermal power plant
- Analyse the chemical and physical characteristics of marine water of the selected coastal sites
- To be supportive in the ecological monitoring of the ecosystem

MATERIALS AND METHODS

Study area

Thoothukudi Thermal Power Station (TTPS) plant is located along the Thoothukudi coast (Lat. 08° 46! 20" N; Long. 78° 10! 46" E). Environmental Impact Assessment study was carried out by collecting water samples from six stations fixed around 300 km point of power plant during December (2019) (Figure 1 and 2).

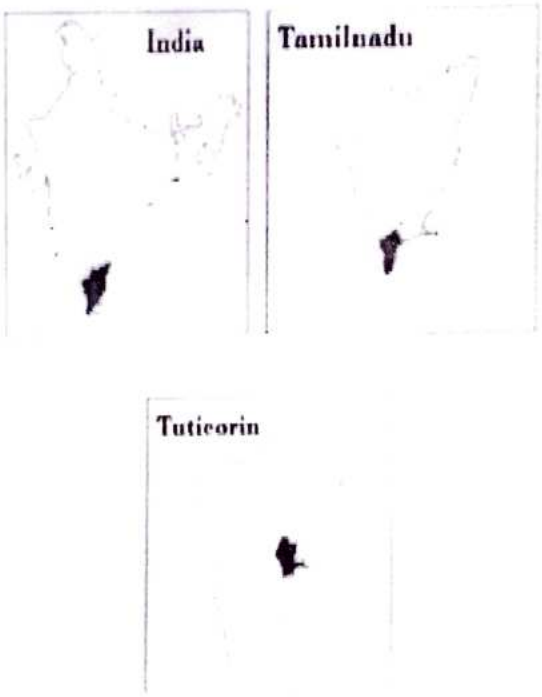


Figure 1: Location map of Thoothukudi district

Method for sample collection

The sample collection was done as per standard method (APHA, 1995). The sample container was cleaned by 1.0 mol/L of nitric acid and left it for 2 days followed by thorough rinsing of distilled water. The samples were collected in clean polythene bottles without any air bubbles. The bottles were rinsed before sampling and tightly sealed after collection and labeled in the field.

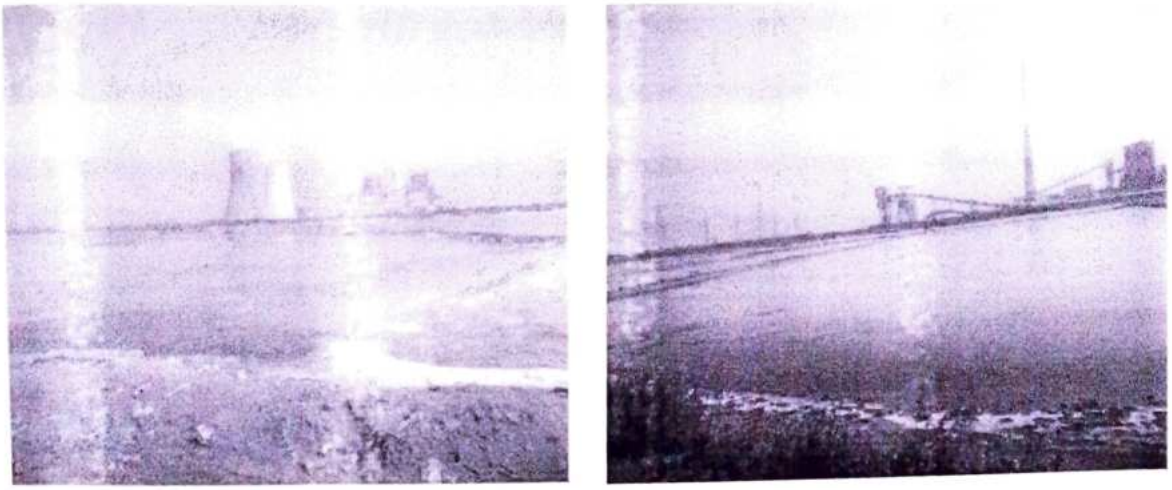


Figure 2. Effluent discharge from Thermal power plant

Collection of the water samples and analysis

During fieldwork, water samples were collected from six stations fixed from the entry point of salt pan to infer the different physical and chemical parameters, to understand the nature and extent of pollution using "standard methods for the examination of water and waste water" APHA (1995), Surface water temperature was measured using thermometer and pH was measured using Elico pH meter respectively.

Parameters like pH, temperature, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, acidity, alkalinity, total hardness, salinity, nitrite, phosphate, copper, lead, zinc and cadmium. are recorded after collecting the samples. Dissolved oxygen, is estimated by Winkler's method. The water samples were analyzed for the parameters such as dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, acidity, alkalinity, total hardness and salinity using titrimetric methods. Nitrate and phosphate were analyzed by spectrophotometric methods. The trace elements were estimated under GBC702 atomic absorption spectrophotometry (APHA, 1995).

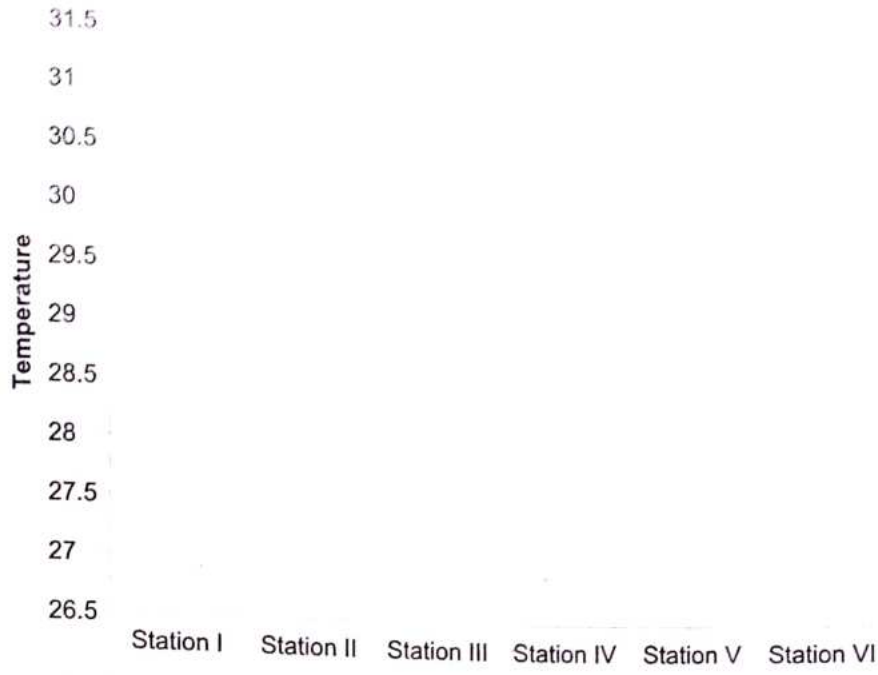
RESULTS AND DISCUSSION

The data may allow us to understand the effects of biological variations in the physico-chemical and organic situations in the coastal seawaters, Thoothukudi region of Tamil Nadu. The discrepancies of the climate and neighboring areas like Thermal power plant and other activities that produce the contaminants. Hence, the current study has analyzed quality of water changes through deliberate data collections related to the seasonal hydrographical environment of Thoothukudi region of Tamil Nadu. Physical-chemical parameters are measured one of the most significant characteristics that have the ability to impact marine ecosystem and show wider progressive and spatial variations. The physicochemical parameters of the water samples were recorded and are tabulated in Table 1.

Table 1 Surface water quality for the selected sampling sites of Thoothukudi harbour area

S. No	Parameters	Station I	Station II	Station III	Station IV	Station V	Station VI
1.	Temperature	29	28	31	31	30	30
2.	pH	8.24	7.62	8.60	8	7.78	7.73
3.	DO (mg/L)	3.5	4.6	3.1	2.3	2.5	3.3
4.	BOD (mg/L)	1.7	0.3	2.5	2.8	3.2	2.2
5.	COD (mg/L)	10	7.2	10.6	8	9.3	4.8
6.	Acidity (mg/L)	16	1.04	2.16	2.28	1.9	0.84
7.	Alkalinity (mg/L)	183	213	349	454	434	166
8.	Total hardness (mg/L)	316	316	78	78	342	342
9.	Salinity (mg/L)	0.84	0.84	344	3.44	5.28	0.02
10.	Nitrite (mg/L)	168	181	162	160	123	144
11.	Phosphate (mg/L)	105	123	107	110	130	125
12.	Coppper (mg/L)	6.325	4.833	48	0	0	483
13.	Lead (mg/L)	14.24	0	14.3	14.3	14.3	0
14.	Zinc (mg/L)	0	0	3	3	3	3
15.	Cadmiun (mg/L)	0	0	3.8	0	3.8	0

Figure 1. Temperature at various stations in the study area

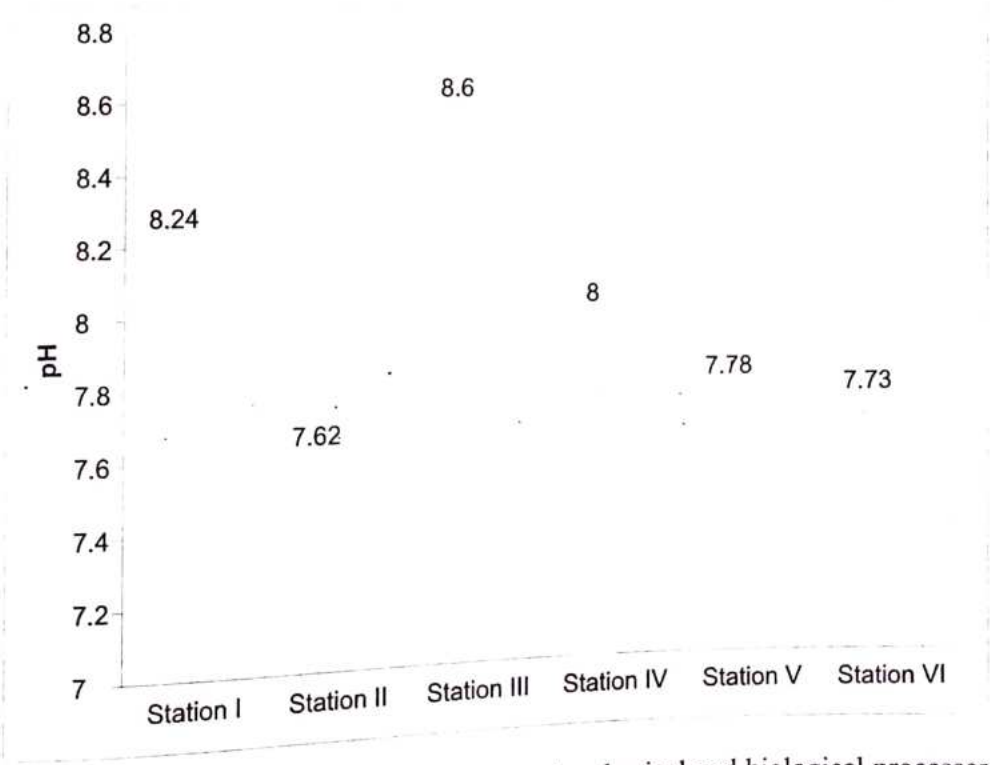


Temperature is one of the important factor in an aquatic environment for its effects on the chemistry and biological reactions in the organisms. The change in atmospheric temperature with change in season brought corresponding changes in water temperature. The average variation in temperature of marine water samples in the study area varied from 29 to 31°C. The minimum temperature was observed in station IV and maximum temperature in station V and VI. The results of the present study is closely related to the results of (Muthuraman *et al.,* 2019). The disposal of fly ash slurry causes the high temperature in station III and IV. Sea surface temperature in station III and IV was high (31) during December month (Table 1, Figure 1). Temperature has been decreasing with increasing the distance. During the study period the temperature varied. Generally, the surface water temperature is influenced by the intensity of solar radiation evaporation, freshwater influx, cooling and mix up with ebb and flow from adjoining neritic water (Govindasamy *et al.,* 2000). Earlier, the temperature of 27-34 °C was recorded in the surface waters of Tuticorin, a part of Gulf of Mannar (Gopinathan and

Rodrigo, 1991). Temperature has been decreasing with increasing the distance (Selvin *et al*., 2010).

The pH is a measure of the intensity of acidity or alkalinity and measures the concentration of hydrogen ions in water. In the present study, the fluctuation of pH in the samples was from 7.62 to 8.60 (Table 1, Figure 2). The pH below 7 indicates that the sample water were slightly acidic may be due to the presence of minerals in the water. These variations in pH may be due to the condition of earth and minerals present. Meiaraj and Jeyapriya (2019) has recorded high alkalinity in marine water along Tuticorin harbour coastal area. High pH values observed may cause sea water deprivation and high density phytoplankton effect (Prabu *et al.*, 2008).

Figure 2. pH at various stations in the study area



Dissolved Oxygen content in water reflects the physical and biological processes prevailing in water and is influenced by aquatic vegetation and plankton population apart from the temperature and organic matters present. Low oxygen content in water is

usually associated with organic pollution. DO content was ranged from 2.3 – 4.6 mg/L in the study area. The variation of the DO is estimated by the oxygen consumption by bacteria when disintegrating organic matter in aerobic conditions. This is due to the large amount of drainage and waste water released into the marine environment (Balakrishnan *et al.*, 2017). The levels of DO recorded in the present study were compared with the work and carried out by Santhanam *et al.* (1994) clearly indicates that the water at all the stations studied is polluted by the effluent discharge from the surrounding industries.

BOD gives a quantitative index of the degradable organic substances in water and is used as a measure of waste strength. The low BOD value in water samples showed good sanitary condition of the water. It varied from 0.3 to 3.2 mg/L in the study areas (Table 1). COD is the oxygen required by the organic substances in water to oxidize them by a strong chemical oxidant. It ranged from 4.8 to 10.6 mg/L. High acidity might due to the fact that industrial wastes which contain mineral acidity are let out into the sea.

The Alkalinity of water is a measure of its capacity to neutralize acids. Alkalinity values provide guidance in applying proper doses of chemicals in water and wastewater treatment processes particularly in coagulation, softening and operational control of anaerobic digestion. The Alkalinity in natural water is caused by bicarbonates, carbonates and hydroxides and can be ranked in order of their association with high pH values. However, bicarbonates represent the major form since they are formed in considerable amounts due to the action of carbonates with the basic materials in the soil. In the present study Phenolphthalein Alkalinity was absent in all samples and Methyl Orange Alkalinity ranged from 166.0 mg/L to 454 mg/L (Table 1), this indicates the absence of hydroxyl and carbonate alkalinity and presence of bicarbonate.

Hardness is caused by multivalent metallic cations. The principal hardness causing cations are the divalent Calcium, Magnesium, Strontium, Ferrous and Manganese ions. The Hardness in water is derived largely from contact with the soil and rock formations. Total hardness was less in station III and IV, station I and II, station V and VI showed similar results.

The amount of dissolved material irrespective of the constituents is quantified as salinity. The values of salinity varied from 0.02 to 5.28 mg/L. Station VI recorded the minimum salinity and maximum salinity was recorded in station V. Previous studies by Muthuraman *et al.* (2019) reveals that the salinity is very than the results of current study.

Due to the dumping of various wastes from Thermal power plant, which contain some heavy metals, there is possibility of leaching of heavy metals into the soil and thereby contaminating the marine water. The copper concentration of the study area varies from 4.8 to 6.3 mg/L (Figure 3). The water samples at station I, III, IV and V indicated alarming figure of lead. The concentration of Zinc as obtained from the analysis of water sample collected is 3.0 mg/L at station III, IV, V and VI. Result indicates leaching of Zinc from the waste dumping site confirming the presence of Zinc in the waste dumped (Figure 4). The concentration of Lead varied from 0 to 14.3 mg/L (Figure 5). Such high values of Lead in those areas might have been caused due to the leaching from industrial waste dumped containing battery. The concentration of Cadmium varied from 0.0 to 3.8 mg/L (Figure 6).

Figure 3. Copper content at various stations in the study area

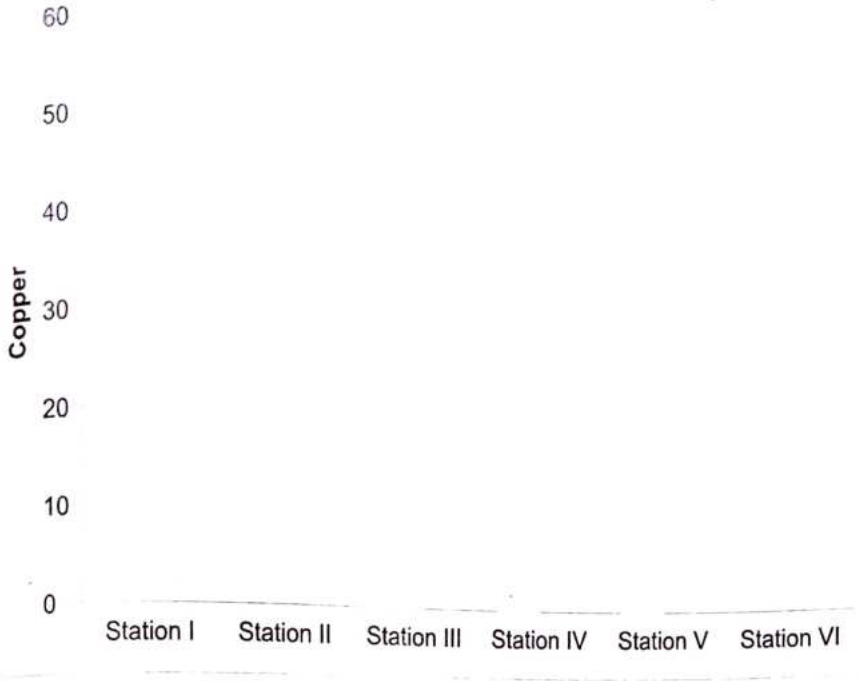


Figure 4. Zinc content at various stations in the study area

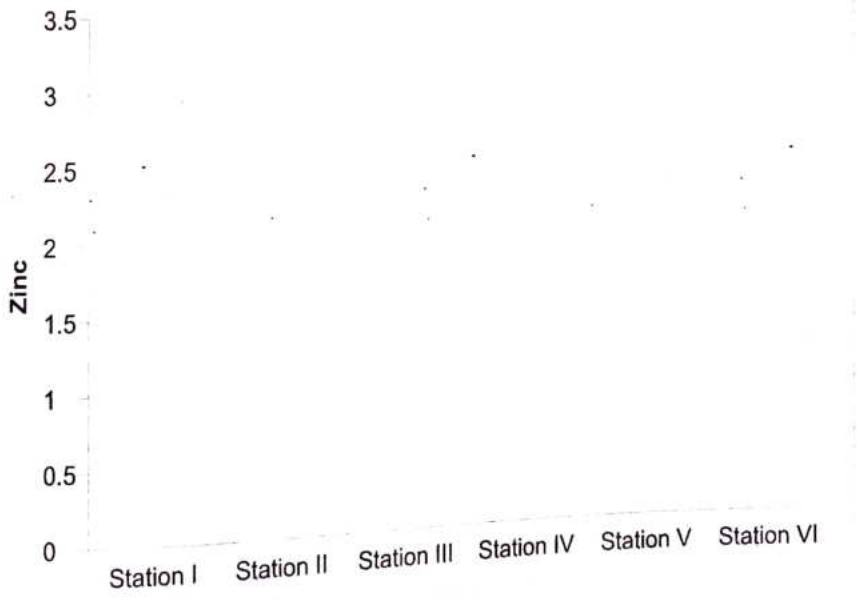


Figure 5. Lead at various stations in the study area

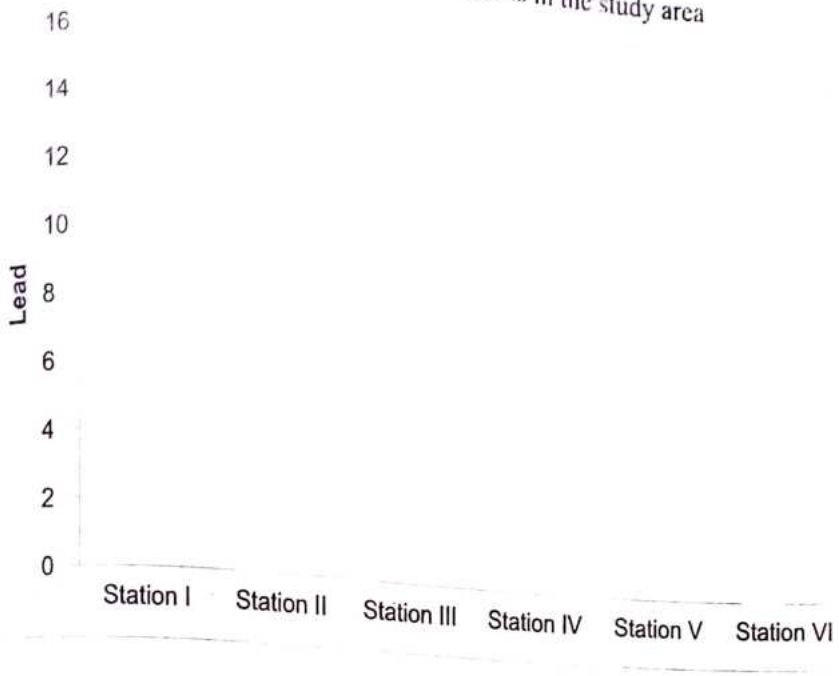
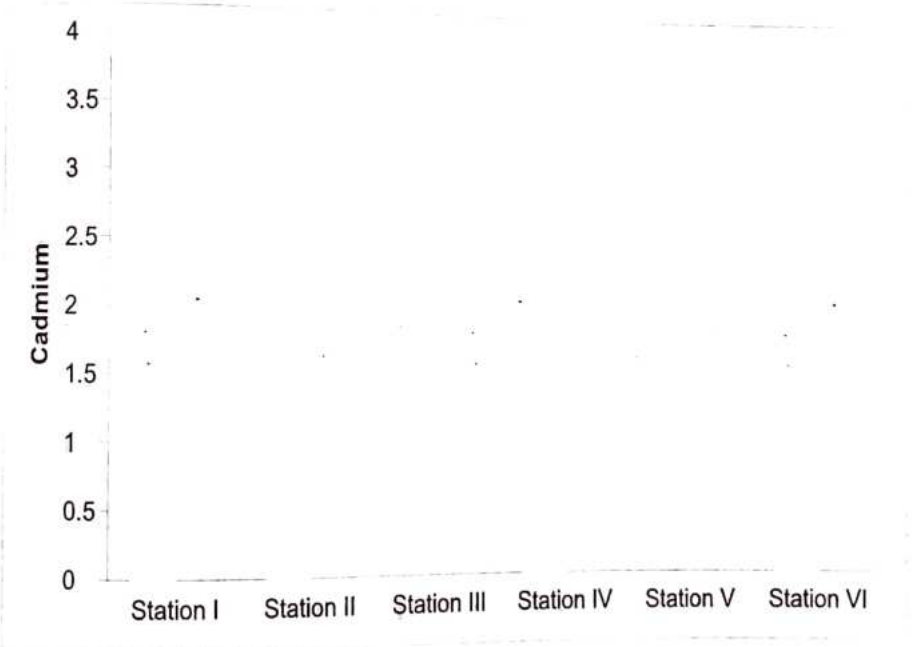


Figure 6. Cadmium content at various stations in the study area



Nitrite ranges from 123 to 181 mg/L. The high nitrite content in station 1 is an index of the balance of active biological oxidation by nitrifying bacteria and phytoplankton (Sekar *et al.*, 2009). The amount of nitrogenous compounds from industrial wastes also contribute to the increase of nitrites in seawater. The amount of

phosphate ranges from 105 to 130 mg/L. The results of the current study reveals the contamination of the study areas with the effluents discharged from thermal power plant.

CONCLUSION

On the basis of the field observations and laboratory study it has been concluded that the water both surface and subsurface existing in the area is polluted and the levels of pollution are unacceptable. It is one of the effects of the presence of a chemical industry which makes the coastal environment in the area highly vulnerable. The practice of letting the effluents without any pretreatment affects the soil and water of the study area which cannot be rectified.

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ISOLATION AND SCREENING OF MICROALGAE FROM NATURAL HABITATS IN THE THOOTHUKUDI COAST FOR ANTIBACTERIAL SOURCES

A short term project work submitted to

St. Mary's College (Autonomous)

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY

in partial fulfillment of the requirement for the Degree of Bachelor of science in Botany

BY

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DEPARTMENT OF BOTANY

ST. MARY'S COLLEGE (AUTONOMOUS)

THOOTHUKUDI- 628001

SEPTEMBER- 2019- 2020

CERTIFICATE

It is certified that this short term project work entitled **"ISOLATION AND SCREENING OF MICROALGAE FROM NATURAL HABITATS IN THE THOOTHUKUDI COAST FOR ANTIBACTERIAL SOURCES"** submitted to St. Mary's college (Autonomous) affiliated to MANONMANIAM SUNDARANAR UNIVERSITY in partial fulfilment of the requirements for the degree of Bachelor of science in Botany, and is a record of work done in the Department of Botany, St. Mary's College (Autonomous). Thoothukudi during the year 2019 – 2020 by the following students.

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INTRODUCTION

Marine microalgae are the microscopic unicellular plants of the sea. They are generally free living pelagic forms and their size ranges from 2 to 20 m. The important component of the microalgae are the diatoms and dinoflagellates. The microalgae play a critical role in the coastal and marine aquaculture of fish, molluses, shrimps and oysters.

Microalgae have been suggested as a potential feedstock for fuel production due to a number of advantages including higher photosynthetic efficiency, higher biomass production and higher growth rates when compared to other energy crops. Microalgae represent an exceptionally diverse but highly specialized group of microorganism adapted to various ecological habitats. Many microalgae have the ability to produce substantial amounts (eg: 20-50% dry cell weight) of tri acyl glycerols (TAG) as a storage lipid under photo-oxidative stress or other adverse environmental conditions. Microalgae with high oil productivities are desired for producing bio-diesel. Depending on the species, microalgae produce many different kinds of lipids, hydrocarbons and other complex oils.

The primary producers of oxygen in aquatic environments are algae, especially planktonic microalgae. These microorganisms are widely distributed in nature and have adapted to different environments with great diversity in size, morphology, life cycle, pigments and metabolism.Recent developments in the biotechnology of microalgae have been focused on their production of useful materials applicable to the cosmetic and medical fields.

Microalgae are one of the important compounds in aquatic food chains and have been used for human consumption as food and as medicines. The wide diversity of compounds synthesized form different metabolic pathway of fresh and marine water algae provide promising sources of fatty acid , steroid , carotenoids , polysaccharides , lectines , micosporine like aminoacid and halogenated compounds polyketides , toxins ,agar agar , alginic acid and carrageenan.

The microalgae thrive in a wide range of habitats including fresh and salt water, blackish, marine and soil environments. They are photosynthetic organisms that grow in a range of aquatic habitats , including lakes , ponds, rivers , oceans , and even waste water. They can tolerate a wide range of temperature , salinities and pH value , different light intensities ,and conditions. They can grow alone or in symbiosis with other organisms. They are the most efficient primarily biomass producesr , accounting for the fixation of 30-50% of

the inorganic carbon from the atmosphere (Longhurst *et al* ., 1995). They play an important role in CO2 recycling through photosynthesis, which is similar to higher plants in O2-evolved systems (PSI and PSII).Research in microalgae has been carried out not only on physiological aspects but also to develop production of useful biomaterials.

Considering the above facts the present work is concentrated on isolation, identification and culture and their potential in antimicrobial activity of microalgae collected from Thoothukudi sea.

SCOPE

&

OBJECTIVES

Scope and Objective

The Scope and objective of this study is to provide scientific information on basic aspects to be taken into account to achieve the successful isolation, identification, culture and then to evaluate the antibacterial potential of native microalgae of the Thoothukudi Sea.

LITERATURE REVIEW

Microalgae are microscopic unicellular organisms capable to convert solar energy to chemical energy via photosynthesis. The first use of microalgae by humans dates back 2000 years to the Chinese. Who used nostoc to survive during famine. Microalgae is gaining worldwide popularity as a food supplements. It has been shown to be an excellent source of proteins.(colla *et al.*, 2007). Polyunsaturated fatty acids (Sajilata, 2008), pigments (Rangel-Yogui *et al.*, 2004; Madhyastha and Valsala, 2007), vitamins and phenolics (colla *et al.*, 2001;Ogloondo *et, al.*, 2007)

Today the major use of spirulina is for the extraction of phycocyanin, a blue photosynthetic pigment. Microalgae major economic important by-products that are used in fruit and vegetables preservatives (Hills and Nakamura , 1976). The biomass of these algae is marketed as tablets. Capsules and liquids which are used as nutritional supplement. (Pulz and Gross, 2004, Spolaore *et al.*, 2006 and Hallmann. 2007).

Microalgae are also added to pasta, snack foods or drinks either as nutritional supplements or natural food colourants.(Becker, 2004). Microalgal biomass contains three main component proteins. Carbohydrates, and lipids.(Um and Kim, 2009).

Microalgal species are established in the skin care market. The main ones being (Slolz and Obermayer, 2005). Food additive to enhance the colour of the flesh of fish and the yolk of eggs, and to improve the health and fertility of grain-fed cattle (Sea survey by Borowitzka and Borowitzka ,1987). Some species of microalgae produced high concentration of valuable compounds such as lipids, proteins and pigments(Abe *et al.*, 1999; El-Baz *et al.*, 2002; Abd El-Baky *et al.*, 2002)

Oil content of microalgae (Chisti 2007).Microalgae are employed in agriculture as biofertilizers and soil conditioners (Song et al., 2005). The plant growth in many fields and deficiency of this element is met by fertilizers(Malik *et al.*, 2001).

Algal organisms are rich source of novel and biologically active primary and secondary metabolites. These metabolites may be potential bioactive compounds of interest in the pharmaceutical industry (Rania and Hala ,2008). Temperature of incubation, pH of the culture medium. Incubation period, medium constituents and light intensity are the important factors influencing antimicrobial agent production(Noaman *et al.*, 2004).

Microalgae feeds are currently used mainly for the culture of larvae and juvenile shelland as well as for vaising the zooplankton required for feeding of juvenile animals (chen.

2003). Microalgae species particularly rich in protein were tested in shrimp dietsda Silva *et al*., 2008). Microalgae are a diverse group of microscopic plants with the wide range of physiological and biochemical characteristics high concentration of vitamins B1,B2,B3,B6,B12,E,K,D,etc (Avagyan, 2008).

Recent studies have shown that various microalgae may have antiproliferative and anticancer activities. In their review, Martinez Andrade et al., reported microalgae which have shown activities against human cancer cell lines, such as the diatoms *Phaeodactylum tricornutum, Skeletonema marinoi* and *Chaetoceros calcitrans,* and the dinoflagellates *Ostreopsis ovata* and *Amphidinium operculatum.* Driven by the increasing rate of antibiotic-resistant bacteria and infections, microalgae have been also recently studied to identify new anti-infective compounds. For example, Lauritano et al., found for the first time that extracts of two diatoms, *Skeletonema costatum and Chaetocerous pseudocurvietus* had antituberculosis activity.

Cancer, inflammation, and the evolution of antibiotic—resistant pathologies, together with other human diseases, are continuously stimulating the search for new bioactive molecules from natural sources. Unlike drug discovery on land, marine drug discovery is a relatively new field which began in the 1940s with the advent of scuba diving and new sampling technologies that allowed scientists to systematically probe the oceans for useful therapeutics. The number of potential compounds isolated from marine organisms now exceeds 28,000 with hundreds of new compounds being discovered every year (Blunt *et al.*, 2015). However, despite the number of compounds isolated from marine organisms and the biological activities attributed to many of these, those that have either been marketed or are under development are relatively few (Jaspars *et al.*, 2016). To natural populations. More recently, there is great interest in exploring the biotechnological potential of microorganisms such as microalgae since they are easier to cultivate, have short generation times and represent a renewable and still poorly explored resource for drug discovery. However, although a range of pharmacological activities have been observed from microalgal extracts, the active principles are often unknown (Mimouni *et al*., 2012; Guedes *et al*., 2013; Nigjeh *et al*., 2013; Samarakoon *et al*., 2013).

Microalgae are photosynthetic eukaryotes that constitute one of the major components of marine and freshwater phytoplankton; they are primary producers, a food source for other marine organisms, and are also excellent sources/producers of pigments, lipids, carotenoids,

ω−3 fatty acids and other fine chemicals (Mimouni *et al.*,2012). Their long evolutionary and adaptive diversification to a multitude of habitats and extreme conditions (e.g., cold/hot environments, hydrothermal vents) make them good candidates for drug discovery, because they may have evolved compounds for communication, defense and survival that are often unique and may not have any terrestrial counterparts(Landsberg, 2002, Wolfe *et al.*, 2002,Caldwell, 2009).

Diatoms are one of the most important groups of microalgae with over 100,000 species, that occur in virtually every environment that contains water, including not only oceans and lakes, but also soil. Diatoms have already found important applications as biofuels, health foods, biomolecules, materials relevant to nanotechnology, and as bioremediators of contaminated water (Bozarth *et al.*, 2009).

However not much is known on the potential applications of diatoms as pharmaceuticals. Various studies have shown that some diatoms are rich in bioactive compounds such as the sulfated polysaccharide named naviculan isolated from Navicula directa, with antiviral activity (Lee *et al.*, 2006), adenosine from *Phaeodactylum tricornutum*, anantiarrhythmic agent to treat tachycardia (Prestegard *et al.*, 2009, 2014), and marennine, a blue pigment identified in the marine diatom Haslea ostrearia that has shown allelopathic, antioxidant ,antibacterial, antiviral, and growth-inhibiting properties (Gastineau *et al.*, 2014).

Other studies have shown that the marine carotenoid fucoxanthin found in brown seaweeds and several diatom species has antioxidant, anti-inflammatory, anticancer, anti-obese, antidiabetic, antiangiogenic, and antimalarial activities (Peng *et al.*, 2011), and that the fatty alcohol ester nonyl 8-acetoxy—G-methyloctanoate(NAMO), isolated from the diatom P.tricornutum, has anticancer effects on three different cancer cell lines including human leukemia (BL-60)and lung carcinoma (A549), and a mouse melanoma (B16F10;Samarakoon *et al.*, 2014).

In addition to these, diatom unsaturated aldehydes such as decadienal, octadienal, and heptadienal isolated from *Skeletonema marinoi* have shown anticancer effects in lungcancer A549 and colon COLO 205 tumor cells, without affecting the Viability of normal cells from the same tissue type (Sansone *et al.*, 2014).

In addition to diatoms, other microalgae (e.g., green algae, flagellates, and dinoflagellates) have been screened for possible biotechnological applications (MacKinnon *et al.*, 2006; Kobayashi, 2008; Samarakoon *et al.*, 2013; Blunt *et al.*, 2015).

Digalactosyldiacylglycerolsand other monogalactosyl analogs isolated from the green algae *Nannochloropsis granulata* exhibited strong No inhibitory activity against LPS-induced No production in RAW264.7 macrophage cells suggesting a strong anti-inflammatory potential (Blunt *et al.*, 2015).

Samarakoon *et al.*, (2013) evaluated the anti—inflammatory activity of two species of green algae, Chlorella ovalis and *Nainchloropsis oculata*, and one species of dinoflagellates, *Amphidinium carterae*. Even if all the species showed activity, N. oculata hexane and chloroform fractions showed the strongest ant Iinflammatory activity.

The green alga C. ovalis and the dinoflagellate A.carterae suppressed the growth of HL-60 cells (human promyelocytic leukemia cell line; Samarakoon *et al.*, 2013) also demonstrating anticancer properties. On dinoflagellates, there are also various studies reporting functional based metagenomic approaches (as reviewed by Kellmann *et al.*, 2010).

Given the fact that polyketides are an important class of bioactive secondary metabolites, major efforts have been major efforts have been focused on isolating polyketide synthase (PKS) genes and new polyketides. PKSs have been found in several microalge, such as *Chlamydomonas reinhardtii, Ostreococcus spp., Emiliania huxleyz', Karenia brevis, Ostreopsis cf. ovata, Coolia monotis, Prorocentrum lima, Gymnodinium catenatum, Heterocapsatriqueta, Azadinium spinosum, and Alexandrium ostenfeldii* (Eicholz *et al.*, 2012; Van Dolah *et al.*, 2013; Meyer *et al.*, 2015).

PKSs very often synthetiz epotent toxins in microalgae responsible for harmful algal blooms (HABs; Meyer *et al.*, 2015), impacting humans through consumption of contaminated shell fish, fin fish and through water or aerosol exposure.

Over vecent years, many new compounds have been identified in sediments deposited in marine and lacustrine environments. Deposite the fact that our knowledge of algal lipid is still far from comprehensive, microalgal sources have now been identified for many of the lipids that are widely distributed in recent sediments. (Boon *et al.*, 1979; Albaiges *et al.*, 1984; Volkman, 1986; ten Haven *et al.*, 1987; Conte *et al.*, 1994).

Microalgae were obtained from the CSIRO culture collection of living microalgae (Jeffrey, 1980). All species of microalgae contain sterols and fatty acids and most contain small amounts of [illegible] (Volkman et al., 1989; [illegible] and [illegible], 1991).

Microalgae are [illegible] photosynthetic microorganisms which have the ability to transform carbon dioxide into biochemicals that can be further processed into biofuels, food, feed and bioactive compounds (Walker et al., 2005). Microalgae are present in both aquatic as well as terrestrial environment (Richmond, 2004). Microalgae have the ability to fix CO_2 using solar energy with efficiency 10 times more than that of the terrestrial plants (Brennan and Owende, 2010).

Biodiesel production from microalgae is a proven technology it still overcomes numerous technical and economical constraints that are need to be mentioned (Pienkos and Darzins, 2009) to increase the production and thus lower the final production coasts (Oltra, 2011).

Microalgae are single celled photosynthetic organisms which have relatively simple requirements for growth, which need sunlight to convert water and carbon dioxide into protein, aminoacid, lipids, polysaccharides, carotenoids (Asha et al., 2011; Mulbry et al., 2008). Microalgae are a promising biomass feedstock owing to their faster growth, higher reproduction rate and reduced greenhouse gas emission. Further, microalgae have many broad applications in biotechnology (Walker et al., 2005), including biofuels (Chisti, 2007), pharmaceuticals (Lorenz, 2003; [illegible], 2003), aquaculture etc. (Mulbry et al., 2008).

Among the compound extracted from microalgae most of them have antimicrobial, antiviral, anticoagulant, [illegible], antioxidant, antifungal, anti-inflammatory and anticancer activity (Priyadarshani and Rath, 2012; [illegible] et al., 2006; Mayer and Hamann, 2005; plaza et al., 2010; Rodriguez-Meizoso et al., 2008; Carvalho et al., 2011). Some of the microalgae have been consumed as food due to their high nutritional value (Barsanti and Gualtieri, 2006).

Microalgae are used in bioassay of organisms to analyse the toxicity of harmful pollutants such as heavy metals, pesticides and pharmaceuticals. Heavy metals have been detected vastly in the aquatic ecosystem and even in marine ecosystems such as fish and mussel (Irwandi and Farida, 2009).

Similarly, Brunson *et al.*, studied domoicacid (DA) biosynthetic genes, by using growth conditions known to induce DA production in P.multiseries. They suggested that the gene candidates for DA biosynthetic (Dab) enzymes are dabA (terpenecyclase), dabB (hypotheticalprotein), dabC [ketoglutarate(aKG)—dependent dioxygenase], and dabD (cytochrome P450, CYP450).

In these days, world wide production of biogas from biomass is gaining importance. An anaerobic digester contains synergistic microbial populations, which convert algal organic compounds (lipid, protein, carbohydrate) to methane and carbon dioxide. Methane is widely used both as a fuel and chemical feedstock. Chynoweth *et al.*, investigated the potential of different feedstocks such as algae, wood, grass, soild waste for biomethane production and found algae biomass as potential candidate for production of biomethane. The biomass productivity of algae is generally higher than land plants, but its growth is influenced by limiting of different nutrients. Wang et al., examined the thermal pre treatment of microalgae for pent of biomethane by using Chlorella sp..

Antiproliferative activity on human melanoma A2058 cells was assessed for all microalgal extracts. Results showed that S. marinoi (FE60/2), A.minutum (FE126/1), A. tanuttum (FE107/1 and FE107/3) and A.andersoni (FE108/1) induced a significant reduction in cell survival at100 g/mL Successively, a dilution series was performed and the active extracts were tested at 100, 50, 25,12.5, 10, 2.5, and 10 ng/mL. Figure 3 shows that S. marinoi FE60/2 inhibits cell survival in a dose-dependent manner while for the other species, the pattern was not clear.

However, A.minutum, A. tanuttum, and A.andersoni also showed toxicity in the cytotoxicity assay. We also tested two different 5. marinoi clones, FE6 isolated in 1997 and FE60 isolated in 2005 in the Adriatic Sea (Mediterranean Sea). Both strains are known to produce secondary metabolites such as short-chain polyunsaturated aldehydes (PUAS) and other oxygenated fatty acid degradation products such ashydroxides, oxoacids, epoxy alcohols, and hydroperoxides that induce reproductive failure in zooplankton grazers (Miralto *et al.*, 1999; Ianora *et al.*, 2004; Fontana *et al.* 2007; Romano *et al.*, 2010; Gerecht *et al.*, 2011). PUAsisolated from F126 have been shown to have antiproliferative activity on colon carcinoma cells (Miralto *et al.*,1999), while FE60 has never been tested before for anticancer properties. Interestingly extracts of FE6 did not show activity for human melanoma cells (A2058), suggesting that the anticancer activity was specific for a particular cancer cell line.

Considering the great market interest of MNPs, the transcriptomic approach has been used in order to reveal the gene pathways responsible for their synthesis. An example is the case of A. catenella . Considering that little is known about the biosynthetic genes responsible for toxin production in dinoflagellates, Zhang *et al*., 2009.

Compared transcriptome profiles of a toxin-producing dinoflagellate, A.catenella, and its non-toxic mutant form. 101 putative homologs of 12cyanobacterial sxt genes (genes involved in the synthesis of saxitoxin and its derivatives) were identified. Among the down-regulated genes in the non-toxic mutant, expression of the transcript assigned to sxtA. the initiater of mutant, expression of the transcript assigned to sxtA, the initiator of toxin biosynthesis in cyanobacteria, was significantly down—regulated, suggesting that it might be directly involved in toxin biosynthesis. In addition, the authors identified sxtO and sxtz genes in dinoflagellates for the first time.

Ueno *et al*.,2004. 44 has examined the potential of marine green alga Chlorococcumlittoraleto produce ethanol under dark fermentation and about 27% of starch contained in algal cells was used within 24 hrs at 25 0C.John *et al*.,2000.

Assessed the feedstock potential of algal biomass to produce bio ethanol and to encourage its use as renewable biofuel for providing tenable option. Examples of green algae employed for bioethanol are: *Dunaliella, Chlorella, Chlamydomonas, Arthrospira, Sargassum, Spirulina, Gracilaria, Prymnesium parvum, Euglena gracili sand Scenedesmus*. El-Sayed *et al*., 2002.

Performed a proteomics analysis of the lipid droplets organelles in the green microalgae Dunaliella bardawil and D. salina. Their analysis revealed the accumulation of two different types of lipids, cytoplasmic lipid droplets (CLD) and B-carotene-rich (BC) plastoglobuli. In particular, the high nutritional and pharmacological value of B-carotene for humans has promoted intensive research aimed to clarify its biosynthesis and regulation . Other studies focused mostly on a proteomic analysis of oleaginous microalgae in the nitrogen-deprived growth condition that tends to increase lipid production. Examples are the cases reported by Longworth *et al*.,2004.

Who examined, in the model diatom P. tricornutum, the proteome response of lipid accumulation induced during nitrogen depletion and by Garibay-Hernandez and collaborators

who performed the first membrane proteome of the non-sequenced microalga *Ettlia oleoabundans* from nitrogen-deprived cultures .

The Gram-negative bacteria E. coli (ATCC 25922) and *P. aeruginosa* (ATCC27853) and the Gram-positive bacteria *S.aureus* (ATCC 9144), *E. faecalis* (ATCC 29212), and Streptococcus B(ATCC 12386) were used as test organisms and antibacterial tests were performed as in Ingebrigtsen *et al.* (2016).

Chlorella vulgaris and Chlorella protothecoides are two main species; containing high oil content has been studied by many workers for production of biodiesel. Gulyurt *et al.,*

Rai and colleagues investigated the proteome dynamics of Chlorella sp.FC2 IITG in nitrogen starvation, using two high-throughput complementary proteomics platforms: DIGE (2D differential in-gel electrophoresis) and iTRAQ(isobaric tagging for relative and absolute quantitation), two methods widely used in ecotoxicology studies.

However, Chlamydomonas alone certainly is not representative of the physiology of all species of biotechnological interest. In the last decade, proteomics approaches have been mostly focused on the modeldiatom *P. tricornutum* and on oleaginous microalgae, among others *Chlorella vulgaris* and *Fistulifera solaris*, species favorable, in particular, for biofuel and biodiesel production (see as example: [39,75,76,77,78]). One example is the case of Siegler and collaborators who, using a semi-quantitative proteomics approach, Iinvestigated the composition of the lipid bodies (LBs) in the oleaginous microalga *Lobosphaera incisa* .Similarly,(Davidi *et al* ., 20006).

The production of primary and secondary metabolites in microalgae can vary depending on eg., growth phases (Vidoudex and Polvert , 2012) , clones (Gerecht *et al.*, 2011), light (Depauw *et al.*, 2012), temperature (Husby *et al.*, 2013), culturing media(Alkhamis and Qin, 2015), extraction method (Juttner, 2001) and probably many other factors (Chen *et al.*, 2011).

Microalgae species responsible for toxic blooms worldwide (Gobri *et al.*, 2013, Sampetro *et al.*, 2013). Diatoms and Flagellates were grown in Guillard's F\2 medium (Guillard, 1975). Screening of marine microalgae in three different nutrient culturing conditions for the treatment of human (Guedes *et al.*, 2013).

Chlorophyll is an essential compound in many every day products. It used not only as an additive in pharmaceutical and cosmetic products but also as a natural food colouring agent. additionally , it has antioxidant and anti mutagenic properties .this review discusses the process engineering of chlorophyll extraction from micro algae. different chlorophyll extraction methods and chlorophyll purification techniques are evaluated. Our preliminary analysis suggests to organic solvent extraction. When compared to spectroscopic technique, high performance liquid chromatography was shown to be more accurate and sensitive for chlorophyll analysis. Finally, through capture and wastewater treatment, micro algae cultivation process was shown to have strong potential for mitigation of environmental impacts.(Aris Hosikian *et al.*, 2003).

Marine microalgae are a diverse group of organisms that have been targeted to figure out their secondary metabolites and broad spectrum of natural bio activities for beneficial health effects in many decades. Recently , increasing attention has been paid on the pronouncement of bio functional proteins and some peptides possess specific biological properties due to these potential components having health promoting effects. Therefore , this review will provide an overview on the protein based research literatures from marine micro algae with the conditions of gaining access to peptides from parent proteins by proteolytic enzymes or fermentations. moreover, this covers most of the proteins and protein derivatives including peptides with the range from di- peptides. Specific bio activities, including anti oxidative, anti hypertensive, anti tumor properties are also discussed. In this review, identified bio activities and potentialities of marine algal protein sources will be discussed for future pharmaceutical, nutraceutical and cosmeceutical applications.(Kalpa Samarakoon *et al.*, 1999).

Microalgal pigment has commercial uses as a natural food coloring and cosmetic ingredient. Some microalgae contain substantial amounts of Carotene (besides beta carotene). Other types of coloring appear in microalgae as well. Beta carotene is used as a food coloring (with a major application in providing the yellow color to margarine), as a food additive to enhance the color of the flesh of fish and the yolk of eggs, and to improve the health and fertility of grain-fed cattle (see survey by Borowitzka and Borowitzka, 1987). Natural Beta Carotene has physical properties that make it superior to synthetic. In particular, natural Beta Carotene is fat soluble. It was announced recently by the National Cancer Institute that Beta Carotene is anticarcinogenic; other studies have found that Beta Carotene is effective in

controlling cholesterol and in reducing risks of heart disease. These new findings make Beta Carotene much more valuable and are likely to increase the demand for the product. By being fat soluble, the natural Beta Carotene is a much superior anticarcinogen and an antiheart disease agent. Thus, the new findings of these desirable medical properties are likely to increase even more the demand and desirability of natural Beta Carotene. The potential of micro-algae as a source of food coloring is limited, however, because algal-derived food coloring is not photostable and the color tends to bleach with cooking. Nevertheless, in spite of this limitation, the potential market for micro-algae-derived food coloring is vast. *Dunaliella salina* is grown for a source of the photosynthetic pigment, beta-carotene. Beta-carotene is used as an orange dye and as a vitamin C supplement. Dunaliella species, Chlorella species and Spirulina species are three major type that have been used successfully to produced high concentrations of valuable compounds such as lipids, protein and pigments (Abe *et al.*, 1999; El-Baz *et al.*, 2002; Abd El-Baky *et al.*, 2002)

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Microalgae are employed in agriculture as biofertilizers and soil conditioners. The majority of cyanobacteria are capable of fixing atmospheric nitrogen and are effectively used as biofertilizers. Cyanobacteria play an important role in maintenance and build-up of soil fertility, consequently increasing rice growth and yield as a natural biofertilizer (Song *et al.*, 2005). The agricultural importance of cyanobacteria in rice cultivation is directly related with their ability to fix nitrogen and other positive effects for plants and soil. After water, nitrogen is the second limiting factor for plant growth in many fields and deficiency of this element is met by fertilizers (Malik *et al.*, 2001)

The microalgae have a significant attraction as natural source of bioactive molecules, because they have the potential to produce bioactive compounds in culture, which are difficult to produce by chemical synthesis. Both cell extracts and extracts of the growth media of various unicellular algae (e.g. *Chlorella vulgaris, Chlamydomonas pyrenoidosa*) have been proved to have antibacterial activity in vitro against both Gram-positive and Gram-negative bacteria. It has also been reported that a wide range of in vitro active antifungal activities are obtained from extracts of green algae, diatoms and dinoflagellates. Microalgae, such as *Ochromonas* sp., *Prymnesium parvum* and a number of blue green algae produce toxins that may have potential pharmaceutical applications(Borowitzka and Borowitzka, 1992; Katircioglu *et al.*, 2006). Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antialgal,

antibacterial, antifungal and antiviral activity. Temperature of incubation, pH of the culture medium, incubation period, medium constituents and light intensity are the important factors affecting antimicrobial agent production (Noaman *et al.*, 2004).

Microalgae feeds are currently used mainly for the culture of larvae and juvenile shellfish and finfish, as well as for raising the zooplankton required for feeding of juvenile animals (Chen, 2003). The most frequently used species in aquaculture are Chlorella, *Tetraselmis*, *Isochrysis*, *Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Nannochloropsis*, *Skeletonema* and *Thalassiosira*. Mainly the microalgae Spirulina and, to some extent, Chlorella are used in this domain for many types of animals: cats, dogs, aquarium fish, ornamental birds, horses, poultry, cows and breeding bulls (Spolaore *et al.*, 2006). . Favoured genera of microalgae for larval feeds include *Chaetoceros*, *Thalassiosira*, *Tetraselmis*, *Isochrysis*, and *Nannochloropsis*. These organisms are fed directly and or indirectly to the cultured larval organism. Indirect means of providing the algae are through artemia, rotifers, and daphnia, which are, in turn, fed to the target larval organisms. Several companies produce aquaculture feeds using Chlorella and Spirulina, or a mixture thereof. Some examples of the use of microalgae for aquaculture includes; Microalgae species Hypneacervicornis and *Cryptonemia crenulata* particularly rich in protein were tested in shrimp diets (da Silva *et al.*, 2008). Microalgae such *as Dunaliella salina*, *Haematococcus pluvialis* and Spirulina are also used as a source of natural pigments for the culture of prawns, salmonid fish and ornamental fish. Over the last four decades, several hundred microalgae species have been tested as food, but probably less than twenty have gained widespread use in aquaculture. Simon and Helliwell , Sartory and Grobbelaar , and Jeffrey et al. found methanol and ethanol to be superior extraction solvents to acetone. Simon and Helliwell conducted their sonication-assisted chlorophyll extractions in an ice bath and in dark conditions to prevent degradation products from forming. They found that, with sonication, methanol removed three times more pigment than 90% acetone. Additionally, when tissue grinding was used, methanol removed 20% more pigment than 90% acetone. Sartory and Grobbelaar similarly found that 90% acetone was an inefficient organic solvent compared to methanol or 95% ethanol. However, it has been shown that the use of methanol as an extraction solvent resulted in an unstable solution and lead to the formation of chlorophyll degradation products. Although 100% acetone was found not to yield the highest amount of chlorophyll from any particular species, its use as an extracting solvent strongly inhibited the formation of degradation products . In studies carried out by Jeffrey *et al.*,2002 and Macías-Sánchez *et*

al., 2005 dimethyl formamide (DMF) was found to be a superior extraction solvent to methanol, 90% ethanol, 100% ethanol and 90% acetone. Extraction using DMF did not require cell disruption as pigments were completely extracted after a few steps of soaking.

In the present scenario, petroleum sourced fuel consumption is unsustainable; therefore, there is a high demand for the development of renewable transport fuels for environmental and economic sustainability. Microalgal fuel, with the significant feature of being carbon neutral, serves as one of the potent tools for tackling the fuel crisis. Enormous researches have been explored using fresh water species on biodiesel production; nevertheless, marine species are still in a grey area, even though reported to have higher lipid content. The current review focuses on a wide spectrum of marine microalgal sources with phycology under the criteria of open pond systems for algal oil production. The discussion on the lipid expression in the marine species have been critically analysed through the vital parameters such as solar irradiation, temperature, pH, nutrient pressure, agitation, CO_2 supply, culture depth, aeration, etc. The parameters are interdependent and, if scrutinized wisely, could result in enhanced lipid productivity. Therefore, the open pond culture of marine microalgae with top prioritized parameters such as nitrogen stress, pH, and light penetration will be a suitable combination for the efficient and effective biodiesel production.(Nidhin Sreekumar *et al.,* 2002).

Microalgae have been vastly investigated throughout the world for possible replacement of fossil fuels, besides utilization in remediation of leachate, disposal of hypersaline effluent and also as feedstock for marine organisms. This research particularly has focused on locally available marine microalgae sample and *Nannochloropsis oculata* for potential mass production of microalgae biomass. Biomass produced by sample 1 and sample 2 is 0.6200 g/L and 0.6450 g/L respectively. Meanwhile, sample 3 and *N. oculata* has obtained maximum biomass concentration of 0.4917 g/L and 0.5183 g/L respectively. This shows that sample 1 and sample 2 has produced approximately 20% higher biomass concentration in comparison to sample 3 and *N. oculata*. Although sample 3 and *N. oculata* is slightly lower than other samples, the maximum biomass was achieved four days earlier. Hence, the specific growth rate of sample 3 and *N. oculata* is higher; meanwhile the specific growth rate of *N. oculata* is the highest. Optical density measurements of all the sample throughout the cultivation period also correlates well with the biomass concentration of microalgae. Therefore, *N. oculata* is finally selected for utilization in mass production of microalgae biomass.(Vijendren Krishnan *et al.,* 2004).

Marine eukaryotic photosynthesis is dominated by a diverse group of unicellular organisms collectively called microalgae. Microalgae include cells derived from a primary endosymbiotic event (similar to land plants)and cells derived from subsequent secondary and or tertiary endosymbioticevents. These latter cells are chimeras of several genomes anddominate primary production in the marine environment. Two consequences of multiple endosymbiotic events include complex targeting mechanisms to allow nuclear encoded proteins to be imported into the plastid and coordination of enzymes, potentially from disparate originator cells, to form complete metabolic pathways.(Micaela S. Parker, *et al*., 2000).

The use of microalgae and cyanobacteria for nutritional purposes dates back thousands of years; during the last few decades, microalgae culture has improved to become one of the modern biotechnologies. This has allowed high amounts of algal biomass to be obtained for use in different applications. Currently, the global production of microalgae and cyanobacteria is predominately aimed at applications with high added value given that algal biomass contains pigments, proteins, essential fatty acids, polysaccharides, vitamins, and minerals, all of which are of great interest in the preparation of natural products, both as food and in cosmetics. Hence, the bioactive components from microalgae can be incorporated in cosmetic and cosmeceutical formulations, and can help achieve benefits including the maintenance of skin structure and function. Tha lass otherapy involves using seawater and all related marine elements, including macroalgae, however, there has been limited use of microalgae. Microalgae and cyanobacteria could be incorporated into health and wellness treatments applied in thalassotherapy centers due to their high concentration of biologically active substances that are of interest in skin care. This paper briefly reviews the current and potential cosmetic and cosmeceutical applications of marine microalgae and cyanobacteria compounds and also recommends its use in thalassotherapy well-being treatments.(M. Lourdes Mourelle *et al*., 2007).

Due to significant lipid and carbohydrate production as well as other useful properties such as high production of useful biomolecular substrates (e.g., lipids) and the ability to grow using non-potable water sources, algae are being explored as a potential high-yield feedstock for biofuels production. In both natural and engineered systems, algae can be exposed to a variety of environmental conditions that affect growth rate and cellular composition. With respect to the latter, the amount of carbon fixed in lipids and carbohydrates (e.g., starch) is highly influenced by environmental factors and nutrient availability. Understanding synergistic interactions between multiple environmental variables

and nutritional factors is required to develop sustainable high productivity bioalgae systems, which are essential for commercial biofuel production. This article reviews the effects of environmental factors (*i.e.*, temperature, light and pH) and nutrient availability (e.g., carbon, nitrogen, phosphorus, potassium, and trace metals) as well as cross-interactions on the biochemical composition of algae with a special focus on carbon fixation and partitioning of carbon from a biofuels perspective.(Ankita Juneja *et al.*, 2003).

in a study on phytoplankton in Antarctic Ocean, Smith and Morris reported that at higher temperatures phytoplankton incorporate more carbon into the protein fraction with a concomitant reduction in lipids. To the contrary, Morris *et al.* reported an increase in the protein fraction of *P. tricornutum* at low temperatures. This variability could be due to the species-specific effects, differences in light intensity, and/or differences in the primary growth conditions.

In another study, Kudo *et al., 2000* observed the effect of iron stress on growth rate and cellular composition of the marine diatom *Phaeodactylum tricornutum*, over the temperature range of 5–30 °C. (Note that the optimum temperature for growth of *P. tricornutum* was 20 °C). The growth rate of Fe-stressed cells was 50% of Fe-replete cell growth rate at the optimum growth temperature. Differences in growth rates diverged significantly at suboptimum temperatures. It was also reported that at optimal temperature, the C:N ratio in the cell decreased by about 5% in cells induced with an iron stress (2 μM to 2 nM Fe). However, an increase of about 4% was demonstrated with the same transition in iron concentration but at a lower temperature (10 °C). Algal growth rates are also affected by light-by-nutrient interactions. Cloern *et al.* developed a model of phytoplankton chlorophyll—specifically, carbon ratio as a function of light and nutrients.

Converti *et al., 2008* studied the effects of temperature and nitrogen concentration on cell growth and lipid content in two strains of algae—*Chlorella vulgaris* and *Nannochloropsis oculata*. Reducing the nitrate concentrations in the growth media by 75% (1.5 to 0.375 g L−1 for *Chlorella vulgaris* and 0.3 to 0.075 L−1 for *Nannochloropsis oculata*), lipid accumulation tripled and doubled respectively, with only a small reduction in growth rate at optimal growth temperature. This result indicates that it may be possible to achieve higher lipid productivity for biofuels production by employing nitrogen limitation with fine temperature control.

METERIALS AND METHODS

Isolation of inoculum

The water samples were collected from shallow regions of Tuticorin coast. Then the water samples were checked for microalgae under the microscope using 1 ml of water sample.

Culture of micro algae

Culture of microalgae by using liquid media

For culturing, 10 ml of sample water containing diatoms was blended with 1000 ml of marine water and this mixture is mixed well. To this solution, TMRL nutrient medium was added in the culture flask and fix the air inlet pump inside the flask for better aeration. This solution mixture is fatherly kept for growth for a week time and the growth is regularly recorded every day.

Standardization based on the OD680 of isolate stocks was carried out previous to growth media, temperature, pH and salinity tests. First, all isolates were centrifuged and supernatant discarded. Next, 45 ml of autoclaved and filter sterilized seawater was added to all stocks and the pellet was resuspended. Then, 500 µl of each were taken and placed in a cuvette to measure their OD600 in an Spectrophotometer, being seawater the blank in all cases.

Preparation of TMRL Medium(Tung Kang Marine Res. Lab.)

Potassium nitrate -10gm/100 ml of DW

Sodium orthophosphate -1gm/100ml of DW

Ferric chloride -0.3gm/100 ml of DW

Sodium silicate -0.1gm/100 ml of DW

The chemicals are kept separately in 100ml reagent bottle.1ml each to 1liter of sterilized seawater is added. This medium canbe used for the mass culture of diatom.

Culture of microalgae by using Agar plate

For the agar plate study, 2.8 g of Nutrient agar medium is measured and poured into 1000 ml of distilled water. This agar mixture is kept for sterilization for obtaining stable and microbe -free solution. After the sterilization process, the room-cooled medium was poured into the agar plates and kept under room temperature for incubation.

After the medium plates comes to hand-bearable condition, 1 ml of inoculum was introduced into the medium plates by spread plate method.

Morphological identification

Micro algal cultures were initially separated based on morphological examination. This general classification method was only used to distinguish isolates on the most basic level. Identification of these isolates to the genus level was based on the morphology of the individual cells following microscopic examination. The strains were identified using the methods similar to reported by Wehr and Sheath (2003). Each isolate in the collection was labeled and photographed at three magnifications (20×, 40×, 60×) using the Nikon Eclipse E800 microscope (Nikon Inc., Tokyo, Japan) with the DXM1200 digital camera and the ACT-1 software program.

Cultivation and harvesting

Each algal culture sample was monitored every day for cellular growth rates by measuring optical density at 680 nm. The cultures were continuously aerated using air pumps with air stones, and the specified media was added to each culture at the end of every week. Constant mixing of the algal culture in the tank was provided by the aeration. The temperature of the mass culture of algae in the tank remained between 21°C and 32°C.

Antibacterial activity of microalgae by Disc diffusion method

Disc diffusion method was used for antibacterial activity. A stock solution of micro algal extract was prepared by dissolving 0.1 g of extract with 10 mL of their respective solvents (distilled water and absolute ethanol) to produce a final concentration of 10 mg/mL. 20 µL of each dilution was impregnated into sterile, blank discs 6 mm in diameter. 5 µL of extract was spotted alternately on both sides of the discs and allowed to dry before the next 5 µL was spotted to ensure precise impregnation. Distilled water and ethanol-loaded discs were used as negative controls for aqueous and ethanol extracts, respectively. All discs were fully dried before the application on bacterial lawn. The positive control used is ampicillin antibiotic discs for all *E. Coli* and *Bacillus Sp.* Antibacterial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the discs. The assay was repeated trice. Antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the leaf extract.

RESULT AND DISCUSSION

Native species of microalgae were isolated from natural water bodies in Thoothukudi District, Tamilnadu and were screened for their potential as antibacterial sources. In agar plate culture, two weeks after initial incubation, most of the plates showed bacterial colonial formations on top of the filter membrane; interestingly, some plates also showed strains growing beneath the filter surface. In some instances fungal growth was observed throughout the filter surface. In some cases the fungal growth was so dense that no colonies could be rescued and those plates were discarded.

The isolated microalgae from sea water sample is considered as inoculum and were cultured into liquid TMRL media for biomass production. After 4 weeks the culture beakers showed decent/high biomass production. The green and yellow microorganism from the medium were used for identification. The microalgae obtained from sampling location is shown in the Plate 1.

Because of time duration and pandemic situation only one micro algae is identified based on morphology is *Chlorella* sp. (Plate 1).

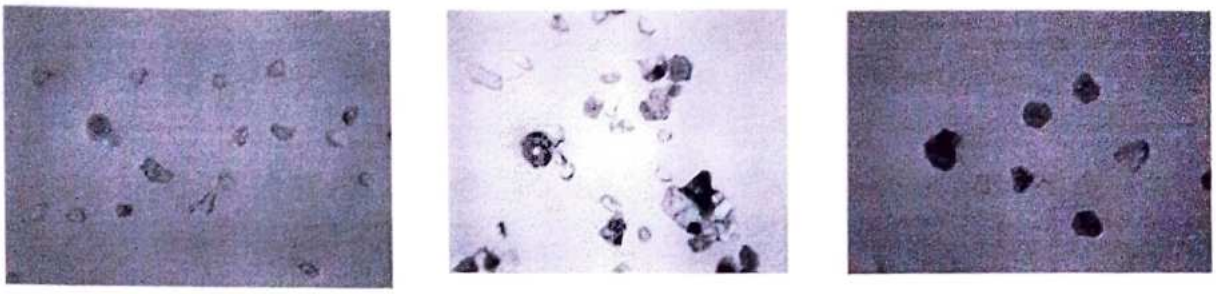
A global growth in energy demand led to the exploitation of nonconventional renewable energy sources. One of these examples of a renewable energy source is a biomass of algae (Bharathiraja *et al.*, 2015) . Its cells live in aquatic environments or in humid spaces, either in salt or fresh water. Since they are autotrophic organisms, they function as producers of organic matter. They are able to efficiently use less fertile areas more than energetic plants such as rapeseed or soya (Schlarb-Ridley and Parker, 2013). They are also capable of growing in wastewater. Algae can assimilate carbon dioxide as well as phosphorous and nitrogen derived from air pollutants, and municipal and industrial sewage. For the proper growth of algae, not only is nonorganic carbon required but also nitrogen and phosphorous. Nitrogen is one of the elements of amino acids as well as nucleic acids (building blocks of proteins), chlorophylls and plant hormones. The presence of phosphorous plays a significant role in metabolic processes of living organisms through the energetic balance regulation and other intracellular processes. Phosphorous deficiency contributes to the decline in the efficiency of marine ecosystems, thus even a small amount of phosphorous in aquatic ecosystem is the cause of an increased growth of algae (Bednarz *et al.*, 2002). Algae biomass is produced in the process of eutrophication in water tanks. In order to avoid an oxygen deficiency, the biomass needs to be constantly removed. Algae can be used in the bio-

sorption and bioaccumulation, in the process of toxic metal ions concentration and in wastewater treatment. The growth of an algal population can be divided into several major phases that follow one after the other – adaptation to environmental conditions, intensive growth of algal biomass, declining growth, stationary phase and algal cell death. These phases are characterized by a specific metabolism and the course of the photosynthesis process.

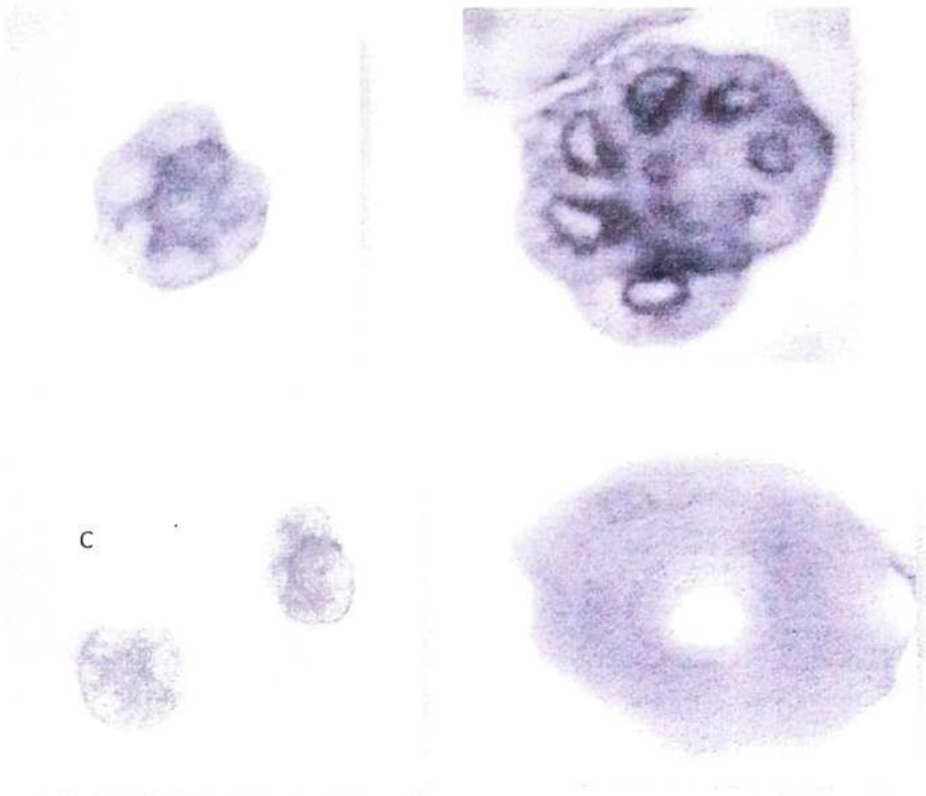
In our study, algae biomass growth assessed based on the optical density OD680. Based on the data presented in Fig. 1, it was found that at the initial stage of the culture algae cells were being adapted to the new environmental conditions, although they earlier stayed in anabiosis state. This led to inhibition of the cell division, which was associated with photoinhibition, particularly during the first three days. At the time, the optical density as well as nitrogen and phosphorous contents varied very slightly. The next step involved the acceleration of metabolic processes. The increase in optical density until the 3rd and 4th day of the culture was relatively low, but the microalgae assimilated the highest amount of nutrient media. This resulted in a significant change in optical density on the following days. Its value increased three-fold (from 0.11 to 0.34) and the nutrient content decreased to 7th and 8th days so it showed again decrease of growth rate. Changes in optical density and nutrient contents were statistically significant.

Antibacterial activity of the aqueous and methanol extracts microalgae isolated fromnatural sea water in Thoothukudi were studied against two bacterial strains (*E. coli* and *Bacillus*sp).In present screening, methanolic extract of microalgae isolated showed the highest antibacterial activity against both the strains (Table 1). The ability to produce antimicrobial agents may be significant not only as a defensive instrument for the algal strains but also as a good source of the new bioactive compounds from a pharmaceutical point- of- view. Screening efforts aimed to identify antimicrobial agents in microalgae have revealed several promising lead compounds. Some of the substances identified include Chlorellin (Metting and Pyne, 1986), Parsiguine (Ghasemi *et al.*, 2004), Nostocyclyne A (Ploutno and Carmeli, 2000), Nostofungicidine (Kajiyama *et al.*, 1998). This study confirms that the supernatant and methanolic extract of micro algae have high activities against all of the test microorganisms.Antibacterial activity of the aqueous and methanol extractsmicroalgae isolated fromnatural sea water in Thoothukudi were studied against two bacterial strains (*E. coli* and *Bacillus* sp). In present screening, methanolic extract of microalgae isolated showed the highest antibacterial activity against both the strains

Plate 1 Micro algae isolated from the Thoothukudi sea



(a) Mixture of Microalgae isolated from the sea water under light microscope



(b) Single micro algae isolated from the culture under light microscope (*Chlorella sp.*)

Table 1: Antibacterial activity of microalgal supernatants against bacteria as presented by inhibition zone diameter (in mm)

Extracts	*E. Coli*	*Bacillus* sp	Ampicillin
Methanol	10	14	15
Aqueous	7	9	10

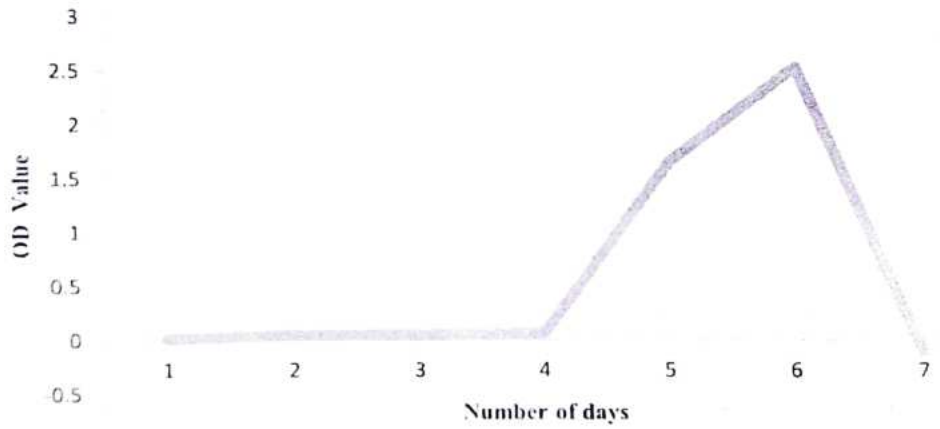


Fig 1 Growth curve of Micro algae isolated from the Thoothukudi Sea in TMRL nutrient medium

SUMMARY AND CONCLUSION

Marine phytoplankton comprises of a complex community of several thousands of floating microalgae in the sea ranging in size from about 1 µm to few millimeters. These microscopic, single-celled organisms are found in greatest abundance in near shore coastal areas, typically within the upper 50 m (160 ft) of the water column. The name "phytoplankton" consists of two Greek words meaning "plant" (phyto) and "wanderer" (plankton). Phytoplankton exhibit a tremendous variety of cell shapes, many with intricate designs and ornamentations. The scientific studies on microalgae is significant because of its dynamic growth characteristics, potent reproducibility, easily available in vitro culture method, distinguished taxonomy, hidden medicinal properties, and possible gene recombination ability. Marine microalgae can be grown in controlled environments on a large scale and can be utilized for the production of substances with medicinal and commercial value. Culture of microalgae on a large scale allows researchers to isolate and purify compounds and also provides enough biomass for rare bioactive compounds extraction. In recent years, microalgal culture technology is a business oriented line owing to their different practical applications. Innovative processes and products have been introduced in microalgal biotechnology to produce vitamins, proteins, cosmetics, and certain foods. For most of these applications, the market is still developing and the biotechnological use of microalgae will extend into new areas.With the development of sophisticated culture and screening techniques, microalgal biotechnology can meet the challenging demands of both the food and pharmaceutical industries. Considering the above facts, the present work details about the micro algae isolation from natural habits in Thoothukudi sea, culture and identification of their antibacterial potentiality. The isolated micro algae were studied under microscope and were used as inoculum for the culture. Two types of culture were tried, Agar Plate culture and Nutrient media respectively. On which in Agar plate, after one week fungal culture were dominated so it's discorded. But in the Nutrient media ie, liquid TMRL media after 4 weeks the culture beakers showed decent/high biomass production. Each algal culture sample was monitored every day for cellular growth rates by measuring optical density at 680 nm. Only one micro algae is identified based on morphology is *Chlorella* sp.

Local weather is one of the major limiting factors when determining which microalgal strains can be grown quickly in an established area. Local species have long been adapted to the prevailing regional abiotic and biotic factors, and thus are evolutionarily primed for local

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PRELIMINARY PHYTOCHEMICAL SCREENING AND PHARMACOGNOSTIC STUDIES ON LEAVES OF SELECTED WEEDS

A short term project work submitted to

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affiliated to **MANONMANIAM SUNDARANAR UNIVERSITY**

in partial fulfillment of the requirements for the Degree of

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INTRODUCTION

Plants are the one of the most important sources of medicines. Plants have been the basis of many traditional medicine systems throughout the world for thousands of years and continue to provide mankind with new remedies. These plants find application in pharmaceutical, cosmetic, agricultural and food industry. Among the plants of medicinal value plant genus like *Tribulus terrestris* and *Ocimum sanctum* are very important for their therapeutic potentials (Gincy *et al.*, 2014)

Traditional knowledge of medicine has long been used since ages for curing various human ailments. About 60-80% of world populations still rely on plant based medicines Santhi.,et al.. (2011). Though the traditional Indian system of medicine has a long history of use, yet they lack adequate scientific documentation, particularly in light of modern scientific knowledge Srivastava and Leelavathi., (2010). The medicinal value of the bioactive phytochemical constituents of the plant and which shows various physiological effects on human body. Nilofer. et al., (2013).

Phytochemical can be defined as any compound found in plants. Phytochemical are certain non-nutritive plant chemicals which have some disease preventive properties. Kokate.et al., (2006) However, the term phytochemical is often used to describe a diverse range of biologically active compounds found in plants.

Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins, sugar and amino acids. Secondary constituents contain terpenoids and alkaloids and phenolic compounds. Nostro.et al., (2000)

Phytochemicals provide plants with colour, flavour and natural protection against pests. Numerous epidemiological studies have indicated that a diet rich in fruit and vegetables offers considerable health benefits to humans. Among these benefits are:

1. Reduction of the risk of developing many forms of cancer (lung, prostate, pancreas, bladder and breast.)
2. Reduction of the risk of cardiovascular disease.

The majority of these beneficial effects are at least in part due to the presence of phytochemicals in vegetable and fruits. In this context phytochemicals may be defined as "non-nutrient" chemicals found in plants that have biological activity against chronic diseases. Kushad.et al., (2003)

In modern science, the importance of medicinal plants and increasing Iordachescu and Dumitriu, (1988). Nowadays, pharmaceutical and cosmetic industries are increasingly using plant resources from rural or unpolluted areas. *Plectranthus amboinicus* (Lour). Spreng, is a large succulent herb. The leaves have many traditional medicinal treatments of cough, sore throats and nasal congestion, but also for a range of other problems such an infection, rheumatism and flatulence. The plant is cultivated in home garden throughout in India for use in tradicinal medicine, being used to treat malarial fever, hepatopathy; renal and vesical calculi, cough, chronic asthma, hiccough, bronchitis, helminthiasis, colic, convulsion and epilepsy. Kaliappan., et al., (2008)

Phytochemical screening is helpful to detect the various important compounds which could be used as the base of modern drugs for curing various diseases keeping this in view the plants *Abutilon indicum* G. Don. Achyranthus aspera L. *Boerhaavia diffusa* L. . Kurz and *Bryophyllum calcycinum* Kurz and *Centella asiatica* Urh Has been taken for preliminary phytochemical screening and pharmacognostic studies

SCOPE AND OBJECTIVES

Medicinal plants have been widely used for thousand years for the treatment of many diseases. Most of the medicinal plants are allelopathic in nature has been used a popular folk and an orient medicines treats against many diseases. The aim of the study was to evaluate the pharmaceutical activity of *Abutilon indicum G.Don. Achyranthus aspera L. Boerhaavia diffusa L. Kurz* and *Bryophyllum calcycinum Kurz* and *Centella asiatica Urh.* used in traditional herbal medicine. The plants were selected on the basis of their reported ethno botanical uses.

- To perform qualitative analysis of phytochemical in various leaf extracts of *Abutilon indicum G.Don. Achyranthus aspera L. Aerva lanata L, Boerhaavia diffusa L. Kurz, Bryophyllum calcycinum Kurz* and *Centella asiatica Urh.*
- To evaluate various pharmacognostical parameters such as macroscopic microscopic characters of *Abutilon indicum G.Don. Achyranthus aspera L. Aerva lanata L, Boerhaavia diffusa L. Kurz, Bryophyllum calcycinum Kurz* and *Centella asiatica Urh.* Which can further lead to provide a beneficial information towards the quality of the drug

LITRATURE REVIEW

Quality can be defined as the status of a drug that is determined by identity, purity, content and other chemical, physical or biological properties or by the manufacturing processes. For the quality control of a traditional medicine, the traditional methods are produced and study, documents and the traditional information about the identity and quality assessment are interpreted in terms if modern assessment, Vikrant,*et al.*, (2012).

Phytochemicals are knows as secondary plant metabolites and have biological properties such as antioxidant activity antimicrobial effect, modulation of detoxification enzymes, stimulation of immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. There are more than thousand known and many unknown Phytochemicals. It is well known that plants produce these to protect themselves, but recent researches demonstrate that many Phytochemicals can also protect human against diseases (Rao,2003).

Phytochemicals accumulate in different parts of the plants, such as in the roots, stem, leaves, flowers, fruits or seeds (Costa et al., 1999).

Medicinal plants are known to produce certain bioactive molecules which inhibit bacterial or fungal growth (antimicrobial activity) (Mothana and Lindequist, 2005; Sharma and Kamar, 2009). Phytochemical studies on *Sopubia delphinifolia* were carried out by Deokule and Patale (2001).

Laily *et al*,(2002) worked out a preliminary phytochemical survey of plants in Crocker range sabbah Malaysia Choudhary. et al., (2009) observed the studies on leaf epidermal micromorphology wood element character and phytochemical screening of three medicinally important taxa of the family convolvulaceae. Farhat and Iqbal (2011) observed the phytochemical screening of some Pakistanian medicinal plants and Chandra shekar and

Rao (2012) observed the phytochemical analysis of ethanolic extracts of leaves of *Clerodendron viscosum*.

Vikrant *et al.*,(2012) worked at a preliminary phytochemical analysis of *Psidium* leaves. Nilofer et al (2013) studied the qualitative phytochemical analysis was done in rat tubers of six species *Dioscorea* found Meghalaya. The test conform the presence of various phytochemicals like flavanoids, saponins, steroids, cardiac, glycosides and terpenoids in two aqueous extract of methanol and ethyl acetate.

Mehta *et al.*, (2013) worked at aphytochemical survey of leaf extract of *Phyllanthus fraternus*. The plant extract contain alkaloids like morphine and boldine. Extracts also contains tannins, saponin, terpenoids and steroid.

Chede (2013) observed the phytochemical screening of fruit pulp of *Citrus sinensis*. The aqueous as well as the ethanolic extracts of the pulp revealed the presence of carbohydrates, alkaloids, tannins, fixed oils and lipids, sugars, proteins, steroids and aminoacids where asterpenoids are present only in the ethanolic pulp extracts.

Oancea *et al.*, 2013 observed the phytochemical screening of the bio active compounds in the most wide spread medicinal plants from Calarasi country. The following categories were identified during the study: aminoacids, protein,polysacharides vitamin A and E.

The phytochemical analysis revealed presence of significant amount of polyphenols and flavonoids (90% and 80%respectively). These findings suggest that *Theprosia purpurea* root extract posses prominent medicinal properties and can be exploited as natural drug to treat the disease anociated with free radical formation oxidative stress and xanthine, oxidase activity Khobra.,(2011).

A large number of medicinal plant are used as alternate medicine disease of man and animals. Since most of them are without side effects when compared when synthedic drug.

identification of the chemical nature of phytochemical compound present in the plants will provide some information on the different functional groups responsible for their medicinal properties. While studying the *in vitro* efficacy of bioactive extracts of 15 medicinal plants against ESBC-Producing multi drug resistant bacteria Iqbal Ahamad and farrukh Aqil ,(2007)detected major group of compound as the most active fraction of four plant extracts by infrared spectroscopy.

Kareru *et al.*,(2008)carried out the spectral analysis for saponins in the crude dry power of 11 plants and detected that *Albizia anthelmintica, senna singuaena,Mytenus senegalenisis,senna didymomatrya Terminalia brownie* and *Prunus Africana* were likely to be bidesmosidic, oleanne type triterpenoids, while those detected in *Entada leptostachya* and *Rapanea rhododendroides* might be monodesmosidic saponins. The different solvent extracts of *Kirganelia reticulata* leaves were screened for their phytochemical constituents by Shruthi et al. (2012). Agnel Ruba et al. (2013) carried out preliminary phytochemical analysis of *Arthocnemum fruticosum* leaf using five different solvents. Packia Lincy et al. (2013) conducted the preliminary phytochemical study of *Ventilago maderaspatana* whole plant, using different solvents.

Majgaine, Shweta and D.L Verma .,2017 studied that *Boerhaavia diffusa* have some pharmacological activities and used as a medicine with multi action such andituretic,entrasthamatic,diaphoretic,anthelmintic,antiasthamatic, diaphoretic antihepatotoxic antiurethritis febrifuge, antiscabies these pharmacological activities are due to the presence of chemical constituents

MATERIALS AND METHODS

MATERIALS

Sample: 1

Botanical Name : *Abutilon indicum* G. Don

Family : Malvaceae

Common Name : Indian mallow

Tamil name : paniyaratutti

Abutilon indicum is a herbaceous or shrubby softly tomentose plant, stem is round often tinged with purple colour. The leaves are petiolate, ovate to orbicular cordate, acuminate and toothed .Flowers are borne solitary in long jointed and axillary pedicels. Calyx lobes divided in the middle ovate and apiculate .Corolla is yellow or orange yellow and opens in the evening. Carpels are 15-20 in number. Fruits are hispid, scarcely longer than the calyx and the awns are erect seeds are three to five kidney shaped dark brown of black tubercle or with minutely stellate hairs (plate 1).

Sample: 2

Botanical Name :*Achyranthes aspera*

Family : Amaranthaceae

Common Name : Pricky chaff flower

Tamil name : Naiyurivi

Achyranthes aspera locally is one of the most important trationally used anti-fertility plants in the indigenous health care delivery system of Ethiopia.It is a stiff erect perennial herb of 1 to 3 feet with simple eliptic leaves. Flowering plant is in summer. The stems are square , leaves elliptic ovate or broadly rhombate. The inflorescences are 8-30cm long, with many single, white or red flowers. (plate 1)

Sample: 3

Botanical Name : *Aerva lanate Linn*

Family : Amaranthaceae

Common Name : Balipoovu

Tamil name : Ciru-pulai

Aerva lanata is a common weed which grows wild everywhere in the planes of India. It is a woody , prostrate, perennial herb. The stems are mostly straggling and sprawling and spread widely, sometimes as much as 6 feet (1.8m) in length. The often stalkless leaves are alternate, oval and 0.5 to 1.5 in (13 to 38 mm) long. They grow from whitish papery sipules with two lobes and red bases. The tiny clusters of two or three flowers grow in the leaf axils. The flowers are about 0.1 in (2.5 mm) long, pink, green or dull white. The flowers are normally self –pollinated. Flowering time is from May to October.

Sample: 4

Botanical Name : *Boerhaavia diffusa* Linn.

Family : Nyctaginaceae

Common Name : Punarnava

Tamil name : Mookiratti

Boerhaavia diffusa is a prostrate herb with very diffuse inflorescences. It is awed found throughout India. Leaves are unequal, ovate, blunt, wavy along margins, flat to somewhat eart shaped at base, leaf stalk is 1cm long . Inflorescence occur at the end if branches , are forked about 3-6 times, Occasionally with sticky intermodal bands, Branches are divergent , terminating in compact subumbellate or capitates 2-5 flowers clusters.flowers have stalk shorter than 0.5. Bract at base of flower tube quickly deciduous, lancelike,0.8-1mm flowers are purplish red to reddish pink or nearly white, bell-shaped beyond the constriction,1-1.5mm stamens 2-3, are inside the flower or protruding .(plate 1)

In this present investigation we selected six different medicinal plants for phytochemical screening and Pharmacognostical studies (*Abutilon indicum G.Don. Achyranthus aspera L. Aerva lanata L. Boerhaavia diffusa L. Kurz, Bryophyllum calcycinum Kurz* and *Centella asiatica Urh.*). Hereafter test samples will be referred as plant 1, Plant 2, plant 3, plant 4, plant 5 and plant 6 respectively.

METHODOLOGY

Collection and processing

The whole plant samples *Abutilon indicum G.Don. Achyranthus aspera L. Aerva lanata L. Boerhaavia diffusa L. Kurz, Bryophyllum calcycinum Kurz* and *Centella asiatica Urh.* was collected from the college sports ground. The collected samples were cut into small fragments and shade dried until the fracture was uniform and smooth. The dried plant material was granulated or powdered by using a blender and sieved to get uniform particles by using sieve NO.60.The final uniform powder was used for the extraction of active constituents of the leaf.

Preparation of extracts for phytochemical screening

Preparation of extracts Cold maceration method

The coarse powder of sample was extracted successively with aceton, benzene, chloroform, ethanol, methanol and petrolium ether. All the extracts were filtered through whatman no.41 filter paper. All the extracts (methanol, chloroform, petroleum ether and ethanol.) were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures

Hot maceration method using Soxhelet apparatus

The coarse powder (100 g) was extracted successively with Aqueous solution, aceton, chloroform, methanol, and petroleum ether each 500ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered through Whatman No.41 filter paper. All the extracts (aqueous

solution, petroleum ether, chloroform, methanol, and ethanol) were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedure Brinda et al., (1993), Lala,(1993).

Qualitative phytochemical analysis of different extract:

The chemical test for various phytochemical in the extracts were carried out as dscribed below

Test for carbohydrate (Benedict's test)

Five ml of Benedict's solution was added to 0.5 mg of extract and boiled in water bath. The appearance of red or yellow or green precipitate indicates the presence of reducing sugars.

Test for protein

(Biuret test (Gahn,1984)

To 1ml of the extract ,one drop of 2 Cuso4 and 1ml of 95% ethanol add KOH pellets .Appearance of pink colour indicates presence of protein .

Test for alkaloid (Wagner's test)

About ten mg of extract was taken and few drops of Wagner's reagent was added and the formation of a reddish brown precipitate indicates the presence of alkaloids.

Test for Terpnoids

Five milliliters of the extract was mixed with 2 ml of chloroform and concentrated sulphuric acid to form a layer. A reddish brown coloration of the interface showed the presence of terpenoids.

Test for Steroid

Five mg of extract was dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring indicates the presence of steroids.

Test for Coumarins

To 1ml of extract, 1 ml of 10% Sodium hydroxide was added .The presence of coumarins is indicated by the formation of yellow colour.

Test for tannin (Ferric chloride test)

Five mg of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish black colour indicates the presence of tannins.

Test for saponin (Foam test)

0.5 mg of extract was diluted with 20 ml distilled water and shaken well in a graduated cylinder for 15 min. The formation of foam to a length of 1cm indicated the presence of saponin.

Test for flavonoides (Shinadow test)

To a few mg of the powder magnesium turnings and 1or2 drops of concentrated hydrochloric acid (Hcl) were added ,formation of colour indicates the presence of flavonoides.

Test for Quionones

To 1ml of extract and 1ml of conc.sulphuric acid was added .Appearence of red indicates presence of Quinone.

Test for Steroid

Five mg of extract was dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring indicates the presence of steroids.

Test for Coumarins

To 1ml of extract, 1 ml of 10% Sodium hydroxide was added .The presence of coumarins is indicated by the formation of yellow colour.

Test for tannin (Ferric chloride test)

Five mg of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish black colour indicates the presence of tannins.

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Test for flavonoides (Shinadow test)

To a few mg of the powder magnesium turnings and 1or2 drops of concentrated hydrochloric acid (Hcl) were added ,formation of colour indicates the presence of flavonoides.

Test for Quionones

To 1ml of extract and 1ml of conc.sulphuric acid was added .Appearence of red indicates presence of Quinone.

Test for glycosides

0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

Test for Cardiac Glycosides (Keller-Killani Test)

Two milli liters of plant extract were treated with 2 ml glacial acetic acid containing a drop of FeCl3. A brown coloured ring or brown-violet under a brown greenish layer indicated the presence of cardiac glycosides.

Test for Phenol (Lead acetate test):

Ten mg of extract was taken and 0.5 ml of 1% lead acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolic compounds.

Test for phlobaphene

Sample was boiled with 1% aqueous hydrochloric acid to produce red precipitate indicating the presence of phlobaphene

Test for anthroquinones:

Add 1 of plant extract and 10% of ammonium solution will gives the appearance of pink colour precipitate indicates the presence of anthroruinones.

Pharmacognostic study

Morphology of Leaf

Morphology of Leaf was studied by naked eye and microscope.

Microscopic Study

Transverse sections of leaf were taken, stained with safranin and mounted in glycerin. Semi-permanent slides were prepared and observed under compound microscope. Photographs of the sections were taken by photomicroscope under ×10 and ×45 magnifications.

Pattern and Distribution of Stomata

Pattern and distribution of stomata were studied by stomata peel method. The peels of the epidermis were stained with safranin and mounted in glycerin. Semi-permanent slides were prepared and observed under compound microscope.

Powder microscopy: Shehla Akbar et al., (2014)

Shade dried leaves, stem, fruit and roots were finely powdered and studied under microscope. Small quantity of different plant parts powder was placed separately on slides and each slide was mounted 2-3 drops of chloral hydrate and each slide was covered with cover slip then examined under microscope. Different cell components i.e. cork cells, sieve tubes fibers, lignified fibers, cortex cells, calcium oxalate crystals, mesocarp, endocarp and stomatal cells were noted and photography was done by using digital camera.

RESULT AND DISCUSSIONS

There have been global interests in scientifically validating the therapeutic efficacy of the medicinal plants. The therapeutic properties of medicinal plants are due to the presence of some secondary metabolites. The secondary metabolites of the plans species specific and can be widely used in pharmaceutical industries. On the basis of literature obtained from ethno medical documentation *Abutilon indicum, Achyranthes aspera, Aerva lanata , Boerheavia diffusa, Bryophyllum pinnatum, Centella asiatica,*,an important medicinal plant of possess various pharmacological activities and used as a medicine with multi actions such as anti-inflammatory, antioxidant, diuretic, insecticide, and pheromone, antidiuretic, anti HIV, analgesis ,anthelmintic febrifuge.

The plants are rich in a variety of secondary metabolites such as tannins, flavonoids, terpenoids, alkaloids, phenol,steroids, glycosides and volatile oil. It is necessary to identify the phytochemicalComponents of local medicinal plants usually employed herbalists in the treatment of disease.

The preliminary photochemical screening was carried out on different extract of leaf sample of plant1, 2,3,4,5 and 6. The result of the preliminary phytochemical screening of leaf, sample of plant1,2,3,4,5is shown in table 1-26. The cold extracts were prepared by using different solvents like Acetone, benzene, chloroform, ethanol, Methanol and petroleum ether. The hot extracts were prepared by aqueous solution, Acetone, Chloroform, Methanol and Petroleum ether.

Anthroquinone, Glycosides, phlobaphene were absent in all the extracts (cold and hot). Protein is totally absent in cold extract in hot aqueous solution it is present in all plants. Steroids were absent in cold extracts of all plants. Hot extracts shows maximum than cold extracts.

The phytochemical screening of plants were consist with the result found in Alaghazeer and El-Saltani(2012)and partial agreement with previous studies Ramawat and Dass (2009) Ravishanker and Bhagyalakshmi(2007) Serrano and Puuponen (2009)Yadav and Kumar (2010).It is difficult to compare the data with the literature because several variables influence the results. According to some authours, the quantity and the composition of bio active compounds present in plant are influenced by the genotype, extraction, procedure, geographic and climatic conditions, and the growth phase of the plants. Ciulei and Istodor. (1995).Trease and Evans(2002).

The therapeutic benefits of secondary metabolism of plant origin have been researched in several recent studies, Nayak.et.,al(2006). The phytochemical screening results of plants are consistent with the results found in Alghzeer and Saltani, (2012) where authors mentioned the presence of tannins alkaloids, saponin and terpenoids in this plant. Similar analyses were conducted in areas that have a long tradition in the cultivation and utilization of medicinal plants, such as Pakistan Dai and Mumper (2010) and India Ravishankar and Bhayalakshmi (2007). Phytochemical screening results can be found in a database with the most important medicinal and aromatic plants in Calarasi-Silistra region.

Leaf Morphology

Abutilon indicum

The leaves simple, alternate,broadly ovate to suborbicular, about 4-12 X 3.5-8.5 cm across, base cordate, margins irregularly sparsely crenate-dentate, apex acute to shallow acuminate, densely or sparsely velutinous with minute stellate simple hairs both above and beneath, petiole, densely or sparsely velutinous with minute stellate simple hairs, about 2-18 cm long, stipules subulate.

Achyranthus aspera

Leaves are simple, usually thick, subsessile, Exstipulate, opposite, decussate, wavy margin, ellipticobovate, Petiolate and slightly acuminate. Petiole is very Short and texture is pubescent due to the presence of thick Coat of long simple hairs (trichomes).

Aerva lanata

Leafs are oval in shape, they are 0.5 to 1.5 in length, are alternately arranged. The leaves are present in the main stem.

Boerhavia diffusa

Teh leaves are borne on stalks of very varied length and are 3-5 cm longx2-4cm wide, broadly ovate in shape and blunt at tip, with a somewhat wavy margin. The under surface of the leaves is pale than the upper.

Bryophyllum pinnatum

The leaves were opposite, decussate, succulent, 10-20 cm in length. The lower leaf were simple, whereas, The upper leaf is 3-4 foliate with long petiole with dark Green in colour and fleshy, which are distinctively scallope. And trimmed in red. The leaves are furnished with rooted Vegetative buds, and leaf apex is obtuse. Petiole was 2-4 cm
In length; leaflet blades were oblong to elliptic, margin Crenate with each notch bearing a dormant bud competent To develop into a healthy plantlet.

Centella asiatica

Leaf simple, alternate, exstipulate, petiolate, all leaves arising from rhizomes, 7-8 in numbers. Variabile styles, laminar reniform (or) orbuscular- reniform; Marginal shallow crenate dentate green in colour, 1.2 to 5.0 cm in diameters, pubescent abaxially, veneation palmatous actinodromous with six primary veins. Petioles quite long 3 to 5 cm with a sheathing base, cylindrical above, adaxially groved, hairy, feebly hairy above.

Leaf Anatomy

Abutilon indicum

T. S of *Abutilon* leaf consists of midrib and lamina. The midrib consist of single row ovoid short cells the outer cell wall contain cuticle and three different type of trichomes .The cortex is several cell rows parenchymatous cell in abaxial side of the midrib but in adaxial side the cortex cells are consists of 3-4 rows of angular collenchyma and 2-3 rows of parenchymatous cell. The vascular bundle consist of phloem and xylem the xylem surrounded by phloem. Phloem cell are several rows with phloem fibers.Each rows of xylem cells are differentiated with xylem parenchyma. Xylemparenchyma. Xylem parenchyma cells containing, simple ovoid starch grains. (Plate 2)

Achyranthes aspera

T.S of *Achyranthes aspera* leaves have covering and glandular trichrome, slightly straight walled single layer upper epidermis and wavy walled single layer lower epidermis.Both the adaxial and abaxial is stomatiferous stomata type is anisocytic and anomocytic and distinct subsidiary cells are evident.3-5 layered collenchyma on upper side and 2-3 layered on lower side .Ground tissue consisting of thin layered parenchymatous cells having 3 vascular bundles.(Plate 2)

Aerva lanata

T.S of *Aerva lanata* leaf is dorsiventral with a prominent midrib and thick lamina. The lamina is amphistomatic, that is, stomata are on both upper and lower surfaces. The adaxial epidermis has wide, rectangular thin walled cells with a prominent cuticle; the cells 20 Nano m thick. The mesophyll consists of an adaxial zone of Palisade tissue of a single row of vertically oblong cylindrically cells. The spongy mesophyll is comprised of 6 or 7 lobed cells

that from a filamentous structure.The vascular bundle of the midrib is single and collateral.It is consists of dense cluster of thick walled, narrow angular Xylem elements and narrow arc of phloem with there sclerenchymal layer beneath vascular bundle.(Plate 2)

Boerhaavia diffusa

T. S of leaf showed uniseriate epidermis covered with a thin cuticle. It consists of stomata and trichomes on both, upper and lower surface. Epidermis is followed by mesophyll tissue. It revealed dorsiventral organization, comprising one – two stratum of palisade and about 3- 4 layers of spongy tissue. Collateral vascular bundle along with thick walled parenchyma cells adjoining the phloem were embedded in the ground parenchyma tissue.(Plate 3)

Bryophyllum pinnatum

T.S of *Bryophyllum pinnatum* shows upper and lower epidermis with cuticle. Midrib region was broad with distinct upper and lower epidermis. The cells between upper and lower epidermis were homogenous and parenchymatous deposited with starch grains and chlorophyll with two vascular bundles found in the center. Each vascular bundle was conjoint, collateral with xylem facing the upper side. The mesophyll region of the lamina was homogeneous and chlorenhymatous and showed spongy parenchyma. Lamina showed distinct upper and lower epidermis.(Plate 3)

Centella asiatica

Leaf T.S of Centella asiatica leaves had an average length of about 3.5 cm and with of 1 cm. Lamina was dorsiventrally differentiated and made up of epidermis mesophyll and vascular tissue. Both epidermis were uniseriate, composed of compactly arranged rectangular cells with moderately striated outer walls. Some of the upper and lower epidermis located at the midrib portion was provided with uniseriate, trichomes. The abaxial epidermis contained

a patch or band of sclerenhymatous tissue made up of 4- 5 layer. Midrib was composed of epidermis collenchyma, mesophyll and vascular bundle. Lamina was dorsiventrally differentiated with adaxial mesophyll having a compact palisade parenchyma with one layer of elongateand barrel shaped cells. A parenchymatous bundle sheath was encircled the vascular strand. Lamina also possessed vascular traces, but and differentiated into distinct metaxylem and protoxylem .(Plate 3)

Pattern and Distribution of Stomata

In *Abutilon indicum* stomata occurs mostly on the lower surface of the leaf. They are anomocytic type, lacking distinct subsidiary cells. *Achyranthus aspera* are amphistomatic and posses an amocutic stomata. The stomata are well developed with two guard cells. Stomata of *Aerva lanata* are anomocytic with no distinct subsidiary cells. Stomata are circular with slitlike stomata pores. Epidermal cells are wide with thin, wavy anticlinal walls.In the case of Boerhaavia and Bryophyllum the stomata areanamocytic. *Centella asiatica* Stomata are described to be mostly rubeaceous (Dutta and Mukherji, 1952). Presently they are found to be mostly anisotricytic and are monocyclic. (Plate 4).

Powder microscopy

Evaluation of the crude drug is an integral part of establishing correct identity of the drug. Pharmacognostical parameters are necessary for the confirmation of the identity of the crude drug. The powder microscopic evaluation of leaf of *Abutilon indicum ,Boerhaavia diffusa,Centella asiatica, Achyranthus aspera , Bryophyllum calcycinum* sample is useful for setting standards for identification and authentication of the drug not only in crude form but also in finished products.

Under microscopical examination, the leaf of *Abutilon indicum, Boerhaavia diffusa,Centella asiatica, Achyranthus aspera , Bryophyllum pinnatum* leaf powder shows

the fibers, cortical cells, xylem vessels, tracheid cells, trichomes and stellate hairs. The photographs were taken and shown Plate (5). These results were supported by the work done by Maria Sumathi, 2014 and Sheela, 2014

Table-1 Carbohydrate in the cold leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	**Acetone**	–	–	–	–	–	–
2.	**Benzene**	-	–	–	–	–	–
3.	**Chloroform**	-	–	+	-	–	–
4.	**Ethanol**	–	–	+	–	–	–
5.	**Methanol**	–	–	–	–	+	–
6	**Petroleum Ether**	-	-	+	-	–	–

Table-2 Carbohydrate in the hot leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	**Aqueous Solution**	+	+	+	+	+	+
2.	**Acetone**	–	–	–	–	–	–
3.	**Chloroform**	-	–	+	-	–	–
4.	**Methanol**	–	–	–	–	+	–
5.	**Petroleum Ether**	-	-	+	-	–	–

Table-3 Protein in the cold leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	**Acetone**	–	–	–	–	–	–
2.	**Benzene**	-	–	–	–	–	–
3.	**Chloroform**	-	–	–	-	–	–
4.	**Ethanol**	–	–	–	–	–	–
5.	**Methanol**	–	–	–	–	–	–
6	**Petroleum Ether**	-	-	–	-	–	–

Table-4 Protein in the hot leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	**Aqueous Solution**	+	+	+	+	+	+
2.	**Acetone**	–	–	–	–	–	–
3.	**Chloroform**	-	–	–	-	–	–
5.	**Methanol**	–	–	–	–	–	–
6	**Petroleum Ether**	-	-	–	-	–	+

Table-5 Alkaloids in the cold leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	**Acetone**	–	–	–	–	–	–
2.	**Benzene**	-	–	–	–	–	–
3.	**Chloroform**	+	+	+	+	–	–
4.	**Ethanol**	–	–	+	–	–	–
5.	**Methanol**	+	+	+	+	+	+
6	**Petroleum Ether**	-	+	–	-	–	–

Table-6 Alkaloids in the hot leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	Aqueous solution	+	–	–	–	–	–
2.	**Acetone**	–	+	–	+	–	–
3.	**Chloroform**	-	+	+	+	–	–
4.	**Methanol**	–	+	–	+	–	–
5.	**Petroleum Ether**	-	+	–	+	–	–

Table-7 Terpenoids in the cold leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	Acetone	–	–	–	–	–	–
2.	**Benzene**	-	–	–	–	–	–
3.	**Chloroform**	-	–	–	-	–	–
4.	**Ethanol**	+	–	+	–	–	–
5.	**Methanol**	+	–	–	–	–	–
6	**Petroleum Ether**	-	-	–	-	–	–

Table-8 Terpenoids in the hot leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	Aqueous solution	–	–	–	–	–	–
2.	**Acetone**	–	+	–	+	–	–
3.	**Chloroform**	-	+	–	+	–	–
4.	**Methanol**	–	+	–	+	–	–
5.	**Petroleum Ether**	-	+	–	+	–	–

Table-9 Steroid in the cold leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1	**Acetone**						
2	**Benzene**						
3	**Chloroform**						
4	**Ethanol**						
5	**Methanol**					+	+
6	**Petroleum Ether**						

Table-10 Steroid in the hot leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	Aqueous solution	–	–	+	–	–	–
2.	**Acetone**	–	+	–	+	–	–
3.	**Chloroform**	+	+	–	+	–	–
4.	**Methanol**	–	–	–	–	–	+
5.	**Petroleum Ether**	-	+	–	+	–	–

Table-11 Coumarin in the cold leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	**Acetone**	–	–	–	–	–	+
2.	**Benzene**	-	–	–	–	–	–
3.	**Chloroform**	-	–	–	-	–	–
4.	**Ethanol**	–	–	–	–	–	–
5.	**Methanol**	–	–	–	–	+	+
6	**Petroleum Ether**	+	+	–	+		–

Table-12 Coumarin in the hot leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	Aqueous solution	+	+	+	–	+	–
2.	**Acetone**	+	+	+	+	+	+
3.	**Chloroform**	+	+	–	+	+	–
4.	**Methanol**	–	+	–	+	+	–
5.	**Petroleum Ether**	+	–	–	+	–	–

Table-13 Tannins in the cold leaf extracts of different plant samples

S No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1	**Acetone**	–	–	–	–	–	+
2	**Benzene**	+	+	–	+	+	–
3	**Chloroform**	+	+	-	+	+	+
4	**Ethanol**	+	–	–	–	–	+
5	**Methanol**	+	+	+	+	–	+
6	**Petroleum Ether**		+	+	+	–	+

Table-14 Tannins in the hot leaf extracts of different plant samples

S No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	Aqueous solution	+	+	-	–	+	+
2.	**Acetone**	+	+	-	+	+	-
3.	**Chloroform**	+	+	–	+	+	–
4.	**Methanol**	–	-	–	-	-	+
5.	**Petroleum Ether**	+	–	–	-	+	–

Table-15 Saponin in the cold leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	**Acetone**	-	+	–	+	-	–
2.	**Benzene**	-	+	–	+	–	–
3.	**Chloroform**	+	+	-	+	+	–
4.	**Ethanol**	–	–	-	–	+	–
5.	**Methanol**	–	–	+	–	-	+
6	**Petroleum Ether**	+	+	+	+	+	+

Table-16 Saponin in the hot leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	Aqueous solution	-	-	+	–	-	-
2.	**Acetone**	-	-	-	-	-	-
3.	**Chloroform**	-	-	–	-	-	–
4.	**Methanol**	–	-	–	+	-	+
5.	**Petroleum Ether**	-	–	–	-	-	–

Table-17 Flavonoids in the cold leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	**Acetone**	–	–	–	–	–	–
2.	**Benzene**	-	–	–	–	–	–
3.	**Chloroform**	-	+	+	-	–	+
4.	**Ethanol**	–	+	-	+	–	+
5.	**Methanol**	–	–	+	+	+	+
6	**Petroleum Ether**	-	+	-	+	+	–

Table-18 Flavonoids in the hot leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	Aqueous solution	+	+	+	–	+	+
2.	**Acetone**	+	-	-	-	+	-
3.	**Chloroform**	-	-	–	-	-	–
4.	**Methanol**	+	+	–	+	-	-
5.	**Petroleum Ether**	+	–	–	-	-	–

Table-19 Quinine in the cold leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	**Acetone**	-	-	-	-	-	-
2.	**Benzene**	-	-	-	-	-	-
3.	**Chloroform**	-	-	-	-	-	-
4.	**Ethanol**	-	+	-	-	-	-
5.	**Methanol**	-	-	-	-	-	-
6.	**Petroleum Ether**	-	+	-	-	-	-

Table-20 Quinine in the hot leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	Aqueous solution	-	+	+	-	-	-
2.	**Acetone**	+	-	-	-	-	-
3.	**Chloroform**	-	-	-	-	-	-
4.	**Methanol**	+	-	-	-	-	-
5.	**Petroleum Ether**	-	-	-	-	-	-

Table-21 Glycoside in the cold leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	**Acetone**	–	–	–	–	–	–
2.	**Benzene**	-	–	–	–	–	–
3.	**Chloroform**	-	–	-	-	–	–
4.	**Ethanol**	–	-	-	-	–	–
5.	**Methanol**	–	–	–	–	-	–
6	**Petroleum Ether**	-	-	-	-	-	–

Table-22 Glycosides in the hot leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	Aqueous solution	-	-	-	–	-	-
2.	**Acetone**	-	-	-	-	-	-
3.	**Chloroform**	-	-	–	-	-	–
4.	**Methanol**	-	-	–	-	-	-
5.	**Petroleum Ether**	-	–	–	-	-	–

Table-23 Cardio Glycoside in the cold leaf extracts of different plant samples

S No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	**Acetone**	–	–	–	–	–	–
2.	**Benzene**	-	–	–	–	–	–
3.	**Chloroform**	+	–	-	+	–	–
4.	**Ethanol**	–	+	-	+	–	–
5.	**Methanol**	–	–	–	–	-	–
6.	**Petroleum Ether**	-	-	-	+	+	–

Table-24 Cardio Glycosides in the hot leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	Aqueous solution	-	+	-	–	-	-
2.	**Acetone**	-	-	-	-	-	-
3.	**Chloroform**	-	-	–	-	-	–
4.	**Methanol**	-	-	–	-	-	-
5.	**Petroleum Ether**	-	–	–	-	-	–

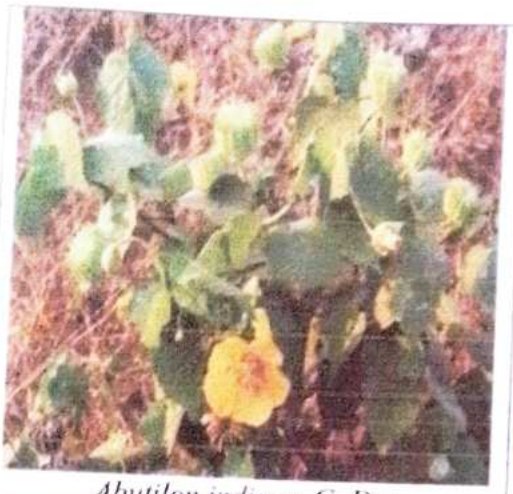
Table-25 Phenols in the cold leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	**Acetone**	–	–	–	–	–	–
2.	**Benzene**	+	–	–	+	–	–
3.	**Chloroform**	+	–	-	+	+	–
4.	**Ethanol**	–	+	-	+	–	–
5.	**Methanol**	–	–	–	–	+	+
6	**Petroleum Ether**	+	+	-	-	-	–

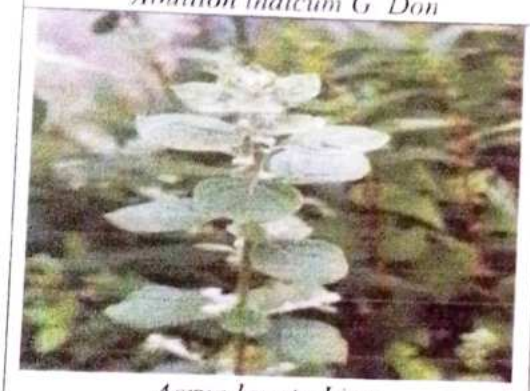
Table-26 Phenols in the hot leaf extracts of different plant samples

S.No	Solvent	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5	Plant 6
1.	Aqueous solution	-	-	-	–	-	-
2.	**Acetone**	-	-	-	-	+	-
3.	**Chloroform**	-	-	–	-	-	–
4.	**Methanol**	-	-	–	+	-	-
5.	**Petroleum Ether**	-	–	–	-	-	–

Plate - 1



Abutilon indicum G. Don



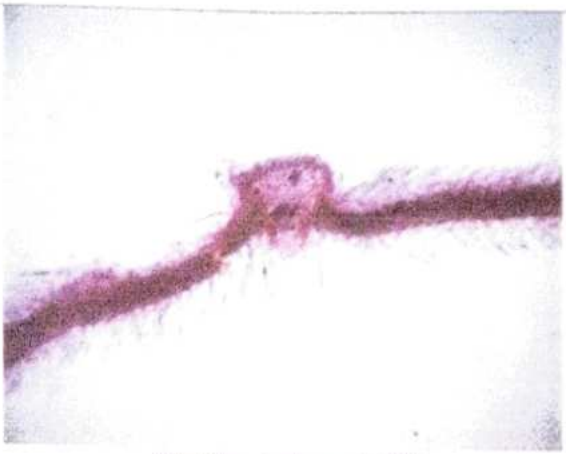
Aerva lanata Linn.

Boerhaavia diffusa Linn

Bryophyllum calcycinum Kurz

Centella asiatica Urh

Plate-2



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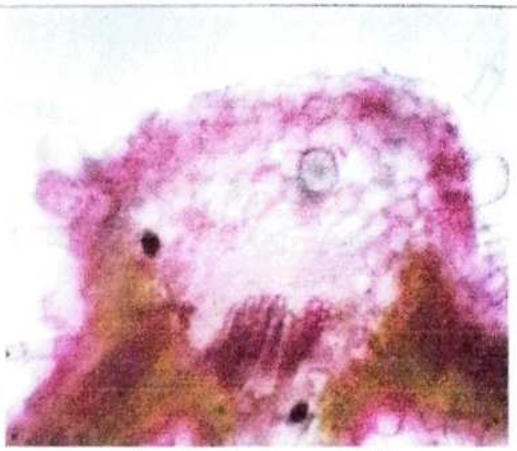

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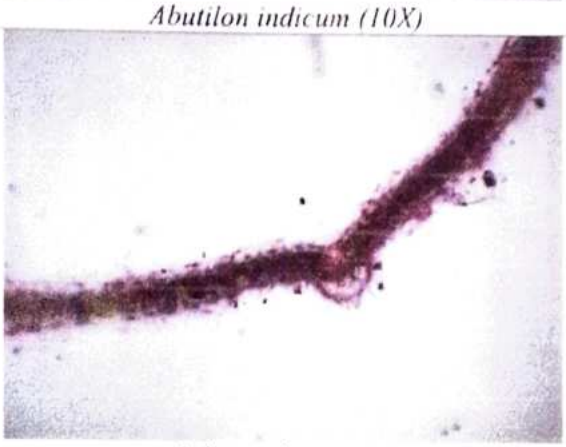
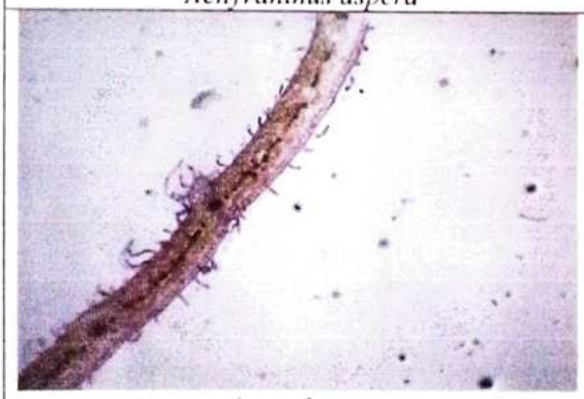
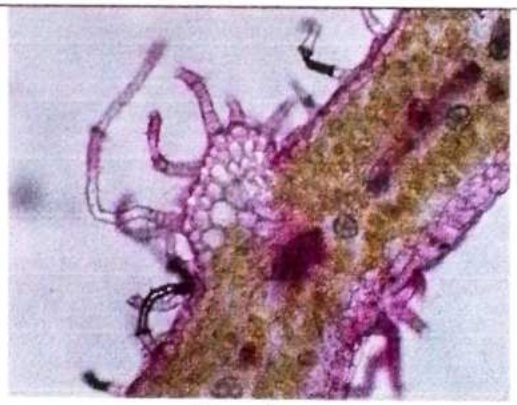


Achyranthus aspera

Achyranthus aspera

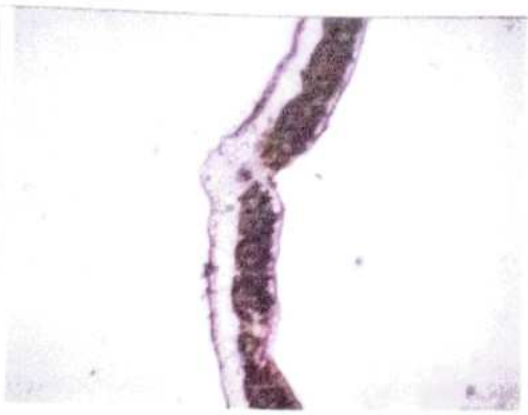


Aerva lanata

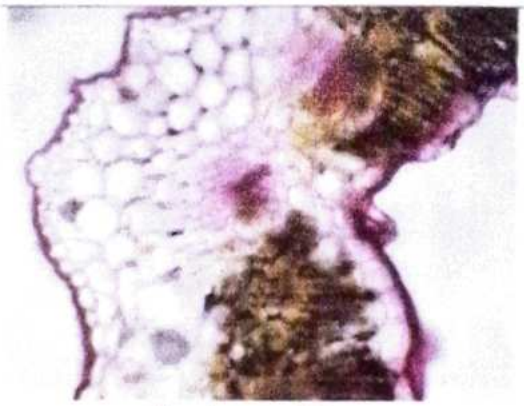


Aerva lanata

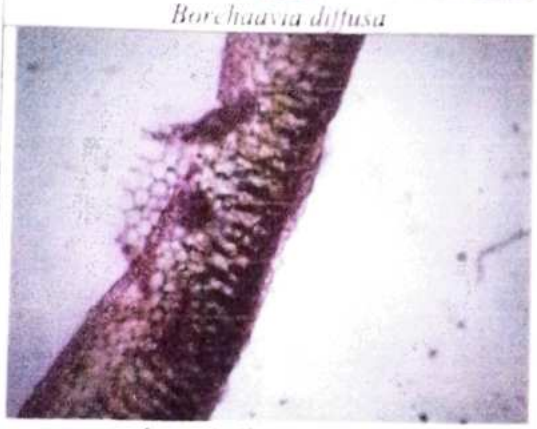
Plate-



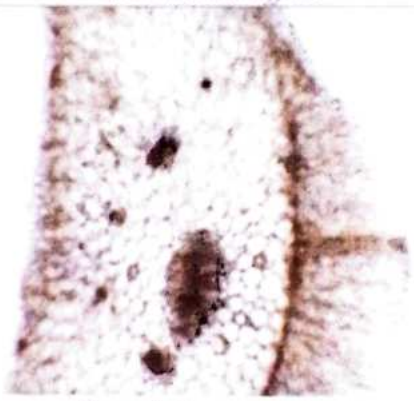
Borchaavia diffusa



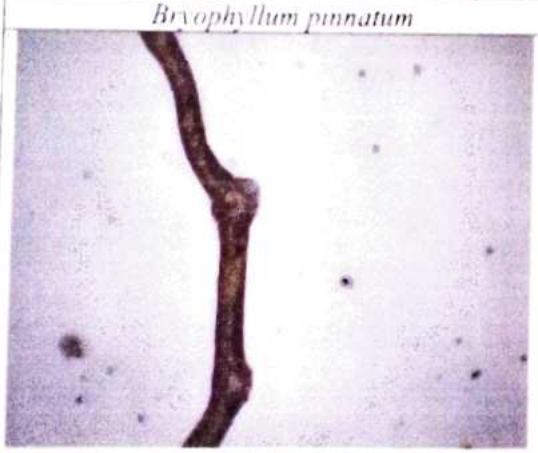
Boerhaavia diffusa



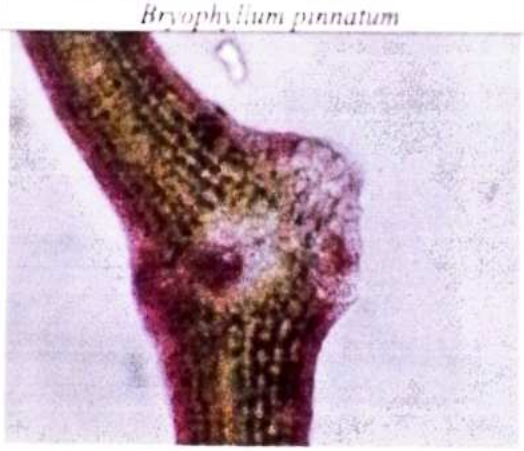
Bryophyllum pinnatum



Bryophyllum pinnatum

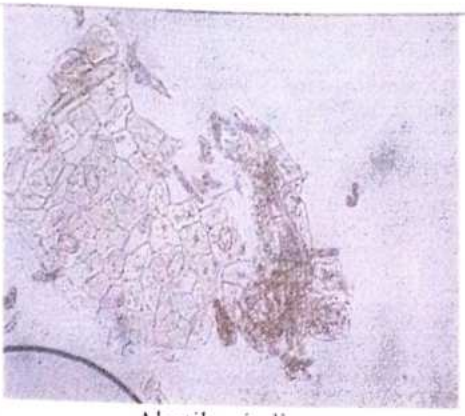


Centella asiatica

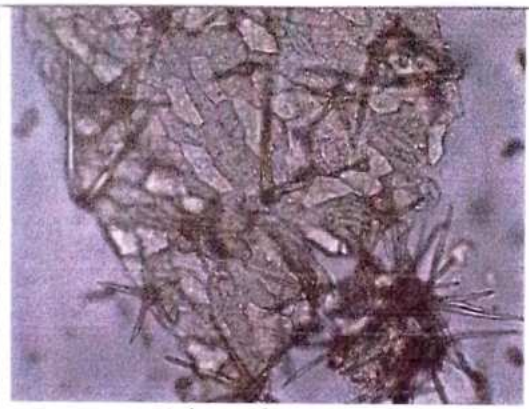


Centella asiatica

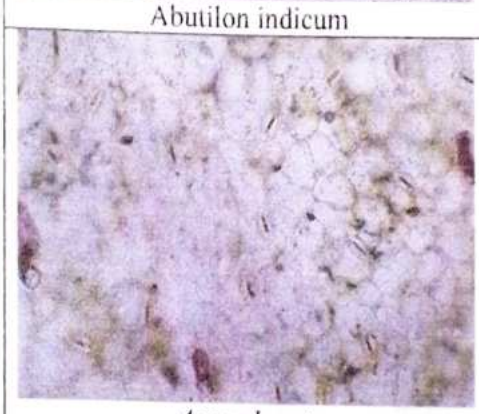
Plate-4



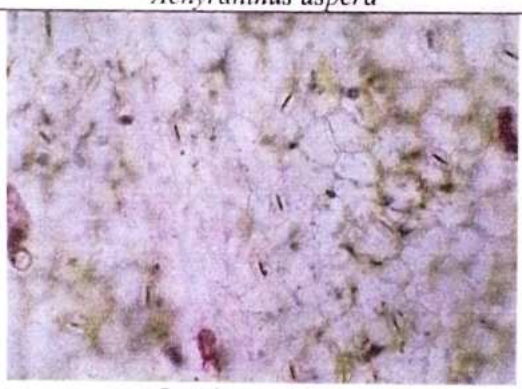
Abutilon indicum



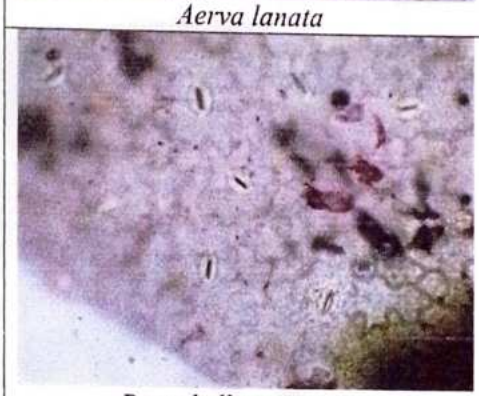
Achyranthus aspera



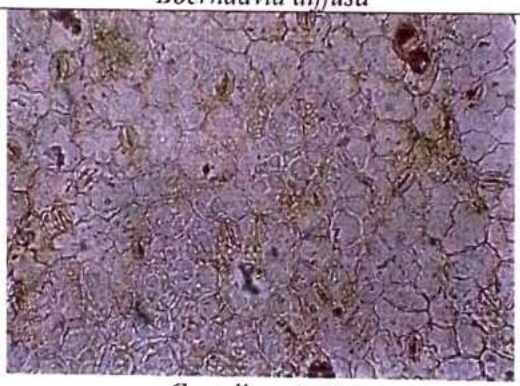
Aerva lanata



Boerhaavia diffusa

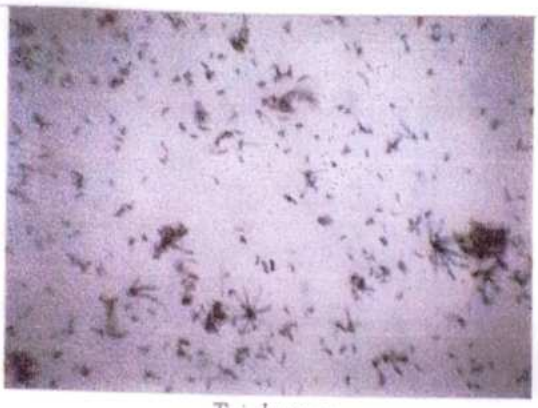


Bryophyllum pinnatum

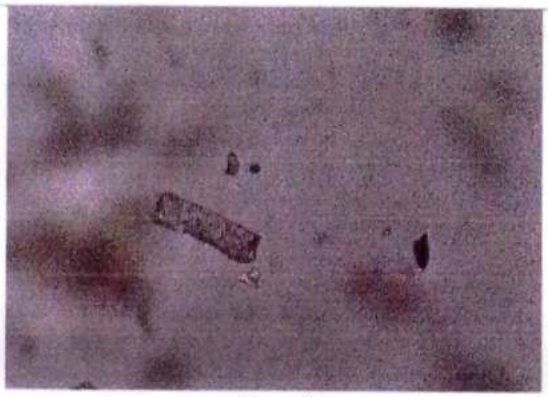


Centella asiatica

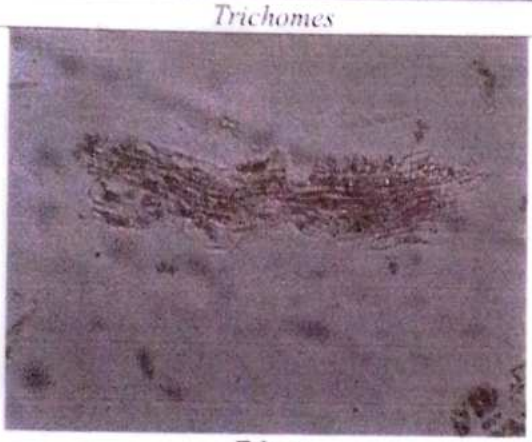
Plate-



Trichomes



Vessel



Fibers

SUMMARY AND CONCULSION

The plants are rich source of secondary metabolites with interesting biological activities In general these secondary metabolites are an important source with a variety of structural arrangements and properties.

The preliminary pytochemical screeing was carried out in the different extract of leafs of *Abutilon indicum* G. Don. Achyranthus aspera L. *Aerva lanata L.Boerhaavia diffusa* L. *Bryophyllum calcycinum* Kurz and *Centella asiatica* Urh After performing an analysis the following phytochemicals such as alkaloids, terpenoids, steroids, coumarin, tannins, saponin, flavones, phenols, protein, carbohydrate and quinones have screened. It is concluded that the phytochemicals present in this plant extracts may also be useful for the treatment of different type of acute and chronic disease. Which are correlated with the ethanobotanical datas on the use of this plant in Indian Folk core and Ayurveda.

The present work summarizes some important botanical microscopic characters of the leaf powder of herbal drug of *Abutilon indicum* G. Don. *Achyranthus aspera* L. *Aerva lanata L.Boerhaavia diffusa* L. *Bryophyllum calcycinum* Kurz and *Centella asiatica* Urh. These quality standards might be incorporated in quality control monographs for establishing the correct identity and quality of the crude drug. The plant can be used to discover bioactive products that may serves leads for the development of the new pharmaceuticals that address hither to unmet therapeutic needs.

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MACROSCOPIC AND MICROSCOPIC STUDIES ON SELECTED SPECIES OF LAMIACEAE

A short term project work submitted to

St. Mary's College (Autonomous)

Re accreditation with A++ Grade by NAAC affiliated to

MANONMANIAM SUNDARNAR UNIVERSITY

In partial fulfilment of the requirement for the

Degree of Bachelor of Science in Botany,

St. Mary's College (Autonomous), Thoothukudi.

By

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DEPARTMENT OF BOTANY

ST. MARY'S COLLEGE (AUTONOMOUS)

THOOTHUKUDI-628 001

SEPTEMBER - 2019-2020

CERTIFICATE

It is certified that this short term project work **"MACROSCOPIC AND MICROSCOPIC STUDIES ON SELECTED SPECIES OF LAMIACEAE"** submitted to St. Mary's College (Autonomous) affiliated to **MANONMANIAM SUNDARNAR UNIVERSITY** in partial fulfilment of the requirements for the degree of bachelor of science in Botany, and is record of work done in the Department of Botany, St. Mary's College (Autonomous), Thoothukudi during the year 2019 – 2020 by the following students.

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EXAMINER

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INTRODUCTION

Plants are the backbone of all life on Earth and an essential resource for human well-being. Plant is an important source of medicine and plays a key role in world health. Herbal medicine proved to be the major remedy in traditional system of medicine. Traditional system of medicine continue to be widely practised on many accounts. They have been used extensively in medical practices since ancient times. This promots the development in the practices of medicinal plants. The reasons are because of their biomedical benefits as well as place in cultural beliefs in many parts of world in the development of potent therapeutic agents.

Modern medicine depends on biological materials as an incomparable source of molecular diversity. One-quarter of all prescription drugs come directly from or are derivatives of plants. Recently however, attention is turning back to natural products as drug sources, since they have been so successful in the pat. These dats the term "Alternative Medicine" became very common in western culture, it focus on the idea of using the plants for medicinal purpose.

Medicinal herbs or plants have been known to be an important potanical source of therapeutics or curaive aids. Throughout the ages, humans have relied on plants as asource of food, flavours, fragrance and medicines. Even today a large number of people use traditional medicinal plants contaning mixtures of various compounds acting individually, additively or in synergy to improve health. Historically, species of the family Lamiaceae have enjoyed a rich tradition of use for flavouring, food, preservation and medicinal purposes, due to both their curative and their prevention properties. In Lamiaceae each species has a special, complex mixture compounds in which each component contributes to its overall bioactivity. The important bioactive components in plants are usually the secondary metabolites such as alkaloids, flavonoids, tannins and other phenolic compound.

The medicinal plants of Lamiaceae has an important value in the socio-cultural spritual and medicinal use in rural and tribal lives of the developing countries. They are known to be used by 70% to 80% of global population for their medicinal therspeutic effects as estimated by WHO. Throughout the ages, humans have relied on plants as a source of food, flavours, fragrance and medicines. Even today a large number of people use traditional medicinal plants contaning mixtures of various compounds acting individually, additively or in synergy to improve health.

In Lamiaceae the aromatic essential oils are mostly present in leaves, however they can be found in all above ground parts of the plants. They are valuable in cosmetic, flavouring, fragrance, perfumery, pesticide and pharmaceutical industries. The members of Lamiaceae family attracted particular unterest as many of them have domonstrated to be antiallergenics, antimicrobial and anti-inflammatory agents, enhancers of the gastrointestinal function, immune-modulates and stimulators as well as blood pressure and cholesterol orduring agent.

Lamiaceae is the sixth largest angiosperm family with about 236 genera and 7173 species distributed throughout the world in both temperate and tropical regions; its main distribution is in the mediterraean (Harley *et al.*, 2004). Lamiaceae is one of the most impottant families due to use in folk medicine and for the commercial production of essential oils. It includes 23 endemic species (A L Khulaidi, 2013). The members of lamiaceae are generally aromatic, includding anumber of widely used culinary herbs, such as basil, lavender, marjoram, mint, oregano, rosemary, sage, savory and thyme (Wink, 2003).

Medicinal plants have been identfied and used throughout human history.Plants have the ability to synthesize a wide varity of chemical compounds that are important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. The use of plants as medicine predates written human history. Ethnobotany is

recognized as an effective way to discover future medicine (Fabricant and Farnsworth, 2001). All plants produce chemical compounds as part of their normal metabolic activities. These phytochemicals are divided into primary metabolites such as sugars and fats, which are found in all plants; and secondary mrtabolites compounds which are found in a smaller range of plants, serving a more specific function. (Meskin and Mark, 2002).

Tulsi is described as a "holy medicinal plant" in ancient literature. The medicinal values of Tulsi are well documented in the Hindu Mythology and considering the health beneficial effect of Tulsi our ancestors in India has insisted, to plant a Tulsi sapling in each and every household. While recognizing the importance of broadenting western medicinal perspective, the WHO organization has recommended that, by integrating the traditional health and folk medicine systems with modern medicine therapies, can address the health problems more effectively worldwide (Priyabrata Pattnaik *et al.*, 2010).

According to estimations, up to 70,000 plant species are used ethanomedicinally worldwide. Effects of herbal extractes have been studied by different pain tests, including writhing test, light tail flick test, tail immersion test, hot-plate test, and formalin test (Faizul haq F *et al.*, 2012). Plants characterize a vast natural supply of appreciated compounds that might achieve primary importance for the expansion of novel drugs. The survey of the effectiveness of the plant-based remedies used in the folk medicine has given great reflections because they are cheep ad have reduced side effects (Kakosti B *et al.*, 2013).

The Lamiaceae family, one of the most important herbal families, incorporates a wide variety of plants with biological and medical applications. The most known members of this family are a variety of aromatic spices like thyme, mint, oregano, basil, sage, savory, rosemary, self-heal, hyssop, lemon balm, and some others with more limited use (Bekut M et al., 2017). Undeniably, medicinal plants have been widely utilized as healing modalities for both

preventive and curative purposes. They play an extremely crucial role in human health. In recent years, there has been a growing trend in the world population with as many as 80% of people globally relying on the use of herbal medicinal products and supplements for their primary healthcare needs (Schuster, 2001).

This increasing demand and interest in the use of herbal medicinal products has encouraged new drug discoveries and developments (Ekor M, 20113). In fact, many active ingredients of new drugs are derived from medicinal plants proven to be remarkably important in aiding drug discovery and development (Katiyar et al., 2012). Hence, studies need to be actively conducted on plants in order to identify possible candidates as safer and effective anti-allergic agents in future.It is also an important herbal family which comprises a wide array of plants with biological and medical applications (Uritu *et al.*, 2018).

The Lmiaceae or labiatae are a family of flowering plants commenly know as mint family. The enlarged Lamiaceae contain about 236 genera and more than 7,000 species. The family is particularly important to humans for herb plants useful for flavour, fragrance, or medicinal properties. *Leucas aspera* and *Anisomelus malabarica* are ethnomedicinal plants of Lamiaceae, commenly used in Inian tradional system of medicines. Traditionally these plants were used in the form of extreacts, powder, paste by tribal populations of India for treating common ailments like cough and cold, fever, skin disease, pains, antimicrobial, antifungal, analgesic, etc.

In view of these fact, the present invegstigation is undertaken.

- To elucidate the macroscopic characteristic of *Leucas aspera* and *Anisomelus malabarica*
- To observe the floral characteristic of *Leucas aspera* and *Anisomelus malabarica*

- To study the microscopic characteristic of stem and leaves of *Leucas aspera* and *Anisomelus malabarica*

REVIEW OF LITERATURE

Plants have been shown to have genuine utility and about 80% of the rural population depands on them as primary health care (Akinyemi, 2000). In developing countries, notably in West Africa, new drugs are not often affordable. Thus, up to 80% of the population uses medicinal plants as remedies (Kirby, 1996). The important bioactive components in plants are usually the secondary metabolites such as alkaloids, flavonoids, tannins and other phenolic compounds (Raj et al., 2013). The Lamiaceae famiy has an almost cosmopolitan distribtion and is one of the major sources of culinary, vegetable and medicinal plants all over the world (Naghibi et al., 2005).

Viroj, 2011 analysed that Dengue fever and malaria are the two common mosquito in tropical and sub-tropical regions that are very important and cause high morbidity and infections mortality for many patients. Since ancient times, several plants and plant products have been used locally to repel or kill or control mosquitoes which case fever and malaria. agent. plants have such important biological and pharmacological activities also due to the triterpene acids that also exhibit anti- inflammatory, antiviral, cytotoxic and cardiovascular effect (Silvia *et al*., 2012).

Choudhary *et al*. (2011) analysed Medicinal plants used for the therapy of epilepsy in traditional medicine havebeen shown to possess promising anticonvulsant activities in animal models of anticonvulsant screening can be an invaluable source for search of new antiepileptic compounds. A. malabarica has numeroustherapeutic utilities in folk medicine. The ethylacetate extract of A. malabarica leaves. It was founded that the extract of A. malabarica leaves has antiepileptic capability.

Leucas aspera is a well known medicinal herb belonging to the family Lamiaceae (Labiatae). It is commonly known as 'Thumbai' and distributed throughout India. At some

places it was also known as 'Dhronpushpi'. It is used as antipyretic, stimulant, expectorant, aperient, diaphoretic, antirheumatic and insecticidal (Chopra et al., 1956). It is also used for snake bite and Migraine (Revathi and parimalazhagan, 2010). Leucas plant is useful in bronchitis, inflammation, asthma, dyspepsia, pparalysis and leucoma. The leavs are useful in fever and urinary discharge.

Plant is also used by tribes of Tamil Nadu (southern India) for treating bronchitis dysentery, diarrhoea and skin diseases (Ignacimuthu, 2006). Besides India, other Asian (Sri Lanka, Taiwan); Central and South American (Mexico, Venzuela, colombia, Cuba, Nicaragua, Guatemala) and African countries (Nigeria, Togo, Ivory, Coast, Kenya) also use this ethanomedical plant for treating dysentery, hemorrhoids, malaria, venereal diseases, ulcers, renal inflammations fever and asthma (Dinda *et al.,* 2015*).*

Jeyachandran *et al.* (2007) showed essential oil of Anisomeles malabarica was found to have anti-allergic properties. Anisomeles malabarica has crucial chemical compounds which are produce antispasmodic agent. Choudhary et al. (2011) analysed medical plants used for the therapy of epilepsy in traditional medicine have been shown to possess promising anticonvulsant activities in animal models of anticonvulsant screening can be an invaluable source for search of new antiepileptic compounds. A. malabarica has numeroustherapeutic utilities in folk medicine. The ethylacetate extract of A. malabarica leaves. It was founded that the extract of A. malabarica leaves has antiepileptic capability. Krishnappa and D.G Basavaraj (1982) reported that the base chromosome number of anisomelous malabarica is 16.

Subba reddy *et al.* (1989) anisomelous appears to be capable on spontaneous autogamy by recurving stigmatic lobes into the pollen remained in underlying anthers shortly before the stigma becomes inactive and pollen becomes inviable in the absence of pollinator mediated cross -pollination. This was experimentally demonstated in Anisomelous malabarica and Anisomelous indica by bagging unmanipulated flowers. Plants are both self and cross

compatible and self and cross pollinating. Fruit and seed production higher cross-pollinated flowers.

ATOMICANAL STUDIES

Anatomy along with plant morphology always treated as the bakebone of plant taxonomy and systematic elucidated the plant diversity, phylogeny and evolution following these traits (Endress *et al.*, 2000). Morphological and microscopic studies of leaves act as are reliable aid for detecting adulteration. These simble but reliable standards will be useful to a lay person in using the drug as a home remedy (Khandelwal, 2007). Microscopy is an important tool for authentification of crude drugs and study of powdered drugs (Ponnudurai *et al.*, 2011).

Morphological and anatomic characters of leaf are used as taxonamic markers to assist in the correct identification of the plant species. Some particular groups of plants or taxa seem to be characterized by specific type of cross sectional anatomy, epidermal features, which are the epidermis, stomata, gland and trichomes (Park, 1994).Glandular trichomes producing essential oils of commercial value are widespread on leaves and flowers of lamiaceace (Akcin et al., 2011 and Kaya et al., 2007). Martin *et al.* (2009) examined the number of secretary cells, the length of stalk cells , density , location and arrangement of these glandular trichomes in the epidermis can vary. Their structure and ultrastructure of vegetative and reproductive organs have been widely spread on literature .

Pandey and Misra, 2014 reported that traditionally, plant taxonomy has depended mainly upon comparative morphological features because the help in taxa delimitation and identification. Cantino, 1990 mentioned that both hypostomatic and amphistomatic leaves are found in the members of the family, the presence of the latter type of leaves being slightly more frequent. Ascensao *et al.* 1995 identified that glandular the morphology, distribution and

frequency of glandular trichomes are used as discriminative characters at subfamillier level in the Lamiaceae.

Anatomical data are applied to improve classificationschemes and it is often used for identification. Wide range of anatomical data is used by systematists including anatomy ftom stem, lesf, petiole, stipule, node, flower, fruit, seed etc. Often these anatomical features are correlated with environmental factors (Naskar, 2016). Establishment of the pharmacognostic, morphological and microscopical characters of leaves and bark of the plant will assist in standardization, which can guarantee quality, purity and identification of sample(Karthikayan, 2012).

It is important to interpret morphological and anatomical descriptions of crude drugs as well as characteristic features of drugs and adulterants of commercial significance (Dharmesh *et al.*, 2010). Cutler *et al.* (2007) reported that in systematic anatomy has a long histery since the invention of microscope. Taxonomists found anatomical similarities among related plant groups. The leaf epidermis is generally a valuable character for the classification and delimitation of species and genera, and/or for the discussion of relevant phylogenetic problems (Jones, 1986).

Trichomes are widely distributed in the vegetative and reproductive parts of plants of lamiaceae and distinguished as and nonglandular trichomes (Navarro and Elqualidi, 2000). Venkateshappa and Sreenath, 2013 discribed that the glandular hairs and their distribution , stomatal distribution and other anatomical features provide significant taxonomic information. The usefulness of the structure of the vascular bundles in petioles for specie identification in the family lamiaceae has been demonstrated (Metcalfe and Chalk, 1972).

The anatomy, morphology and trichome distripution on the aerial parts of Lamium truncatum boiss. Were studied in order to understand the usefulness of these characteristics for

systematic purposes. Aytas akcent chamilli and camili B. 2018.reported that micromorphological and anatomical characters of the Turkish endemic Marrubium trachydium boss.

Many species of lamiaceae have been used as herbal teas in Turkey and most of them have great importance due to their economic values and many of them are used as raw material in cosmetic industry.Some species are used in official and traditional medicines in Anatolia,Europre and China (Van whk *et al*., 2003). Lamium species are used in official and treditional medicine in Anatolia , Europe and china possessing antioxidant, anti-inflammatory, blood tonic, uterotonic, antiplasmodic, antiseptic, trauma, hypertension,Chronic bronchitis, pharyngitis and other properties.(Bremness, 1995; Baytop, 1999).

Alkhulaidi, 2013 reported that In Yemen, Lamiaceae is one of the most important families due to use in folk medicine andfor the commercial production of essential oils. The members of Lamiaceae are generally aromatic, including a number of widely used culinary herbs, such as basil, lavender, marjorum, mint, oregano, rosemary, sage, savory and thyme (Hussain *et al*., 2008).Marrubium has been used as a treditional medicine for asthma, pulmmonary infections, inflammation and hypotension and also as pain reliver (Meyer-silva and Cechinel-filho, 2010).

MATERIALS AND METHODS

COLLECTION AND IDENTIFICTION OF PLANT MATERIALS

The fresh plant materials of *Leucas aspera* and *Anisomeles malabarica* are colleted from the Harbour beach road and college campus in the month of January 2020. The plants are identified with the help of local floras. The collected plants are presreved as per the standard procedure (Jain and Rao, 1977). Voucher specimens of all the selected taxa are deposited and preserved in the St. Mary's College Herbarium (SMCH), Research Centre for plant sciences, St. Mary's College, Thoothukudi, Tamil Nadu, India.

Macroscopic studies

Macroscopic evaluation is the method of qualitative evaluation established on the study of morphological and sensory profiles of whole plant. Fresh, full-grown and healthy plant of both species are collected and washed in pure water to remove all the impurities. The samples are subjected to macroscopic evaluation by abservation with naked eyes. A magnifying lens with a dissecting microscope is used for a better evaluation of surface characters.

Morphology

Study of plant morphology is the first step in the medicinal plant research and is useful in the identification of plants. The morphological characters like plant height, leaf size, shape, phyllotaxy, inflorescence type, flower colour, floral feature, fruit, seed characters and other important features of selected plants are noted.

Floral description

To study the floral charctersitics of *Leucas aspera* and *Anisomeles malabarica* the flowers are cut and separated with the help of needle. Floral parts are examined under simple microscope.

Organoleptic character (Khandelwal, 2003)

Oraganoleptic evaluation can be done by means of organs of sense which includes the characters like colour, shape, odour, taste, surface characteristics and texture and there by some specific characteristics of the material which can be considered as the first step towards establishment of identity and degree of purity of the drug are observed and noted.

Microscopic studies

The microscopic evaluation is used for studying the anatomical features of transverse section of stem and leaf of *Leucas aspera* and *Anisomeles malabarica*. Enough number of sections are taken by hand using razor blade. The sections are carefully transfered to a petridish containg water and few thin sections that floated in water are selected. Then selected sections are stained in saffranin. A stained section is carefully transferred on a clean glass microslide using this brush. With the help of a forceps and a needle a clean cover slip is placed gently over the section. With the help of a bloting paper excess glycerine is removed and the slide is observed under a digital microscope.

Epidermal peel

Healthy leaf from the selectd plants are removed and with help of forceps the peel is removed from the upper and lower surface of the leaves. The peel is allowed to remain in a watch glass holding water for some time. The peel is stained by adding some drops of safranin through a dropper. After 2-3 minute the peel is taken out and placed on the clear glass slide.

Slide is examined first under low power and then under high power magnification of a compound microscope.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs whenever necessary. Photographs of different magnifications are taken with Nikon Laphot 2 microscopic unit. For normal observations bright field is used. Magnifications of figures are indicated by scale bars.

RESULT AND DISCUSSION

Indigenous knowledge had a role to play understanding coexistence of man with fauna and flora over centuries. In spite of great importance biological diversity and the amount of attention is currently being given to traditional medicine at both national and international levels. Many of these biological diversities are used in local tradicional medicine and have been reputed through experience inherited from one generation to the other.(Ibrahim *et al., 2007*). In the present investigation, *Leucas aspera* and *Anisomeles malabarica* belongs to the family Lamiaceae have been subjected to macroscopic, microscopic and floral character analysis.

Systematic position

In Benthan Hooker's classification, the systematic position of ***Leucas aspera*** and ***Anisomeles malabarica*** is as follows.

Kingdom	-	Plantae
Class	-	Dicotyledons
Sub class	-	Gamopetalae
Order	-	Lamiales
Family	-	Lamiaceae
Genus	-	*Leucas* , *Anisomeles*
Species	-	*L.aspera* , *A.malabarica*

Plate 1 : *Leucas aspera*

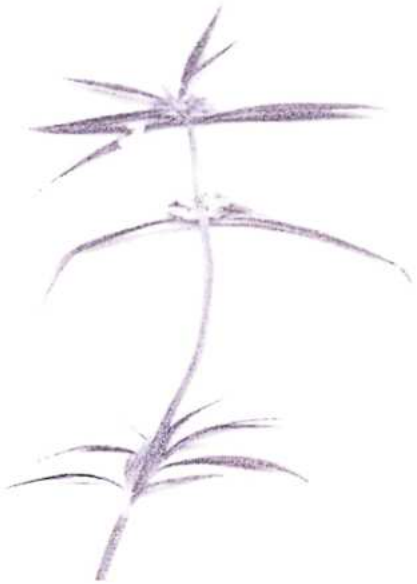
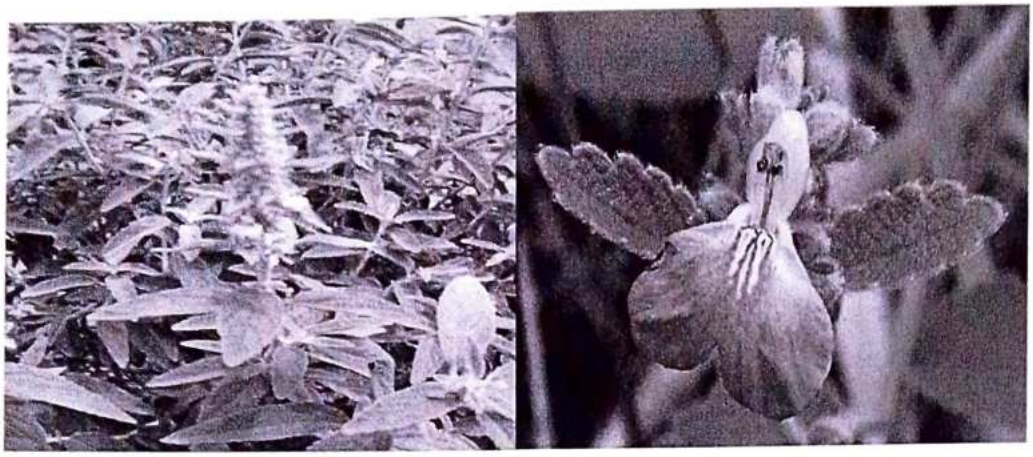


Plate 2 : *Anisomeles malabarica*



Macroscopic Characters

***Leucas aspera* (willd.) Link**

It is an annual herb or undershrub, that can reach heights of 15–60 cm; stem is quadrangular, much branched, hispid or scabrid; Leaves are opposite, short petioled, linear or narrowly oblong- lanceolate, entire or distantly crenate, obtuse, narrowed at the base. They can reach up to lengths of 8 cm and be 1.25 cm broad. The length of petiole is typically 2.5–6 mm. flowers are held together in auxiliary whorls or dense terminals. They contain 6 mm long bracts equaling the calyx that are bristle-tipped, linear, acute and are "ciliate with long slender hairs". Calyx 8-13 mm across,triangular; The corolla is 1 cm in length and the tube is 5 mm in length, "densely white-woolly", upper lip is approximately 3 mm in length and the lower lip is approximately 6 mm in length. Seeds are nutlets that are brown.(Plate 1)

Flowering and Fruiting : February to March

Distribution : in dry, open, sandy soil and is abundant in areas with waste.

Vernacular name : Thumbai

***Anisomeles malabarica* (L.)R.Br ex Sims**

Perennial, semi-shrubby herb; stem to about 2 m hight, much branched from base, subquadrangular, thickened below to 1.8 cm in diam. Leaves ovate-lanceolate to oblong-lanceolate, 3.6-16 x 1.3-7 cm, narrowed and rounded at base, acute, slightly bullate and velvety lanate above, densely so beneath; petioles 0.7-3.5 cm long, lanate. Floral leaves 8-10 mm long, Bracts linear, to 5 mm long. Calyx to 9 mm long; tube to 5 mm long, lanate without, glabrous within; lobes lanceolate, to 4 mm long, acuminate. Corolla 1.4-2 cm long; tube to 9 mm long, white, glabrous without; throat pilose towards base of lower lip; Upper lip oblong, 4-6 mm long, obtuse at apex, slightly arched, whitish; lower lip to 1 cm across, coral pink, with 2 white

streaks towards base, pilulose without with gland-tipped hairs, the lateral lobes shallowly rounded. Style glabrous; unequal. Seeds are Nutlets ovoid, blackish-brown and shining.(Plate 2)

Flowering and fruiting : November to December

Distribtion : widely distributed.

Vernacular Name : Malabar catmint, perumthumpai.

Organoleptic characters

The macroscopic characters such as size, shape, margin, apex, surface, colour, odour, taste, nature, texture are studied for morphological investigation. Organoleptic characters of *Leucas aspera* and *Anisomeles malabarica* are examined and presented in Table 1 and Table 2.

FLORAL CHARACTER

Leucas aspera

Inflorescene

Verticillaster, flowers white, small and directly attached to the base without peduncle or stalk. The flowers are held together in auxiliary whorls or dense terminals. They conatin 6mm long bracts equalling the calyx that are bristle-tipped, linear acute and are "ciliate with long slender hairs".

Flower

Complete, bisexual, irregular, zygomorphic, hypogynous, pentamerous, white.

Table1: Organoleptic character of *Leucas aspera*

Colour	Green,upper side darker then lower side
Shape	Linear-lanceolate
Odour	Slight odour
Texture	Slight smooth
Taste	Slightly bitter
Apex	Acute
Base	Cuneate
Petiole	2.5-6 mm long
Leaf arrangment	Simple, apposite-decussate
Margin	Entire-serrate
Venation	Reticulate

Table2: Organoleptic character of *Anisomeles malabarica*

Colour	Greenish gray in colour
Shape	Ovate
Odour	Strong camphor
Texture	Softly pubescent
Taste	Bitter
Apex	Acute
Base	Asymmentric base
Petiole	0.7-3.5 cm long
Leaf arrangment	Opposite-decussate
Margin	Serrate
Venation	Reticulate venation

Calyx

Sepals 5, gomosepalous, 10 nerved, tubular, curved, 6-10 toothed, contracted at the mouth, glabrous below, ribbed and scabrid above; mouth oblique, produced on the upper side; teeth short, triangular, spinulose, ciliate, the upper one is the longest and 8–13 mm (0.31–0.51 in) in length.(plate 3a)

Corolla

Petals 5, gamopetalous, bilabiate; tube annulate; lower lip 3 fid, spreding, mid lobe large; upper lip 2 fid, erect, concave, villous outside, white. The corolla of *Leucas aspera* is 1 cm (0.39 in) in length and the tube is 5 mm (0.20 in) in length. It is annulate in the middle portion and pubescent on the upper region. The corolla is "densely white-woolly", upper lip is approximately 3 mm (0.12 in) in length and the lower lip is approximately 6 mm (0.24 in) in length. The middle lobe is rounded, obviate and the lateral lobes are subacute and small in size.(Plate 3b)

Androecium

Stamens 4 , epipetalous, didynamous, ascending, the upper pair shorter; anthers connivent, cells divericate, ultimately confluent.

Gynoecium

Carples 2, syncarpous, ovary superior, 2 celled but at maturity four celled due to the formation of septum, axile placentation, 1 ovule in each chamber; style gynobasic, long; stigma bifid subulate, upper lobe minute or obsolete.(Plate 3c and 3d)

Anisomeles malabarica

Inflorecence

Sessile, white, crowded in dense, globose, about 2-3.5 cm across, surrounded by numerous foliaceous bracts, thin, lanceolate, acute, ciliate, 1.2-1.5 cm long and 0.3-0.35 cm

wide: calyx, tubular, slightly curved, 1-2.25 cm long, glabrous in lower part, hairy on upper part, 10 dentate with a villous throat; corolla, white, 1.7-2 cm long, bilipped, upper lip about 4 mm long, wooly, lower lip nearly twice as long as upper one: lateral lobes small.

Flower

Complete, bisexual, irregular, zygomorphic, hypogynous, pentamerous, purple

Corolla

5 petals, gamopetalous, bilabiate, 2 lobes in one whorl and 3 in other, imbricate aestivation, inferior.

Calyx

5 sepals, gamosepalous, bilabiate, 2 lobes consisting one lip and three the other lip, petaloid, imbricate, aestivation, inferior (Plate 4a)

Androecium

4 stamens, posterior, epipetalous, short filament connected to a long connective, lowernend of connective is flat, short and steril, anthers bicelled, introse.

Gynoecium

2 carpels, syncarpous pistil present on necter secreting disc, ovary, superior, bilocular, when young, but becomes tetralocular in the later stage, single ovule in each locules, axile placentation, gynobasic style, bifid stigma.(Plate 4b and 4c)

Plate 3a : Calyx *Leucas aspera*

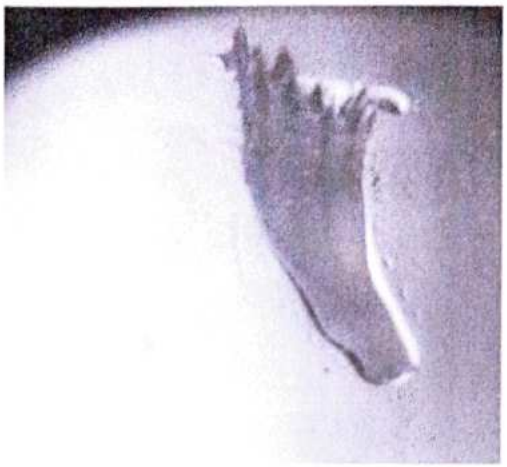


Plate 3b : Corolla Cut open *Leucas aspera*

Plate 3c and 3d : Gynoecium and C.S of ovary *Leucas aspera*

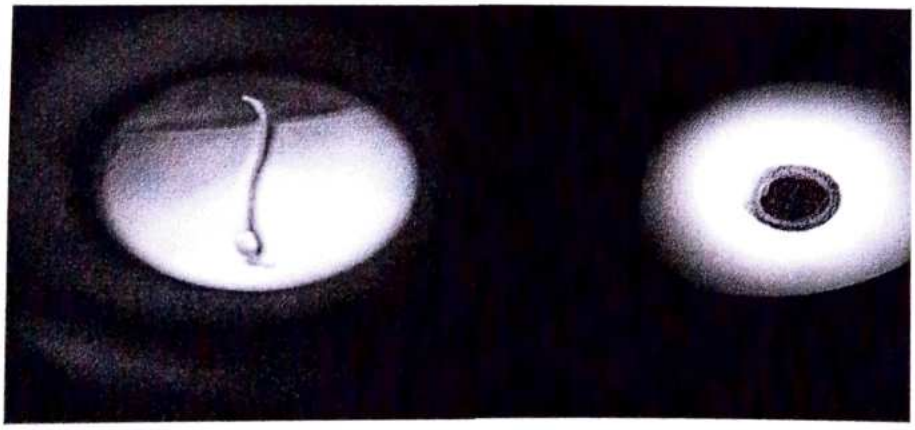


Plate 4a : Corolla cut open *Anisomeles malabarica*

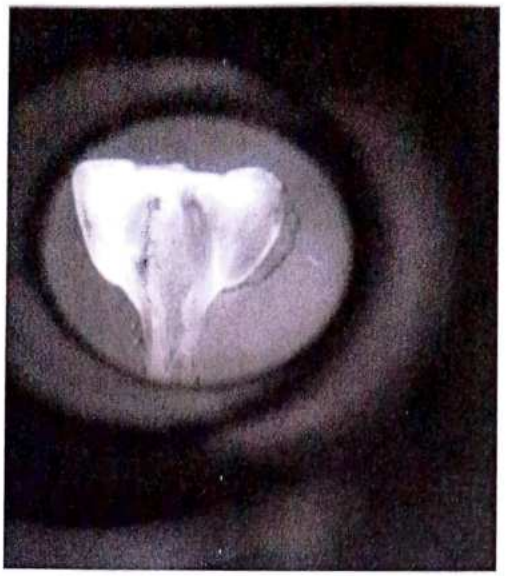
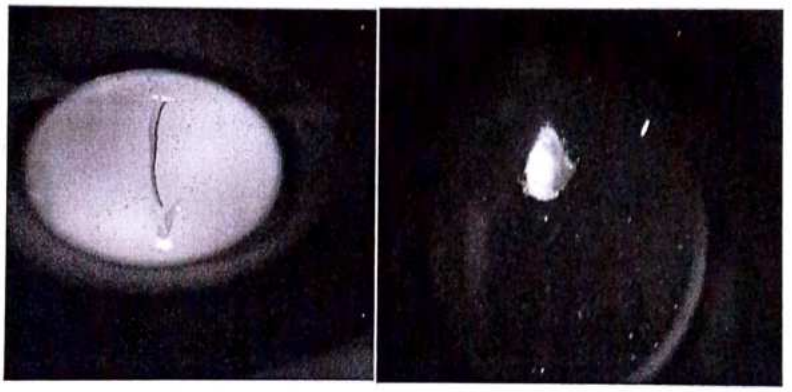


Plate 4b and 4c : Gynoecium and C.S of ovary



Microscopic characters

The microscopic evaluation of transverse section of stem, trichome and leaf of ***Leucas aspera*** and ***Anisomeles malabarica*** are carried out.

Leucas aspera

Stem

Transverse section of stem was quadrangular with clear cut lobes and was longitudinally ribbed and furrowed. Stem surface showed wide opened stomata and numerous hairs. Hairs were glandular, straight and unicellular or whip like multicellular Parenchyma. Endodermis was distinct with barrel shaped cells. Pericycle was in the form of two layered parenchyma. Four large vascular bundles lie underneath the 4 ridges while the small ones were located in the furrows. Pith was of thin walled parenchyma . Crystals of varied shapes – needle shaped , cuboid and rhombohedral were observed in the cells of pith.

Presence of glands and exudates on stem surface indicate the origin of aromatic substances of medicinal value from glands and their subsequent polymerization in the form of exudates. Presence of lenticels and exudates near them also indicate the probability of the production of certain chemical entities within the stem and their polymerization on stem surface after their exit through lenticels. Chemical nature of these exudates, blebs on the hairs and stem and the crystals of varied shape within and on the surface is to be probed further and the same are in progress.(Plate 5a)

Trichome

Hispid, non-glandular, 2-3 celled, base flatted, bent, blunt length 123.80±2.37 µm. breadth 10.16±0.20 µm.(Plate 5b)

Plate 5a : T.S of stem *Leucas aspera*

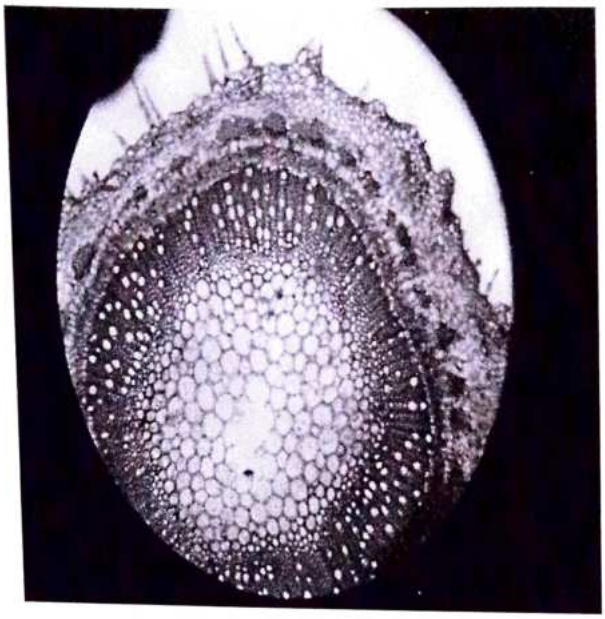


Plate 5b : Trichome *Leucas aspera*

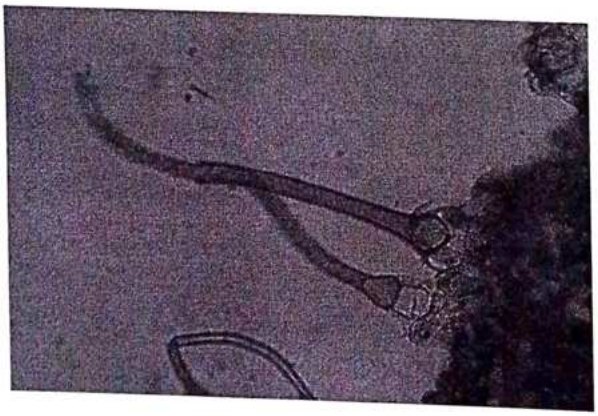


Plate 5c : T.S of leaf *Leucas aspera*

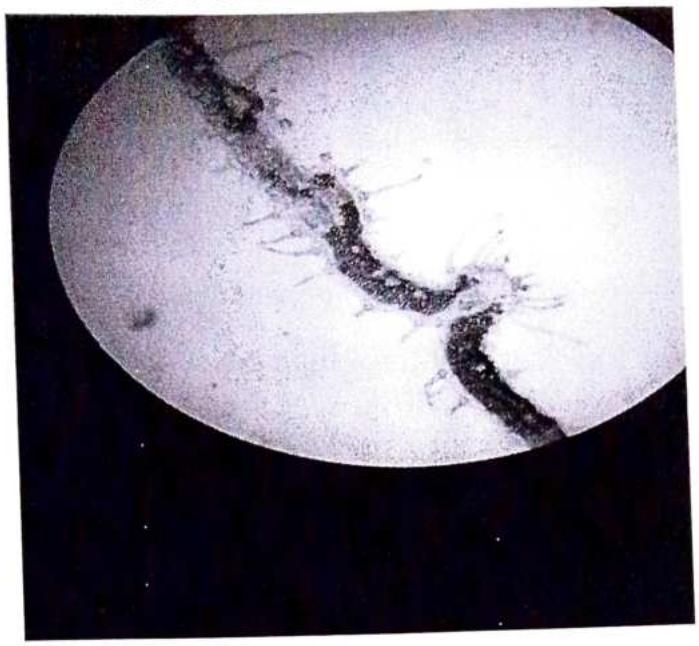
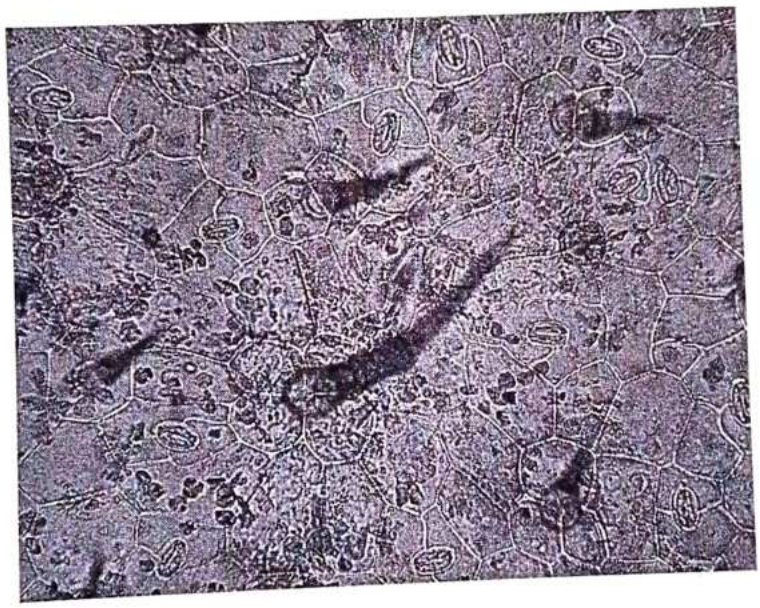


Plate 5d : Epidermal peel (upper) *Leucas aspera*



Leaf

leaf showed distinct midrib. Lamina showed 2-4 side veins. Single vascular bundles were noticed in both the midrib and side veins . Region below vascular bundle was filled with collenchyma. Epidermis was single layered followed by single layered palisade cells and parenchymatous mesophyll. Paracytic stomata were observed on both the surfaces. Isolated needle shaped crystals were noticed near the stomata on leaf surface. Three types of hairs were observed on both the surfaces. One with bulbous base bulgedmiddle region and tapered tip. Few were broad based with tapered tip (likenarrow triangle). The third type were with bulbous base with a curve and tapered tip.(Plate 5c)

Stomata

Traversed stomata.(Plate 5d)

Anisomeles malabarica

Stem

TS of stem is approximately same quadrangular in shape exhibiting 4 equidistantly placed pubescent ridges, central wide parenchymatous 4 angled pith encircled by a ring of xylem, very narrow phloem and collenchymatous hypodermis. The detailed TS of the stem are quadrangular shaped. The epidermis consists of single layer rectangular cells, and is surrounded by a thin cuticle layer, traversed with few stomata and bearing simple covering multicellular (2-3 cell) and glandular (non-covering) trichomes. The sessile glandular (non-covering). Cortex is collenchymatous, 2 to 4 layered but many more; reaching up to 10 beneath the primary rides. Endodermis is distinct. There are lignified sclerenchyma fibers between the cortex and vascular tissue. Cambium is indistinguishable. Central wide pith is parenchymatous.(Plate 6a)

Trichome

Long, uni-to tricellular trichomes with pointed ends.(Plate 6b)

Leaf

In the anatomical characters, the lower epidermis of the leaves are covered by a thick layer of cuticle. The epidermal cells are polygonal, equal in size and shape. Paracytic stomata are present in the both surfaces. The leaf mesophyll consisting of elongated palisade cells arranged in a single layer. The midrip portion of the leaf contains two layers of collenchymatous cells on both epidermis. Cortex consisting of single layered, round to angular collenchyma; parenchyma consists of thin-walled cells containing prismatic crystals of calcium oxalate. Vascular bundle arc-shaped, present in centter. The vessels in the vascular bundles are annular and spirally thickened. Numerous caryophyllaceous or diacytic stomata present in epidermise.(plate 6c)

Stomata

Stomatal index 16.6-40.5 on lower surface, 16.6-30.7 on upper surface; palisade ratio7-9.(Plate 6d)

Plate 6a : T.S of stem *Anisomeles malabarica*

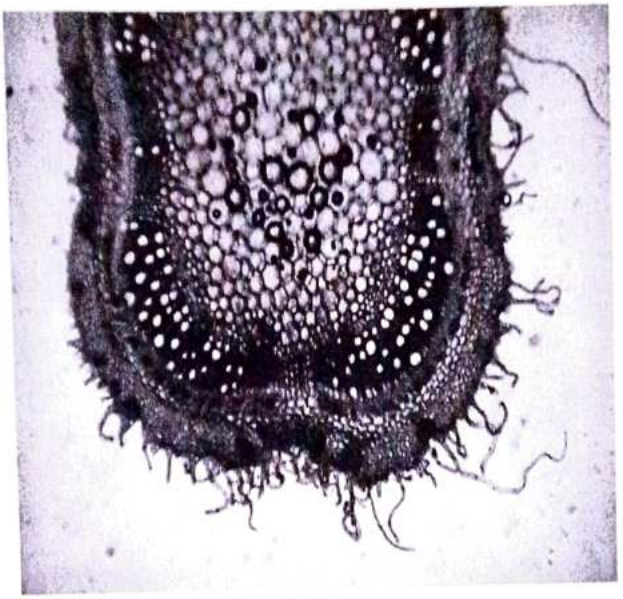


Plate 6b : Trichome of *Anisomeles malabarica*

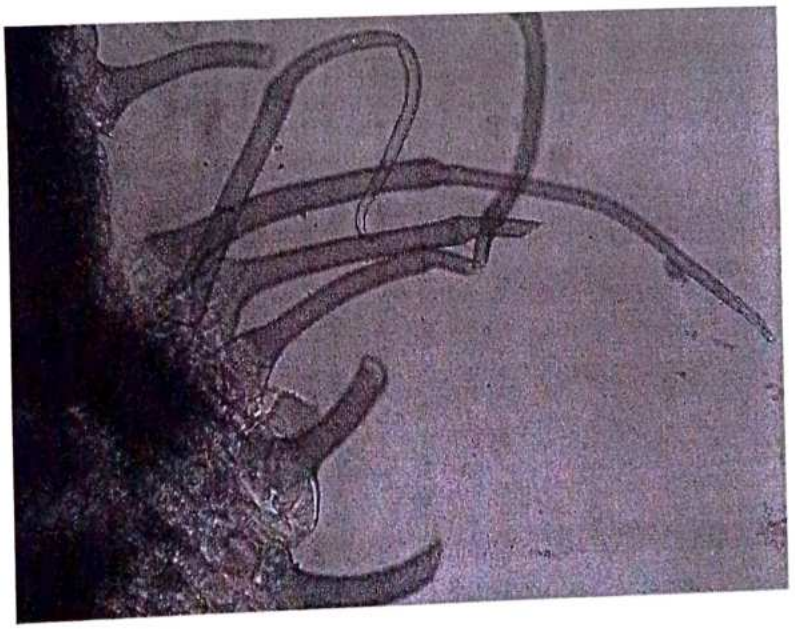


Plate 6c : T.S of leaf *Anisomeles malabarica*

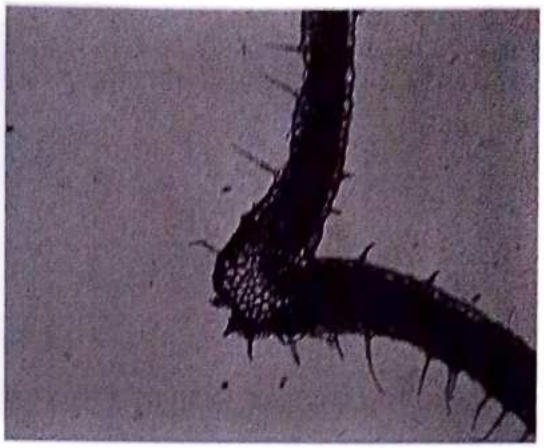


Plate 6d : Epidermal peel (upper) *Anisomeles malabarica*

SUMMARY AND CONCLUSION

The thesis entitled "Macroscopic and microscopic studies on selected species of Lamiaceae" deals with a systematic evalution of morphological and anatomical of *Leucas aspera* and *Anisomeles malabarica* belongs to the family Lamiaceae.

The present work is focused on the following aspects of the two selected plants.

- To elucidate the macroscopic characteristic of *Leucas aspera* and *Anisomelus malabarica*
- To study the floral characteristic of *Leucas aspera* and *Anisomelus malabarica*
- To study the microscopic characteristic of stem and leaves of *Leucas aspera* and *Anisomelus malabarica*

Plants are becoming potential sourse for phytoconstituents with varied pharmacological activities. Identification of such plants of potential use in medicine is of significance and as a prelude to this, it becomes necessary to examine the various pharamacognostical character of the plant before further investigation. In pharmacognostical studies the organoleptic charcters, marphology and anatomy ore carried out.

The various distinguishing features of two selected taxa observed through morpho anatomical study are

- In *Anisomels malabarica* the trichomes are long, uni or tri celled nonglandular trichome.
- The trichome of *Leucas aspera* is non glandular, unicellular short trichome.
- The stomata of *Anisomeles malabarica* is paracytic.
- The stomata of *Leucas aspera* is anomocytic.

The pharmacognostic standardization of the present study can be used as a standard in future research work to identify the two species of *Leucas aspera* and *Anisomeles malabarica*.

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Characterization of sea weeds and Effect of sea weed liquid fertilizer on seed germination potential of leguminous plants and growth parameters of Brassica juncia

A project submitted to

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

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVELI

In partial fulfillment for the award of the degree of

BACHELOR OF SCIENCE IN BOTANY

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THOOTHUKUDI JULY -2020

CERTIFICATE

This is to certify that the project entitled **"characterization of sea weed and effect of sea weed liquid fertilization on seed germination potential of leguminous plants and growth parameters of brassica juncia"** 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 2019-2020 by the following students.

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Introduction

Seaweeds are the macroscopic algae, grow in intertidal and subtidal regions of the sea, serve as an excellent source of food, fodder, fertilizer and industrial raw material for the production of phycocolloids like agar, algin, carrageenan (Chapman and Chapman,1980) and antimicronial agents (Padmakumar, 2002). Several thousand species of macroalge live in the oceans throughout the world, some are free floating and most are found attached to the substratum. All these seaweed are potential source of biofertilizer.

Biofertilizer is 100% natural. The best quality organic fertilizer is known to provide all the nutrient required by the plants and helps to increase the quality of the soil with a natural microorganisum environment. Biofertilizer contains a wide range of naturally chelated plant nutrient and trace elements, carbohydrate, amino acid and other growth promoting substance. The seaweed biofertilizer checks the leaching of minerals from the field to a considerable extent. Potash content is generally poor in sandy soil. Seaweed enhance the potash and mineral contents of the soil. Since they have gelatine substance, they improve the physical and chemical properties of the soils. The calcium carbonate present in the seaweeds increase the pH of the soil. The increase in the yield of barley, potato, coconut, palms, citrus was reported by Mehta et al.,in 1967.

Kelp acts as a soil conditioner by stimulating microbial activity in the soil which result in improved air water relationships in soil, improved fertility and makes soil less prone to compaction and erosion. Organic growers who use kelp in their regular fertility programing report increase in yield quality, self life and resistance to environment stresses such as drought,extreme heat, early frost, pest, and disease problems.

Seaweed are used in different parts of the world as fertilizer for land crops. In India, freshly collected and coastal ashore seaweed are used as manure for coconut plantation either directly or in the from of compost in coastal areas of Tamil nadu and Kerala. Seaweed manure has been found superior to farm yard manure. They are as a store-house of the important potash, ionic sulphate, trace eleents and growth substance, besides having every other element and radical required by plants. Seaweed manure seems to increase resistance to disease. Most of the nutrients including nitrogen compounds are in ionic from and a quick absorption by crops takes place and relative little is left to be broken down by soil microflora, thus preventing acid condition of the soil arising from the fermentation. In general the mineral diffuse out from the seaweed thallus rapidly. Yet another feature is that seaweed manure holds water and air at the same time and improve the soil in bath respect. Like other manures seaweed have a similar role but also contribute the requirement potassium, sulphur, phosphorous and calcium. (Durgude, et al.,1996).

Morden agriculture has managed to give spectacular increase in food production by the application of a variety of inorganic fertilizer added to the soil to boot production has resulted in damage to our soil. All these problems threaten the very basis of our survival. Therefore, there is a constant effort by scientists to use agricultural practice which are eco-friendly. Seaweed biomass can serve as an excellent source of organic manure because of its great potential in improving soil fertility, chealting activity and moisture retaining capacity (Crouch and staden, 1993).

The application of seaweed as manure is practiced in many European countries (Chapman and Chapman,1980). Addition of seaweed to soil has improved the binding properties and the water retaining capacity of the soil (Pachpande, 1991). Seaweed is beneficial as it has been proved to have increased the nitrogen amount in the applied fields and also the soil phosphorus,

potassium, exchangeable sodium and hydrogen ion concentration. Application of seaweed liquid fertilizer to crop has been found to increase seed germination percentage, yield of crops, resistance to frost and fungal attacks and uptake of the inorganic nutrients from the soil (Bhosle et al., 1975; Venkataraman kumar et al., 1993; Mohan et al., 1994; Sekar et al., 1995).

Recent researches established the seaweed liquid fertilizer are better substatitute for chemical fertilizer (Bhosle et al., 1975; Mohan et al.,1994) they not only have N, P, K but also have trace elements and growth regulator (Booth 1969). Today, SLF (Seaweed Liquid Fertilizer) has been widely used with excellent result in all kinds of plants in all countries. Therefore constant effort is taken by scientist to use SLF to agricultural practice as they are eco friendly.

Tuticorin coast situated along the Gulf of mannar is the excellent source of seaweed, which are being not utilized systematically for agricultural purpose, inspite of their significance in determining their function as biofertilizers. Array of literature also indicated that on step was taken about the study on biochemical constituents of seaweed and their role as biofertilizer on crop plants.

Scope and objectives

Scope and objectives

Eco friendly and scientific methods of crop production envisage the addition of SLF of which not only help in increasing the yield of crops, but also act as store-house of nutrients, improving the soil physical condition (Balasubramanuan et al., 1998). Keeping in mind the beneficial and adverse of the seaweed liquid fertilizer and also need of production of species of the socio-economic development of the farmers and the country, the present investigation is undertaken to evaluate the role of seaweed liquid fertilizers (SLF) on the growth and biochemical changes of *Brassica juncia (*L) W.D.J.koch.

The present study pertain to the effect of SLF on the germination growth and yield of Brassica juncia (L) has the following objectives

- Collection of seaweeds from GOM
- Characterization of seaweeds
- To study the effect of SLF on morphological character of Brassica juncia
- To understand the role of SLF on biochemical constituent of Brassica junccia
- Effect of SLF on leguminuos seed germination.

REVIEW OF LITERRATURE

REVIEW OF LITERATURE

Now a days the nutritional value make seaweeds a valuable fresh or dried vegetables or an ingredient for a whole range of healthy food. In Mexico the marine algae are used as emulsifier for a local drink as "Atole" (Espinoza 1994). Because of social and economic benefits, the rock seaweed could provide an alternative fishery at a time when tradional fisheries are experiencing serious problems. In addition to these, seaweeds are used as animal nutrition, as feed or as a supplement to fodder, fertilizer and for industrial production of phycocolloids. In recent years, research on the physiology and ecology of marine macroalgae has increased due to potential commercial usage (Bird and Benson, 1987).

Amongst marine natural product 'seaweeds' belong to the primitive group of non-flowering plants. Based on the pigmentation, seaweeds are broadly classified into green, brown and red algae (Muruga 2005). This plant from an important renewable resources in the marine environment and have been a part of human civilization from the time immoral (Sub Reo et al.,2006).

Seaweed is a multicellular marine algae, it's very important for the marine living renewable source, seaweeds wildly used for gelling and stabilizing agents for many food and pharmaceutical industries. Soil fertility it depends upon the fertilizer requirement of the plant or crop, now a day's lot of chemical fertilizers commercially available but the growth, yield and fertility of the soil are very much reduced/damaged. Recent
studies proved that seaweed fertilizer very much better than other fertilizer. The seaweed fertilizers contained a lot of nutrients such as nitrogen, phosphorus, potash and plant growth hormones and trace element. This seaweed fertilizer is used to enhance the seed germination and plant growth/crop yield.

Seaweed contains all the trace elements and growth hormones required by plants. Recently there is a growing concern over the use of seaweed liquid fertilizer (SLF). Today, there is a high demand for environment friendly agriculture for production of quality and healthy food to nourish the increasing population. The present study investigates the effect of seaweed extract, from Ulvalactuca, Sargusum wightti, Kapphaphycus alvarezii and Gracilaria verrucosa on the seed germination and plant growth of Capsicum annum. The Seaweed Liquid Fertilizer (SLF) was tested both with and without chemical fertilizers.The experiment was arranged in randomized Complete Block design (RCBD) with nine treatments four replications. Once a week a different Seaweed Liquid Fertilizer(SLF) individually and different SLF concentrations in combination with Recommended rate of Chemical Fertilizer (RCF) were applied to plants after germination and transplanting. Their performance was recorded once in two weeks. The average maximum fresh shoot height (15cm) and the dry weight (0.856g) were observed in application of foliar spray SLF75% plus RCF extracted from Sargussum wightti and Kapphaphycus alvarezii respectively.Seaweed extract 75% SLF withRCF increased significantly to root dry weight, number of leaves, number of flowers, number of pods, pod length over the control respectively in seaweed extracts from Sargussum wightti, Gracilaria verrucosa,Sargussum wightti and Ulvalactuca. The seaweed liquid fertilizer of each species with 100% (SLF only) foliar application was less effective on increase in above parameters than combined fertilizers (SLF plus CRF), but also has significant effect on increase in shoot height and other growth parameters when compared to control and recommended rate of chemical Fertilizer (RCF) only. Therefore, it could be concluded that combined effect in (SLF 75% plus CRF) foliar application could have met the requirement of micro nutrients in chili crop than SLF individually. The SLF -75% plus CRF foliar application of Sargussum wightti significantly increased several growth parameters such as shoot weight, root weight, number

of leaves and number of pods when compared to the extracts preparation from other species. SLF applications in combination with CRF are proposed to be used for enhance the growth, yield and quality of Capsicum annum. (Pradeepa Jayasinghe on 05 November 2016).

Seaweeds are used as manure or plant nutrient because relatively high in N2 potash and low in phosphorous content. The plenty of nitrogen available is not readily used by plants, hence used farm yard manure. They are free crops and cabbage (Chapman and Chapman, 1980). Apart from these, plant growth regulators like Auxin, cytokinin and giberelin are also present (Tay et al., Paier et al.,1993, Duan et al., 1995,Sekar et al., 1995, Peez-sanz et al., 1996, strik and staden 1997, moller and smith, 1998a, b and 1999).

In the present study, intensive investigation was made on the effect of seaweed liquid fertilizer (SLF) of *Codium* decorticatum on the seed germination yield biochemical and pigment characteristic of *Capsium annum* under laboratory conditions and in pots. Different concentrations such as 10%, 20%, 30%, 40% and 50% of SLF were prepared using distilled water. The seeds were soaked in 10 h for each SLF concentration then placed in separate Petri plates. Similarly, water soaked seeds were used as controls. Application of a lower concentration (20%) of SLF Showed maximum seed germination, fresh weight, dry weight, root and shoot length, number of branches, leaf area, number of pods andcontent of total chlorophyll, chl a, and chl b, protein, carbohydrate and lipids were observed. Therefore, the results of the present study suggested that the SLF of *C. decortianum* could serve as an alternative bio-fertilizer as is eco-friendly, cost-effective, deliver substantial economic and environmental benefits to farmer. (Acta Ecologica SinicaOctober 2019, Pages 406-410).

Seaweeds contain valuable trace elements viz Mn, Bo, Ba. The manurial value of seaweeds was not only related to NPK content but also of hormonal or trace

element content in view of unusual properties of promotion of seed germination. Increased frost hardness, increased resistance to fungal and insect pests was also reported by Booth in 1969. Generally seaweed extracts are effective positively at low concentration (Bhosie et al., 1975; Mohan and Venkataraman Kumar et al., 1993; Venkataraman Kumar et al., 1993).

Seaweeds constitute one of the commercially important marine living and renewable resources. The abundant, diversified seaweeds have been used in the field of agriculture and industry. The use of seaweeds as manure has been recognized for a long time in other countries . It is mainly the large brown algae, wracks and oar weeds that are used for manure. Drift weed that collects on the shore usually contains an admixture of red algae, green algae and rarely brown algae. There is much utilization of the Fucus frift, so much so that it is even used as litter and afterwards put on the field along with the stable manure. Large red algae are used as organic fertilizers (Kingman and Moore, 1982; Bhosle et al., 1975 Rajeshwari et al., 1983, vijayalakshmi and lakshmanan, 1988).

Along with population rise, there should be a rise in the agro product as well. Though the fertilizer industries are fast growing to compete with the rise in food production, the rise in fertilizer product is not up to the mark (Jeswani,1999). According to the World Economic Outlook report, World fertilizer nutrients consumption is estimated to reach 186 900 000 tones in 2014, up by 2.0 percent over 2013. World demand for total fertilizer nutrients is estimated to grow at 1.8 percent and is forecast to reach 50.21 million tones by 2020.

Seaweeds posses both saturated and unsaturated fatty acids in considerable amount, *Padina boergesenii* contains high amount of plamitic acid and the unsaturated fatty acids, oleic acid and linolleic acid compared to the other seaweeds (Vasanthi et al., 2003; venkatesalu et al ., 2003). Highest lipid content

was reported in *stochospermum marginatum* (Marilh et al., 1983; Parekh et Al., 1983; Sobha et al., 19878, Ganga Devi et al., 1996). The formation of fatty acids in algae is controlled by environmental factors such as light, temperature and availability of nutrients and is dependent on the growth phase, age and also on the nature of the algae (Nichols, 1965).

The seaweeds are used agriculturally and horticulturally in many countries as seaweed meal and liquid extract. While seaweed meal takes months to become fully effective in soil as plant nutrient because of it carbohydrate material which has to be broken down by before it can be used the polysaccharide content is already broken down, becomes effective at once (Stephenson., 1974).

The seaweed *Gracilaria coricota* powder was diluted with distilled water and different concentration were sprayed on the seedlings of *Phaselous mango* and *Pennisetum typhoides* were grown in different soil. The growth parameters such as shoot length, root length, shoot length and dry weight, root fresh and dry weight was increased with the optimum concentration of seaweed extract treatments in both black gram and cumbu. (Murugalakshmikumari, et al., 2020).

The seaweed liquid fertilizer of *Padina povonica* and *Sargassum plagiophyllum* when applied on drought stressed black gram *Vigna munga* accumulating more amount of coluble effect (Venkataraman Kumar and Mohan, 20000. The effect of fresh extracts obtained from green seaweed *Enteromopha intestinalis* showed maximum activity in terms of increase in seed germination, length of main root and shoot system and also increased chlorophyll content in Sesamum indicum (Gandhiyappan and Perumal, 2001).

The seaweed liquid fertilizer of *Sargasssam wightii* increased the protein, amino acid, total sugar content more in the leaf tissues then in the stem and root at 10% concentration at all the samples. The protein content increase at gradually with increased concentration of SLF socked plants of *Vigna radiatea* upto 10%

concentration are other higher concentration should the decreasing trand in the SLF of *Sargassum wightii* promoted the biochemical constituents at the lower concentration of 10% on *Vigna radiatea*. (Sivasankari et al.,2006).

The seaweed liquid fertilizer prepared from *spatoglasum asperum*, *Stoechospermum marginatum* and *sargassum sp*. Promoted the growth and fruitification if chilies, turnips and pineapples (D1hargalkar and Untaale, 1983). Diluted seaweed liquid fertilizer of spatoglassum sp., *Ulva lactuca* and *Entermorpha intestinalis* enhanced the seed germination and seedling growth of grams, groundnut and maize (Bukan and Untewale. 1978). The soil application of seaweed liquid fertilizer of *Chaetomorpha limum* and *Hypnea musciformis* increased the growth characteristics, carbohydrates and protein content of *Vigna eadiatea* (Kannan and Tamil selvan, 1990) and *Hordeum vulgare* (steve4ni et al., 1992).

The seaweeds extract were add in form of crude plant extracts and commercial seaweeds extracts. Generally seaweed extracts are effective, positively at low concentration (Bhosie et al., 1975, Mohan and Venkataraman Kumar et al., 1993, Venkataraman Kumar et al., 1993).

A number of studies had been undertaken on the effect of seaweed extracts on plants.The seaweed extracts are added in the form of crude plant extracts and commercial seaweed extracts, example-SM3 and Algifert.Generally seaweed extract is effective at low concentration (Bhosle et al., 1975, Mohan and venkataraman Kumar, 1993; venkataraman kumar et al., 1993). The role of seaweedliquid fertilizer of *Sargassum* and *Padina sp* on *Cajanus cajan* (Mohan et al 1994) and Cicer arietimum (Venkataraman Kumar and Mohan, 1994) was reported. In *Vigna unguicata* (L) Walp, seaweed liquid fertilizer from *Ulva lacuca* was found to be effective on growth and certain other parameters (Sekar et al., 1995).

Application of seaweed was found to increase levels of available p and Nin a calcareous soil (Mario caiozzi, 1968). Application of seaweeds would be beneficial for increasing growth parameters. Pachpande (191) reported increase in total C and N content of soil on application of seaweed manure. In general seaweed fertilizer have high levels of organic matters which aids in retaining and minerals in the upper soil level available to the plants (Aitken and Senn, 1965).

Application of seaweeds, in soybeans, shows increase in protein content (Senn and Kingman, 1977) and same increase is seen in pasture grass which is reflected in the meat quality of animals that graze grasses. Use of seaweeds improve seed germination of grasses, cereals, vegetables, flowers and related to soil type. (Senn and Skeiton 1969).

The effect of different concentration of liquid extracts of Gracilaria edulis and Caulerpa racemosa and highest value was recorded at 10% Caulerpa recemosa liquid was recorded at 100% Gracilaria edulis treated seedlings. The hightest protein aminiacid contents and reducing total sugar content, were observed at 10% Caulerpa racemosa extract socked plant sand the lowest values of these biochemical compounds were observed at 100%.Gracilaria edukis extract soaked plants (Anantharaj & Venkatesalu., 2001).

The seaweed liquid fartlizer (SLF) treatment increased the phosphorus content in the cucumber leaves. The seaweed extract has induced the uptaken of unavailable nutrients and improved the efficiency of the utilization of available nutrients (Nelson and Van staden, 1984 b). The soil application of SLF transformed and soil into fertile soil, besides enchancing water retention capacity and improving the soil texture. In addition soil application factilitaes nutrients uptake, because ions are in chelated forms (Perez-Sanz et al., 1996).

Wong and wong (1989) reported positive and negative of fly ash and ash water on germination and seeding growth in *Brassica juncia*. High concentration had inhibitory effects on seed germination similar observation have been made by Akoi et al., (1984) on spinach, cucmber and Chinese. Cabbage, Karpate and Choudhory (1997) found the damaging effects on cytological parameters of wheat, negative effects of ash water is reported by many investigators (somashekhar et al., 1984; Shane et al., 1988).

The ash water is collected from the thermal power plant (T,P,P). various concentrations of ash water is made as control, 10, 20, 30, 40, 60, 80, and 100% concentration levels. The nature of soil in blank cotton soil. *Brassica juncia* plants treated with these concentration. From amino acids and oil control were estimated by using the methods of Sandasivam and estimated by the methods of Arnon (1949). Data were analyzed by the methods of Arrora and Malhan (1996).

Seaweed extract of Ulva lactuca is known to certain cytokinin (Sekar et al., 1995). Green seaweeds contained higher amount of cytokinin than that of red algae (El . Sheikh and EL Saied, 2000). Mooney and Van staden (1984) reported pronounced quantitative and qualitative seasonal changes in the cytokinin content of the holdfast, vegetative and reproductive laterals. These changes correspond with the seaeonal periodicity of growth, development and reproduction of these algae.

Auxins are seen in seaweeds like *Sargassum polycystrum* (Sumara and Cajipe, 1981) and Ascophyllum nodosum (Kindman and Moove 1982., Boyer and Dougherty , 1988). Gibberellins have been lacated in Fucus spiralis and Tera selmis sp (mowat 1964 a,b). Endogenous cytokinins are seen *sargassum polycystum* in *Ecklonia maxima* (Featon by – smith and van staden.

Presence of auxin was demonstrated in seaweeds like (surnera and Cajipe, 1981) and Ascophyllum nodosum (Kingman and Moore, 1982; Boyerand Dougherty, 1988). Gibberellins have beenlocated in Fucus spiralis and Tertaselmis spp. (Mowat, 1964). Presence of cytokinins in seaweeds and in commercial seaweed products was established (Brain et al., 1973, Blunden, 1977; Van Staden and Davey, 1981; Tay et al., 1985 and 1987). Endogenous cytokinins in Ecklonia maxima were reported(Featonby – Smith and Van staden, 1984). Stephan et al., (1985) observed trans – zeatin, trans zertin riboside, their dihydro derivatives, isopentenyladenine and isopentenyladenosine have been identified and quantified in seasol, a commercial extract of Tasmanian Giant Bull kelp, Durvillea potatorum.

The highest values of shoot and root length, leaf area and the fres weight shoot and root were obtained after the applications of seaweed liquid fertilizers of *Caulerpa scalpelliformis* and *Gracilaria corticota*. It also observed that the biochemical constituents like carbohydrate, protein and liquid were increased after application (Thiyumal Thagam et al., 2003). Venkataraman Kumar and Mohan (2003), reporded both seaweed liquid fertilizer treated and untreated water stressed plants showed a market increase in soluble protein and soluble protein and soluble sugar contents.

The low concentration of aqueous extract prepared from *Caulerpa racemosa* and *Gracillaria edulls* promoted the seedling growth fresh and dry weight, chlorophyll content, protein, amino acid and total sugar content in *Vigna catajung* and *Dolichos biflorus* (Anantharaj and Venkatesalu, 2001 and 2002).

Increase in leaf area, prtiole length and number of leaves by seaweed extracts was studied on *Psophocaepus tetragonbus* (Vijayalakshmi and lakshmanan 1988). An increase in percentage of seed germination, leaf number, leaf area, texture and pigmentation was observed at low concentration of seaweed extract(Rajeswari et al., 1983). Addition of seaweeds to soil improved binding

properties and water retaining capacity of soil (pachpande 1991) and promoted growth and improved the quality of production and also prevent pests and diseases of agriculture and horticultural crops (Verkleij, 1992).

In cluster beans there is an increase in growth parameters (shoot, root, length, leaf area, fresh eight of shoot and root) and biochemical parameters (chl-a, chl-b, xanthophylls, carotenes, carbohydrates, protein and lipid) due to seaweed liquid fertilizer which induced absorption of essential nutrient and increased enzyme resources (Thirumal Thangam et al., 2003).

Oil content and oil yield of mustard were found enhanced due to the application of FYM that promoter the synthesis of oil. An application of 10uha Fym significantly produced higher seed yield of mustard (Tomer et al., 1996, Thanki et al., 2004)and an rapeseed.

The several reasons, sulphur deficiency in Indian soils is one of the reasons for low productivity. The experiments soil contains 12.7 mg 3/kg soil sulphur is a component of amino acids like Cystein, cystein, methionine and essential for chlorophyll formation. It is also required for protein synthesis. Oilseed crops, therefore need more sulphur for their oil and protein synthesis studies carried out on sulphur nutrition in oilseeds indicated a considerable increase in yield and quality of oil seed (Chauhan,et al., 2002).

The increase inyield attributes with increasing rate of sulphur is ascribed to its role in the synthesis of protein, oil and vitamins. Since the experimental soil was deficient in available sulphur (28kg S/ha), application of sulphur helped in improvement in yield attributes of Indian mustard. These results the findings of Chauhan et al., 1996).

Genotypes did not significantly differ for the seeds/siliqua, length of siliqua, 1,000-seed weight and straw yield. However genotype PC 5-17' produced significantly more siliquae/plant and seed yield that PC 5'. It might be owing to

better genetic potential of the genotype PC 5-17'. The yeid level in second year was lesser than that of the first year, which may be due to occurrence of unusual weather conditions. Punia et al., (1993) also reported significant difference in the seed yield of Brassica juncea driftdue to varieties.

Protein are complex nitrogenous substances. They are biopolymers containing large number of amino acids. All the biochemical reactions in a cell are catalysed by eneymes, all of which are proteins. Several hormones are also proteins. The algae were found to have a number of proteins, peptides and amino acids. They varied in quality from season to season. Each alga has its own aminao acid, protein and peptide (Lewis and Gonzalves Ella, 1962c). Some amino acids were found to increase in concentration with increase in the age of the plant (Lewis and Gonzalves et al., 1962a).Twenty four different amino acids has been reported from cystocarpic and tertrasporic plants of Agardhiella robusta (Lewis and Gonzalves Ella, 1962b).

Protein content in Chlorophyceae, Phaeophyceae and rhodophyceae were documented (Venkataraman Kumar, 1993 a). The protein content was higher in phaeophyceae and chorophyceae than in Rhodophyceace (stella Roslin, 2003 a). On the country, kaliaperumal et al., (1994) reported that the seaweeds of Lakshadweep showed maximum protein content in green algae followed by red algae and brown algae. The protein content also varied among different genera and different species of the same genus (Dhargalkaret al., 1980). Same species collected from different localities during different seasons also showed fluctuating values (Dave and parekh, 1975; Ganesan and Kannan, 1994; Ganga Devi et al., 1996).

In Padina species, twenty-two amino acids were detected. Of these praline, aspartic acid, glutamic acid, leucine, phenyl alanine, histidine and glycine were

found in large quantities when compared to the others (Lewis and Gonzalves Ella, 1962b). The algae on the west coast of India were richer in protein content than the algae from south-east coast of India (Sitakara Reo and tipinis, 1964). The various compound such as phospholipids, ornithine, amino butyric acid, cysteic acid were found in marine algae. These compounds could be as a taxonomic tool (Dave and Chauhan, 1987, Khotimchenko and Titlyanova, 1996).

Carbohydrates of seaweeds have an effect upon the physical nature of soil and are insignificant when food value is concerned composition of seaweeds as a manure, has its effect an chemical composition of soil. Algin and algin decoposing bacteria, have its effect an physical condition of soil, consolidating and binding more sandy soils and lossening more clayey soils (Chapman and Chapman, 1980).

The total sugar and protein contents in *Vigna catajung* and *Dolichos biflorus* showed a significant increase due to seaweed application (Anantharaj and Venkatesalu 2001 &2002). The increase in the protein content was due to the absorption of most of the necessary elements by seedlings (Kannan and Subramaniam, 1999; Rajkumar et al., 1999, Thirumal Thangam et al., 2003).

Alginic acid is a polyuronide found in the cell walls of brown marine algae (Kappanna et al., 1962, Stella Roslin, 2003 b). The alginates are used as stabilizers in food industry due to their polyelectrolytic property and viscocity. It is used in dentistry and pharmaceuticals because of the gelformation during chemical reactions and also used in artificial seed production. As algin has base exchange property, it is used to prevent radiostrontium toxicity (Doig et al., 1973). A B -1-4 linked glucoxylan and beta-1-4 linked glucoran had been isolated from Ulva rigda (Lahaye et al., 1996).

High concentration of tocopherol was seen in the brown algae followed by the green and red algae. *Padina boergasenii*, *Sargassum polycystum* and *Dictyota dichotoma* among the brown algae, *Enteromorpha compressa*, *caulerpa* recemosa and Caulerpa aertuaroides among green algae and the red algae *Grscilaria edulis* and *gracilaria follifera* exhabitedhigh concentration of tocopherolknown to occur in red, green and brown seeweeds except members of fucaceae (Ragan, 1981). Alpha tocopherol is the most active form of vitamin-E which is a powerful biological antioxidant.

Vitamin-C is an antioxidant and protects hydrogen / electron carriers within the cell and maintain suitable redox levels of enzyme systems. Ascorbate is also involoved in the biosynthesis of hormones and deoxyribonucleic acid. The algae belonging to Chlorophyaceae and phaeophyceae have higher annual mean contents of the vitamin-C than Rhaeophyceae Vitamin-C showed significant positive relationship with chlorophyll-a in Rhodophyceae and Chlorophyceae and also with carbohydrates content (Sarojini and Sharma, 1999). The distribution o Vitamin-C is associated with the morphogenetically important vegetative and reproductive phases indicating its key role in metabolic function. Seasonal variations in ascorbic acid content are apparently dependent on hydrographic parameters and solar radiation on one hand and the growth intensity of plants on the other hand (Poppy Mary Vimalabai yet al., 2003; Vasanthi sand Rajamanickam, 2004).

Vitamin are also essential growth factors and in their absence no growth can occur or growth is limited. The food value of algae is not only from the bulk of nutrients that they provide but also from the essential vitamins contained (Mumford and Miura, 1988). Marine algae are an excellent source of vitamins. It was reported that ascorbic acid (Vitamin –B2) tocopherols (Vitamin – E) and Vitamin – K are other constituents of marine algae (Thivy , 1960; Smith, 1961; Subbaramaiah, 1976).

Vegetables play an important role in meeting the needs of human nutrients. Large quantities of cellulose and fibre in vegetables help in digestion and prevent constipation. They have laxative qualities and neutralize acid substances in the digestive tract for maintaining good health, it is nessary to taje vegetables in our daily diet. (Sharma and Nandita pathamra., 1994).

MATERIALS AND METHODS

Materials and methods

The healthy seaweed of *Ulva lactuca*, *Caulerpa scapelliforms*, *Padina tetratromatica* and *Sargassum linearfollium* were collected from December (2007) to Janauary (2008) along the Tuticorin (Lat 9.15'N long 79'E) is situated on the south-east coast of Tamil nadu. It is a rocky coast with sandy bottom in the inshore regions. The rocks are exposed during low tide to a greater extent. Collection of seaweed was done during the low tide period.

Description of the selected seaweeds:

1 *Ulva lactuca*:

Division – Chlorophyceae

Order – Ulvales

Family – Ulvaceae

Genus – Ulva

Species – lactuca

The plant is bright to light green in colour (Fig-1) They are attached to the substratum by holdfast composed of rhizodal outgrowth

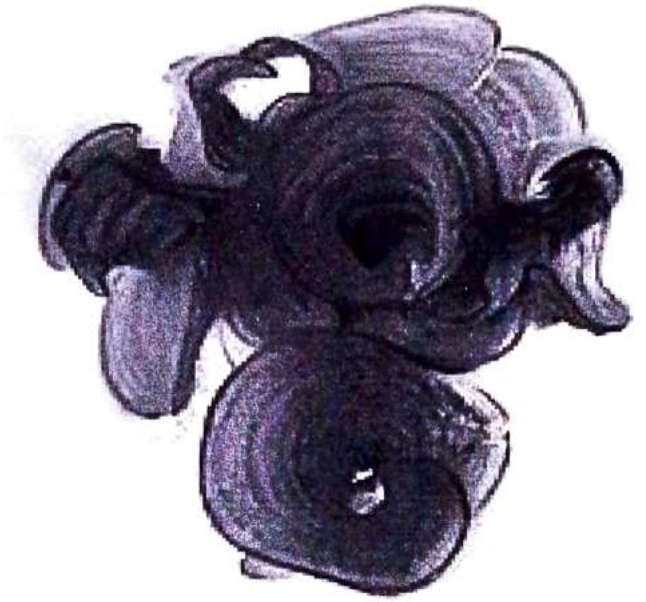
Fig.1

Ulva lactuca

Fig.2

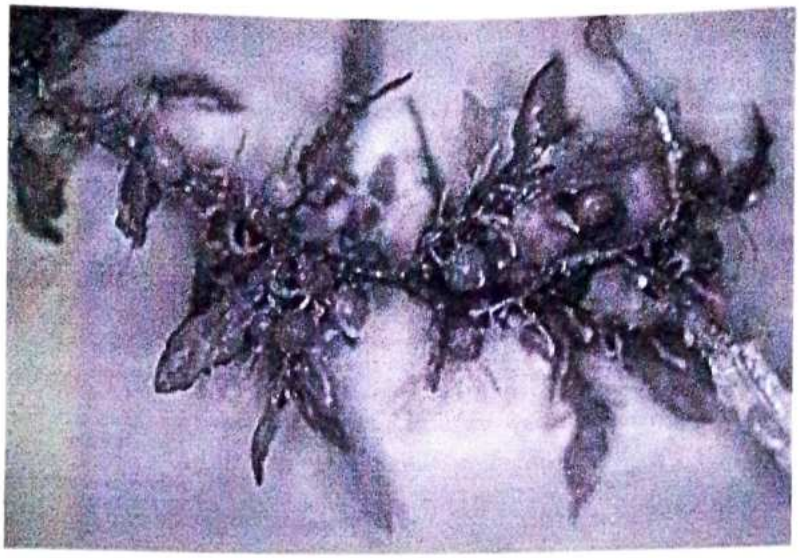
Caulerpa scarpelliformis

Fig.3



Padinia tetratromatica

Fig.4



Sargassum linaerfollium

From the lower cells, the stalk inconspicuous or apparently absent, blade lanceolate to rounded, often some what lobed and undulate or folded, to 6cm long or more and relatively broad; margin of thallus ruffled and wavy and folded, thallus lobes varying in thickness. The rhizoids emrge from thallusnear the point of attachment of the substratum and become closely intermingled to one another forming a pseudo parenchymatous holdfast.

2, *Caulerpa scalpelliformis* - c. Agardh)

Divison - Chlorophyceae

Order - caulerpales

Family - caulerpaceae

Genus - Caulerpa

Species - scalpelliformis

The thallus is brown yellowish green to olive green in colour with extension stoloniferous prostate axes bearing up – right fronds to 20cm in height and 3cm broad(fid -2). Stolons terete naked,1.3mm in diameter attached to the

substratum by the rhizoidal branches.upright braches terete near the base, bearing closely set, deretermine lateral result in an alternate distichous pattern ramulifrom a few mm -1.5cm long flattend,upwardly curved broadest at the distally taperint to a spinous tip. They are mostly seen in interidal zone.

3.*Padina terastromatica*-(hauck)

Division – phaeophyceae

Order – dictyotales

Family – dictyotaceae

Genus – padina

Species – tetrastromatica

Plants are 10-16 cm in height and 5-7 cm in bradth brown and yellow in colour (fig-3). Dried theybecome olive-green, basal portion forming a thick a rhizomatous disc attached into sand accumulated at the bottom of mid littoral region with short stripe. Its thalli are reigon irregularly cleft into narrow lobes, suppose on both. Surface in the basal part of the blade to about into fourth of the length, usually divided into a flabellate lobes in young plants and divided to the base into many cuncate lobes of about 2-4 cm wide; proliferous zone on both surfaces occurring alternately and well developed with globous zone between them upper portion is border and forms the fan-shaped and shaped frond, apical margins are involute, the lower region of thallus becomes gradually narrow.hairs are occurring on the surface of the hallus and form concention row s. which render the thallus stipped.

4,*Sargassum linearifolium*- (c.agardh)

Divison – phaeophyceae

Order – fucales

Family – sargassaceae

Genus – sargassum

Speices – linearfolim

Thallus are 35cm tall, with yellowish brown colour (fig.4) attaced with discoid holdfast, hair axis cylindrical and brought due to the presence of numerous out growth, supporting alternatively arranged branches,bearing leaves and the vesicles; in young the talk and 2.5-11.5 mm wides;leaves are generally oblong slightly tapered retuse (slightly rounded) or emariginted leave, smaller 7-15 mm long including stalk and 17.4 mm wide oblanceolate, oblong with to pared bases, the apices are rounded, obtase to acute outer margin in coarsely 3 errate; prominent midrib at a short distance from the apex of the leaves. Pendulate vesicles are ovule with adiameter of 1.5-33mm vesicles may be solitary or may form clusters attached to the primary and secondary branches.

Description of experimental plants:

Brassica juncia **(L)**

Class – dicotyledons

Sub- class – polypetale

Series – Thalamiflorae

Order – Parietales

Family – Brassicaceae

Brassica juncia is an annual crop plant cultivated for its seeds which are commonly used as aspice. The fruit is asiliqua. It develops from a superior ovary with ovules on parietal placenta. The pericarp splits in to 2 halves from below upward and the replum or false is left in the middle like a frame work and the seeds are attached to either margin. The seeds are small, spherical brown to

back in colour. The seeds were used as condiments in the preparation and also for flavouring curries and vegetables. The oil extract is used as cooking oil, for preparing salad and margine. It is also used in industries for making grease, lubricants, soft-soap and synthetic rubbers. It Is modified from of septifragal capsules. The seeds are small. Spherical brown to back in colour. The leaves of young plants are used as green leafy vegetables. Tha plants is used as greenfodder and also as green manure.

Algal collection and preparation of Seaweed Liquid Fertulizer (SLF):

The algal species were handpicked and washed thoroughly with running tap water to remove all the unwanted impurities, adhering particles and epiphytes. Seaweed were dried in sunlight for 5 days then oven dried for 24 hr at60-64c, hand crushed and made ass coarse powder using a mixer grinder. The coarse powder was soaked in water for 5 minutes before extraction. Seaweeds were cooked in pressure cooker for 2hr. the ratio of material and water was 1:10(w/v). the extract was removed from the pressure cooker and filtered through a double layered cheese cloth. The filrate was centrifuged at 1000 rpm to remove most of the suspended impuritis. The filtrate was dried in ahot air oven at 65c for 48 hr, and the powder was stored in air tight bottles. The dried seaweed sample was considered as 100% seaweed extract (Rama Rao, 1990). Different concentration of Seaweed Liquid Fertilizer (SLF) were prepared (5% and 10%(w/v)) from the 100% seaweed extract obtained from the green seaweed *U.lactuca* (plate-1) .

Plate – 1

SLF with different concentrations of various algae.

C.scalpelliforms and brown seaweed *P.tetratromatica*, *S.lineraiform* using distilled water.

Wet Method of algal specimen preservation:

The sample of the seaweeds were collected from Tuticorin coast, Gulf of mannar during the low tide. The collected specimens were washed thoroughly with fresh water to remove the unwanted debris. sample was preserved in the bottle or in the plastic bags which contained solution of 10% formaldehyde (prepared in seawater). The preserved bottles were closed with tightly to avoid leakage of fumes of formaldehyde.

Protocol for seed germination study:

Seed germination can be defined as a process in which the dormant embryo of the seed resumes active growth and forms a seedling. Seed germination is also controlled by hormonal status of seed. When seed germination promoting hormonce nullify seed germination inhibitors, germination takes place. Auxin has no marked effect on germination

Experimental settings:

Musted seeds were initially surface sterilized with 0.1% mercuric chloride for one minute and repeatedly washed thoroughly with distilled water to removed any trace of mercuric chloride. The seeds were soaked separately in 100ml of two different concentration such as 5%and 10% off SLF (Seaweed Liquid Fertilizer) prepared with *U.lactuca*, *C.scalpellifromis*, *P.tetrastromatica* and *S.linearifolium*. The soaked seeds (15 seeds/pot) were sown in mud pots, filled with garden soil. They started germination on third day onwards. The pots

were regularly irrigated with tap water. Seaweed Liquid Fertilizer were sprinkled over the leaves in the morning after the irrigation except the pots, kept as control. Ten days after treatment the plants were used for analysis. Morphological parameters such as length of root, length, of root, length of shoot, leaf area and number of leaf were studied. Biochemical constituent like soluble sugar, soluble protein, amino acid and vitamin-c were also estimated based on standard methods. The pot not treated with SLF was considered as the control.

Eastimation of protein (Lowry et al.,1951)

Reagent:

Alkaline -A: 2% Na_2co_3 in 0.1 NaOH

Solution -B:2% sodium potassium tartarate

Solution -C: .0.5% $Cuso_4$

Just before the experiment mix 100ml of solution A and 2ml of solution B with 1ml of solution C.one gram of fresh tissue was homogenized in 100ml of distilled water and filtered through a muslin-cloth and was centrifuge at 3000rpm for 10 minutes. To the supernatant 10ml of 10% TCA was added and left in an ice bath for 30 minutes to precipitate protein. Then centrifuge at 3000rom and discared the supernatant.dissolved the precipitate with 10ml of in 0.2N NaOH and diluted to aknown volume.

To 10ml of the protein extract 2ml distilled water was added. To thisadded with 5.5ml alkaline copper reagent and shake well. It was tehnallowed to stand for 15 to 30 minutes then0.5ml of folin-ciocalteau reagent was measurd red filter (650nm)with photoelectric calorimeter 112. A prper blank without protein was used polated, the value in standard graoh and the amount of protein was found out.

Estimation of free sugar (Jayarman, 1981):

One garm of fresh plant tissue were homogenized thoroughly in 10ml of distilled water. Then filtered the homogenous through cloth and centrifuge for 15 minutes.

To 1ml of the extract was added with, 4ml of the anthrone reagent. Then boiled for 10 minutes. Cooled and read the OD at 650 nm using orange filter. Glucose was used as the standard.

Estimation of amino acid- Nin hydrin method (Moore and stein 1948):

One gram of fresh plant tissue was homogenized in 10ml of distilled water. The homogenate was filtered and the filterate was centrifuge at 6000rpm for 5 minutes to sediment the cell debris. The pellet was discarded and the supernatant was to the volume of 10ml.

To 2ml of supernatant and 2ml of Nin-hydrin reagent was added and shaken well and heated in a boiling water bath until bluish colour appeared. Cooled to room temperature and added 3ml of 5% ethanol. Mixed and thoroughly and after 10 minutes read OD at 550 nm. The amount of free amino acid was calculated using lecucine as the standard.

RESULT AND DISSCISION

Result and discussion

With increasing in macrophytes as a source of biological active compounds, phytochemicals such as protein, vitamin-c and manure potential the present

investigation on four algae belonging to different gruopwas made to study their effects onleguminous seed germination and as manure on *Brassica juncia* (L). many attemps have been made on group plants over short period. But comprehensive studies on *B.juncia* is limited. The present investigation may provide the information about the effect of SLF of different algae on morphological and biochemical constituent of *Brassica juncia*(L).

The sample of the seaweeds were collected from Tuticorin coast, Gulf of mannar during the low tide Tuticorin(Lat 9.15'n Long 79'E) is situated on the south-east coast of Tamil nadu. It is a rocky coast with sandy bottam in the inshore regions. The rocks are expose during low tide to a greater extent. Collection of seaweeds was one during the low tide period.

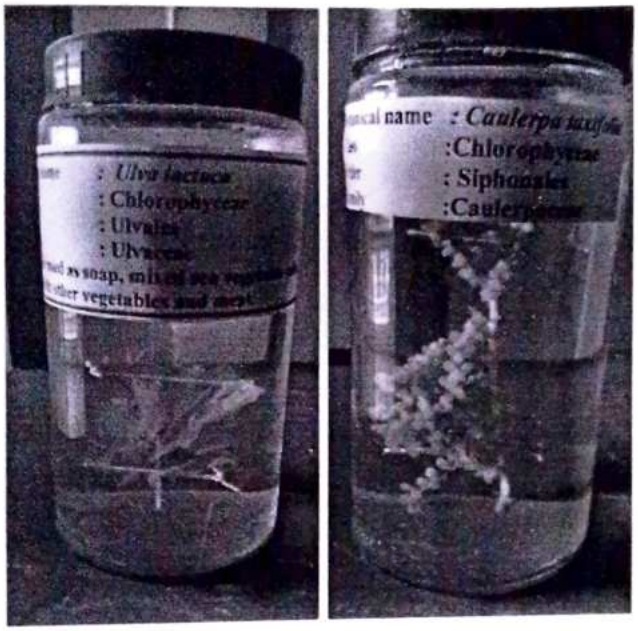
The collected specimens were washed thoroughly with fresh water to remove the unwanted debris and other flora and fauna which were associated with seaweeds later the sample was preserved in the bottle or in the plastic bags which contained solution of 10% formaldehyde (prepared in seawater). The preserved bottles were closed with tightly to avoid leakage of fumes of formaldehyde. The containers were made air tight, labeled with date of collection, locality and time later transported to the laboratory for further identification.

The specimens were preserved in 3-5% buffered formalin, (which can retains the specimen's natural colour i.e., original colour). In case of fleshy specimens, utmost care was taken to remove sand particles, unwanted debris, shells of gastropods and larvae of other microorganisms. The specimens which bears thick hold fast, a small portion were retained to facilitate pressing. In some cases, it was very difficult to remove a part of holdfast, in such condition, the entire hold fast was retained, which made a good herbarium specimen. Calcareous specimens were preserved by treating the materials with 3 to 5% formalin and then soaked in 40% glycerene later in 3% buffered formalin prepared in seawater were dried for 10-15 days. In this way, the calcareous specimens were preserved in dry condition without pressing.

The collected seaweeds were preserved in the glass bottle containing the formalin but corallines are kept in the 3-5 % formalin, prepared in filtred seaw water (3-5% of formalin is a good preservative for algae). To preserve a specimen for a longer period, it was kept in the dark in a sealed container or polythene bags to prevent bleaching.

Prepared bottle spiemens are presented Fig-1

Fig.



Ulva lactuca *Caulerpa taxifolida*

Gracillaria verrucosa(Hub) *Gracillaria corticata*

Padina tetrastromatica *stoechospermum*

Gracilaria corticata(L) *Gracilaria verrucosa (Hub)*

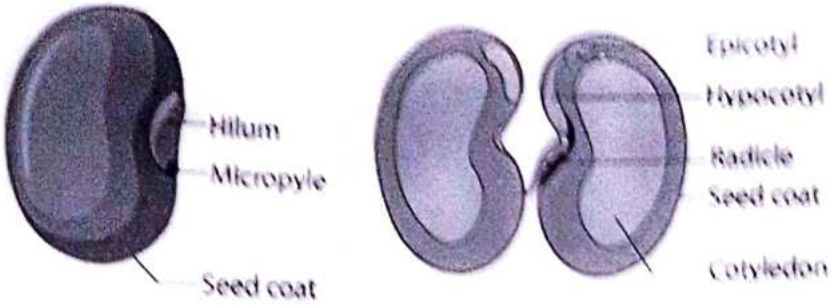
Structure of seeds

Seed is a structure which contains embryo of a plant. It is a small embryonic plant enclosed in a covering called the seed coat, usually with some stored food. The formation of the seed completes the process of reproduction in plants (started with the development of flowers and pollination), with the embryo developed from the zygote and the seed coat from the integuments of the ovule. Seeds are the product of the ripened ovule, after fertilization by pollen and some growth within the mother plant. The embryo is developed from the zygote and the seed coat from the integuments of the ovule.

A typical seed includes two basic parts:

1. Embryo
2. Seed coat

In addition, the endosperm forms a supply of nutrients for the embryo in most monocotyledons and the endospermic dicotyledons.



(a) Lateral view of a seed (b) A mature embryo with its parts

SEED DORMANCY

Seed dormancy has been defined as the failure of a viable seed to germinate under favourable conditions. The seeds of some species are prevented from germination by coat-enhanced dormancy. Embryos isolated from these seeds are not dormant. But in some other species even the embryo itself is dormant.

Dormancy is caused by internal as well as external factors. Internal factors include and testa barrier (hard testa), impermeability of seed coats to moisture and oxygen, immaturity of the embryo and dominance of germinating inhibitors in seeds. External factors include availability of water, appropriate temperature, specific light requirement (in same seeds) When dormancy is caused due to internal factor it is called true dormancy or innate dormancy or primary dormancy When dormancy is caused due to external factor it is called imposed dormancy or quiescent dormancy or secondary dormancy. Both of these (primary &secondary) influences are mutually dependent and cannot be singled out. (Sinha, 2002).

SEED GERMINATION

Seed germination can be defined as a process in which the dormant embryo of the seed resumes active growth and forms a seedling. Germination is a sequential serious of morphogenetic events that result in the transformation of and embryo into a seedling. Formation of seedlings from seed is a reproductive mean of plant propagation (Berlyn, 1972).

Germination commences with the uptake of water by the dry seed through imbibitions. The imbibitions process is completed when a part of the embryo, usually the radicle, extends to penetrate the structures that surround it.

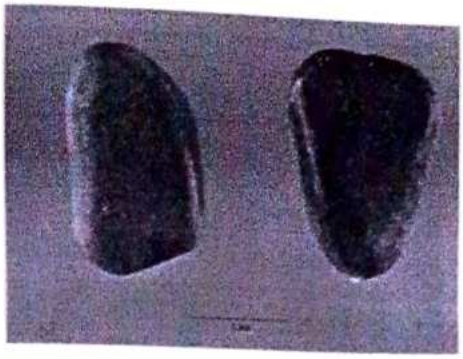
A sequence of physiological and biochemical events takes place during seed germination. These events include imbibitions, hydration of protoplasm, restoration of enzymatic activity, protein synthesis, hydrolysis of food reserves, respiration enhancement and hormonal changes.

Seed germination is also controlled by hormonal status of seed. When seed germination promoting hormonce nullify seed germination inhibitors, germination takes place. Auxin has no marked effect on germination. Gibberlic Acid (GA) promotes germination is barley seeds (creals) but not in cucurbita mexica, hence, GA cannot be regarded as universal seed germination promoter hormone. Cytokinin promotes germination. ABA inhibits germination of seeds. (Sinha, 2002)

The present chapter is dealing with the impact of SLF on seed germination. A preliminary triled carried out with the selected seed from family Leguminosae caesalpina pulcherrima. Bahinia purpurea,adenanthra pavonina, Leucaena leucocephala and their characteristic they are also studying

Caesalpinia pulcherrima:

Fruit and seed :



Fruit shape: pod or pod-like

Fruit length: 3 to 6 inches

Fruit covering: dry or hard

Fruit color: brown

Fruit characteristics: does not attract wildlife; not showy; fruit leaves not a litter problem

Bauhinia purpurea:

Scientific name: Bauhinia purpurea

Common name(s): Purple Orchid-Tree

Family: Leguminosae

Fruit and seed

elongated; pod Fruit length: 6 to 12 inches Fruit covering: dry or hard Fruit color: brownOrchid-Tree should be grown in full sun on welldrained soil.

Adenanthera pavonina

Family-Fabaceae

Scientific Name-*Adenanthera pavonina* L.

Linnaeus, C. von (1753) *Species Plantarum* 2: 384. Type: Habitat in India.

Fruit and seed

Pods about 22 x 1.6 cm. Seeds quite hard. Testa shiny red.

Seedlings

Cotyledons erect, fleshy, obovate, about 10-12 x 8-9 mm. At the tenth leaf stage: leaflet blades elliptic, apexmucronate, base oblique or obtuse; stipules very small, visible only with a lens. A number of very small red glands visible on very young growth at all stages. Seed germination time 11 to 24 days

Leucaena leucocephala:

Division-magnoliophyta

Class- dicotyledons

Order : Fabalese

Family : Fabaceae

Sub family :Mimosoideae

Genus : Leucaena

Species : L. leucocephala

Hindi name :Subabo

Fruit and seed:

Lecocephala is essentially a tropical species requiring warm temperaturesfor optimum growth, with poor cold tolerance and significantly reduced growth during cool winter months in subtropical areas [7]. Shading reduces the growth of leucaenaalthough this plant has moderate tolerance of reduced light when compared with other tree legumes [1]. grows well in subhumid or humid climates with rainfalls between 650 mm and 3000 mm, although it can tolerate moderate dry seeds Seeds: Flat, brown and shiny, 6 -10 mm long.

Usually grown in single rows (or double rows 70 – 90 cm apart), with 4 – 10m between rows; inter-row may be left as native pasture or sown to improved pasture. Inter-row width is increased as average rainfall declines.

Experimental setting for SLF treatement on seed germination

New seeds of above mentioned plant were collected from St.Mary'scolledgeThoothukudi. After collection of seeds they were washed and socked in fresh water 10 hours . Now the seeds are again soacked in SLF (Seaweed Liquid Fertilizer) for 10 hr. After 10 hours the seeds were removed from the SLF and planted them in the seed dary filled with coconut peat.

The number seeds taken form each plant is 30. The result obtained from the study are presented table-1

Effect of 10% slf on germination of leguminous seeds at different perids:

Caesalpinia pulcherrima:

Control:

Name of the plant	6th day	12th day
Caesalpinia pulcherrima	8, 26.6%	13, 43.5%

SLF traeatement

Name of plant	6th day	12th day
Caesalpinia pulcherrima	9,30%	16,53%

Control

Bauhinia purpurae

Name of the plat	6th ady	12th day

Bauhinia purpurea	7,23.3%	12, 40%

SLF treatment:

Name of the plant	6th day	12th day
Bauhinia purpurea	8, 26.6%	14, 46.6%

Adenathra pavonina

control

Name of the plant	6thday	12thday
Adenanthra pavonina	5, 16.7%	10, 33%

SLF treatement:

Name of the plant	6thday	12thday
Adenanthra pavonina	8, 26.6%	12, 40%

Leucana leucocephala

Control

Name of the plant	6th	12thday

Leucena leucocephala	4, 13.3%	8 , 26.6%

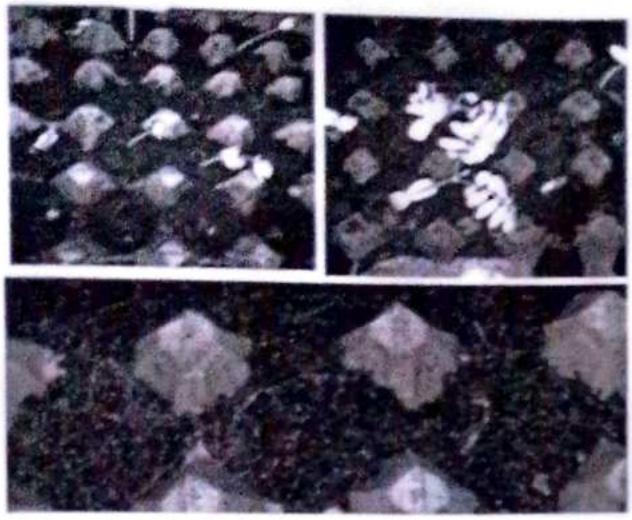
SLFtreatement:

Name of the plant	6^{th}day	12^{th}day
Leucena leucocephala	6, 20%	13 , 43%

Percentage of growing seedling:

Caesalpinia pulcherrima:

6^{nd} day 12^{th} day



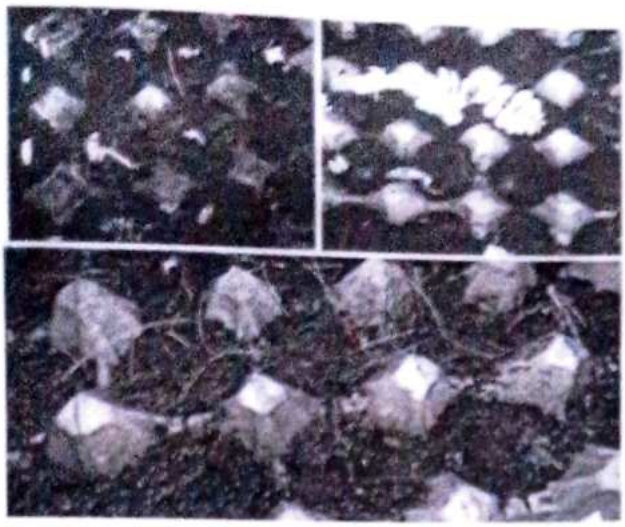
Control

6th day 12th day

SLF

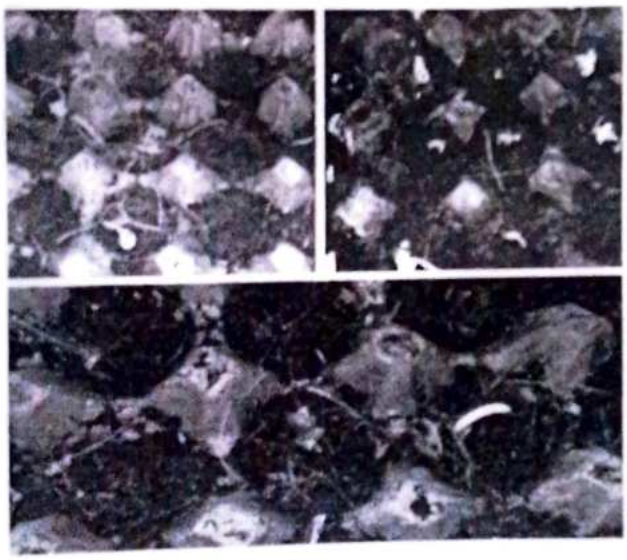
Bhaunia purpurea :

6th day 12th day



Control

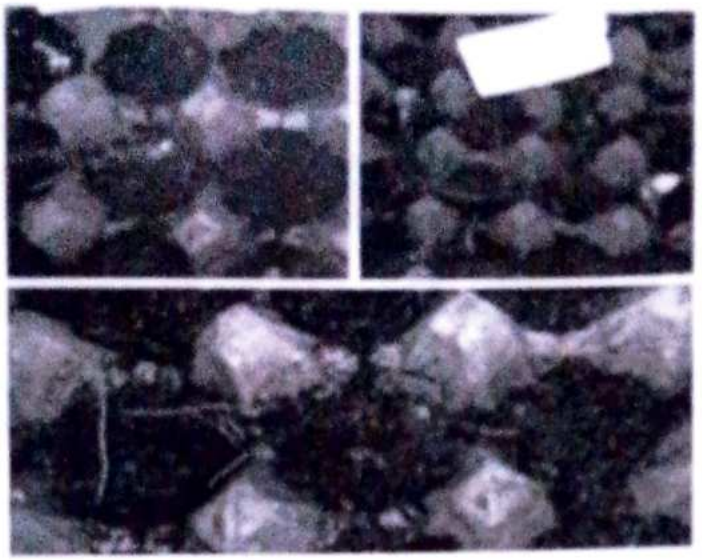
6th day 12th day



SLF

Adenathra pavonina:

6th day 12th day



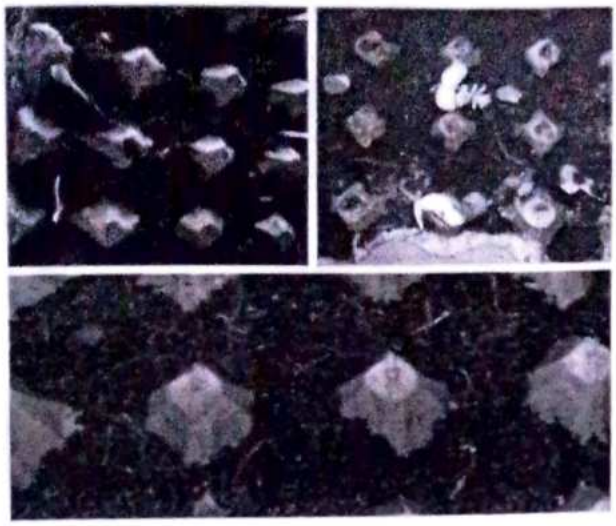
control

6th day 12th day

SLI

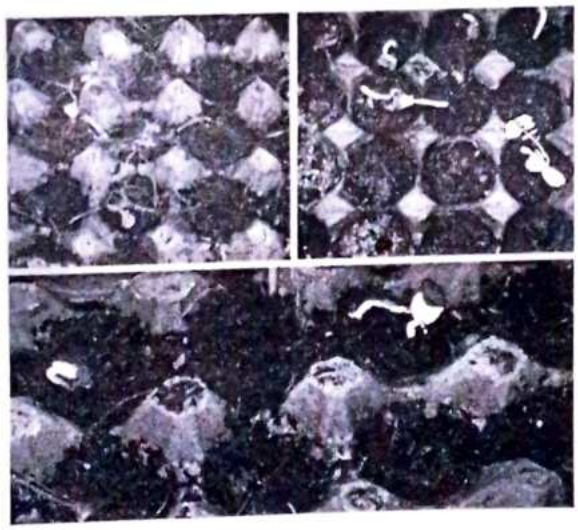
Leucaena leucocephala:

6th day 12th day



Control

6th day 12th day



SLF

Result:

effect of SLF on germination of leguminous seeds the four types of seeds are treated in under slf treatment that seeds are germinate in high percentage in more than the control seeds.

The over all result of SLF treatement on seed germination study clearly reaviled the fact thar SLF treatement has more impact on (caesalpinia pulcherrima, bauhinia purpurea, adenanthra pavonia, leucaena leucacephala.).

Gowth hormones play very important role in breaking seed dormany by chaning the psychological activities that control seed dormancy. Show the present study suggest biochemical constent of SLF may have hormonal effect on seed germination . however, the plants light Adenanthra pavonina showed less response to the SLF. this could be due tosome ingibitor in the seed cat that seed coat that cannot be broken by tha SLF

The result of the germination study on caesalpinia pulcherrima and bauhinia purpurea showed 6th day 80%. Similarly on the 12thday 93% over than the control.

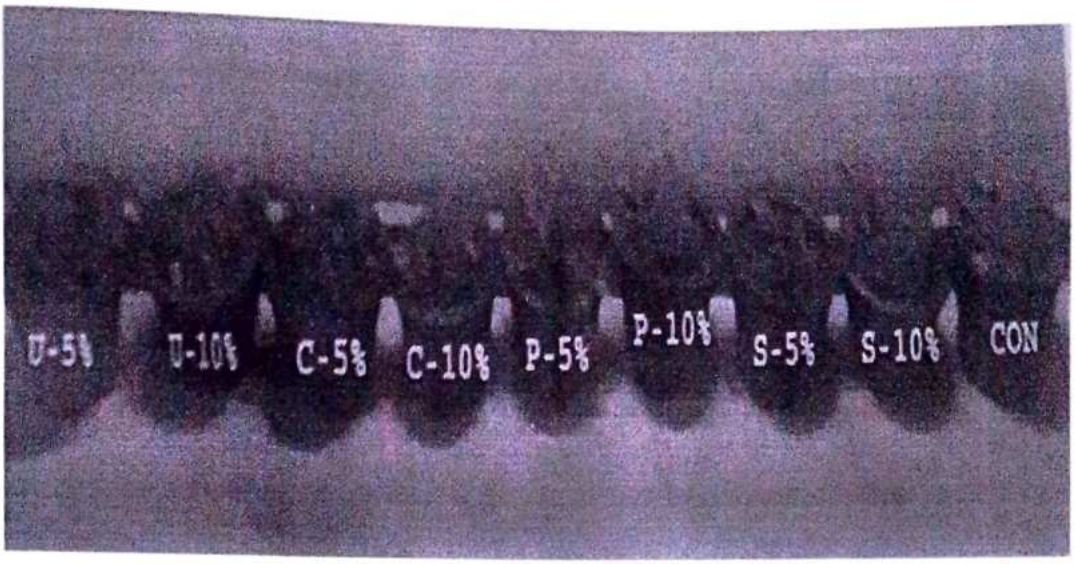
In the Adananthra pavonina and leucana lecocephala less germination 60% over than the control in 53%.

Effect of SLF of various algae on the morphological characters of *B . juncia* :

The effect of SLF on morphological character of *B. juncia* (L)were presented (Table 1 to 3). The study revealed that seaweed liquid fertilizer of *U.lactuca* , *C.scalpellifromis*, *P.tetrastromatica* and *S.linearifolium* have significant effect on various growth parameters like root length, shoot length and leaf area. The increased in growth was found to be concentration dependent. The SLF of *P.tertrastomatica* at 10% concentration showed maximum shoot length (12.1cm) over the control (8cm) on 21st day (Table-1). However the

seedlings showed the pronounced growth effect only in the later period (Fig-5) on 35th day the shoot is significantly more in *Brassica* panda treated with 10% SLF of *P tetrastromatica* (Fig- 6)

Effect of SLF of *Brassica* (L.) at 35th day.



Increasing root length, leaf area and shoot length over the control for all treatements indicate that nutrients in the SLF were absorbed and translocated efficiently by Brasscia juncia.

Effect of SLF of various algae on the biochemical constituent of *B.juncia* :

The effect of SLF on biochemical such as protein, sugar, and amino acid presented (Table 4 to 6) and (Fig -7 to 9).

Protein:

Protein are complex nitrogenous substances. They are biopolymers containing large number of amino acids. All the biochemical reaction in a cell are catalysed by enzymes, all of which are proteins, peptides, and amino acids. They varies in quality and quantity from season to season. Each algae has its own amino acids, protein and peptide pattern (Lewis et al., 1962). The protein rich seaweed could provide supplementary diet and so should be made palatable and popularized. Protein content in marine algae was reported to vary from. 10-33%dry wt..(Dhargaalkar et al., 1980). Seaweed like *Ulva* and *porphyra* are the good source of protein (Kaliaperumal et al., 1987). The protein content estimated from *Brassica juncea* of the present (Table -4). On the 7th day of analysis,the protein content did not show much differences between treated and the control plant. Pronunced variations are noted in the treated plants on 21st and 35th day of analysis (Fig-5). Among the various SLF used, SLF of *P.tetratromatica* at 10% concentration showed a significant increase in protein content over the control at about 45% on 35th day of analysis. Similar trend was also noted for *S.linearformis* (Fig -7). The increase in protein content of *B.jnucea* due to SLF of *P.tetrastromatica* and *S.linearfolium* could be attribute to high content of protein in these seaweeds. (Kaliaperumal et al., 1987).

Amino acid:

Amino acid plays on important role in nitrogen metabolisum by their involment in the biosynthesis of many other nitrogen containing compounds like alkaloids, amides, cyanogenic, glycosides, porphrins, purines, pyrimidines, and cytokinins (Beevers, 1976). Free amino acid and protein amino acid of lage of algae have been studied by Lewis and Gonzolves (1959) The different concentration of SLF treated with Raphansus sativus seeds showed better results in amino acid contents. (Thirumaran, et al., 2007).

The amino acid content of *B.juncea* at various days of analysis are presented (Table -5). The 7thday analysis on the amino acid content of *B.juncea* treated with the 10% SLF of *P.tetrastromatica* is 37% more over the control. Same treatment showed (54%) more over the control on 21st day. Among the various SLF used

SLF of 10% *P.tetrastromatica* and *S.linearifolium* are considered as the best nutrient source of the growth of *B.juncia.* Array of literature indicate that Ulva is the rich source of amino acid whereas in the present study *B.juncia* did not show much response to the SLF of *U.lactuca* (Fig – 8). The reason is yet to be studied.

Sugar:

The seaweed contain various carbohydrate which differs from those in higher land plants (Percival,1968). Carbohydrate are polyhydroxy alcohols having potentially active aldehyde and ketone groups. When carbohydrates are oxidized in the body, they liberate carbondioxide, water and energy. The carbohydrates in a living cell are in constant flux participating in many enzyme catalyzed reations. It is necessary to convert bond energy in to chemical energy for the growth and development of the cell. (Gangadevi et al.,199).

Carbohydrate contents varied among the different groups of algae (Venkataraman and kumar et al.,1993).

The sugar content of present study is tabulated. (Table – 6). Start from the being there is a constant in sugar content in all the treatments over control. The indicates the response of *B.juncia* to various SLF .this is in conformity with the findings of venkataraman kumar and Mohanin 2000, they stated that the SLF of *P.tetrastromatica* and *S.linearifolium* when applied on back gram induced concentration of soluble sugar. The SLF of *P.tetrastromatica* at 10% concentration

Show 21% increase over control on 21st day of analusis (Fig- 9). When compared to the control, the pronounced effects were noted on thee plant treated with SLF of *C.scalpelliformis* at 10% concentration this may be possible due to the increasing photosynthetic pigments and enhancing photosynthetic activity (Thirumal Thangam et al., 2003).

SUMMARY

Investigation were made to find out the effect of SLFs obtained from the seaweed such as *U.lactuca*, *C.scalpelliformis*, *P.tetrastromatica* and *S.linearifolium* occurring abundantly along the coast of Tuticorin. The seaweed were collected from December (2019) to January (2020). The algal species were hand picked and washed thoroughly with running tap water to remove all the impurities. Part of the good, healthy and broken specimens were displayed on the glass plate and tide with thread and preserved under wet method in the gass bottel by using standares algal presaervate (formalin3-5%). The present investigation has preserved 10 algal seaweed for futher application .the remaing Seaweeds were dried in sunlight for 5 days then oven dried, hand crushed an made into powder using a mixer grinder . powder was soaked and cooked in pressure cooker. The extract was removed and filtered through a double layered cheese cloth. The filterate was dried in a hot air oven. The dried seaweed sample was considered as 100% seaweed extract (Rama Rao). From that 100%SLF concentrate, 10% Solution of SlF was prepared and utilized to study is effect on germination . the germination study onlegumious seed reviled that higher percentage of infulese on *caesalpinia pulchrrima* and is followed by *bauhinia purpurea* seed reaviled lower percentage germination .Different concentration of SLF were prepared (5% and 10%), from the 100% seaweed extracts. To study the effects of SLF, an annual crop, *B.juncia* was selected treated with various concentration of SLF. The treated plants were analysed to known the effect of SLF on morphological and biochemical parameters.

The result of the investigation revealed the positive effect of SLF on various parameters studied and was found to be concentration dependent. The significant effect of SLF were well pronounced only after 14 days of analysis. Among the various SLF used, of *P.tetrastromatica* at 10% concentration showed result for root length, shoot length and leaf area.

The amount of protein was found to be more when the plant received 10%SLF of *P.tetrastromatica* and *S. linearifolium*. Similarly the amino acid content was also more in *B.juncia* treated with 10% SLF of *P.tetrastromatica* and S.*linearifolium*. But it showed poor response to the SLF of *U.lactuca*. the result of sugar content indicated that the SLF of *C.scalpelliformis* were more effective than the others.

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PHYTOCHEMICAL SCREENING ANDANTIOXIDANTACTIVITY OF TECOMA STANS(L.) JUSS. EX KUNTH

A Short term projectsubmitted to

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in partial fulfilment of the requirements for the degree of

BACHELOR OF SCIENCE IN BOTANY

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APRIL 2019-20

CERTIFICATE

It is certified that this short-term project work entitled "**Phytochemical Screening and Antioxidant Activity of *Tecomastans* (L.) Juss. Ex Kunth**" submitted to **St. Mary's College (Autonomous)** affiliated to **Manonmaniam Sundaranar University** in partial fulfilment of the requirements for the degree of Bachelor of Science in Botany, and is a record of work done in the Department of Botany, St. Mary's College (Autonomous), Thoothukudi during the year 2019-2020 by the following students.

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INTRODUCTION

INTRODUCTION

India is one of the richest countries in the world in terms of biodiversity, has 15 agro-climatic zones. Plants have been used for medicinal purposes since time immemorial, and to this day, many of the important and familiar remedies originate in plants. Out of the 17000-18000 species of flowering plants, more than 7000 are estimated to have medicinal usage in folk and documented systems of medicine like Ayurveda, Unani, Siddha & Homoeopathy.

Before the introduction of chemical medicines, man relied on the healing properties of medicinal plants. Some people value these plants due to the ancient belief which says plants are created to supply man with food, medical treatment, and other effects. It is thought that about 80% of the 5.2 billion people of the world live in the less developed countries and the World Health Organization estimates that about 80% of these people rely almost exclusively on traditional medicine for their primary healthcare needs. Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis (Davidson-Hunt, 2000).

Medicinal and Aromatic Plants (MAPs) have been utilized in various forms since the earliest days of mankind. They have maintained their traditional basic curative role even in our modern societies. Apart from their traditional culinary and food industry uses, MAPs are intensively consumed as food supplements (food additives) and in animal husbandry, where feed additives are used to replace synthetic chemicals and production-increasing hormones. Importantly medicinal plants and their chemical ingredients can serve as starting and/or model materials for

pharmaceutical research and medicine production. Current areas of utilization constitute powerful drivers for the exploitation of these natural resources. Today's demands, coupled with the already rather limited availability and potential exhaustion of these natural resources, make it necessary to take stock of them and our knowledge regarding research and development, production, trade and utilization, and especially from the viewpoint of sustainability. The series Medicinal and Aromatic Plants of the World is aimed to look carefully at our present knowledge of this vast interdisciplinary domain on a global scale. In the era of global climatic change, the series is expected to make an important contribution to the better knowledge and understanding of MAPs.

Although there is no doubting the predominance of chemical research in modern medicine, there is a notably increasing interest, within both medical circles and the general public alike, in plant-oriented folk medicine. Further research into the biochemical mechanisms of herbal medicines will enable a synthesis of traditional and modern methods of health care, to the benefit of all.

In the development of human culture medicinal plants have played an essential role, for example religions and different ceremonies (Hosseinzadeh *et al.*, 2015). Among the variety of modern medicines, many of them are produced indirectly from medicinal plants, for example aspirin. Many food crops have medicinal effects, for example garlic. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. The medicinal effects of plants are due to secondary metabolite production of the plants. Keeping this in consideration there have been increased waves of interest in the field of research in natural product chemistry. This interest can be due to several factors, including therapeutic needs, the remarkable diversity of both chemical structure and biological activities of naturally

occurring secondary metabolites, the utility of novel bioactive natural compounds as biochemical probes, the development of novel and sensitive techniques to detect biologically active natural products, improved techniques to isolate, purify, and structurally characterize these active constituents, and advances in solving the demand for supply of complex natural products (Clark, 1996). The importance of traditional medicine has also recognized by World Health Organization (WHO) and has created strategies, guidelines and standards for botanical medicines. For the cultivation, processing of medicinal plants and the manufacture of herbal medicines agro-industrial technologies need to be applied (WHO, 1993).. Medicinal plants are resources of new drugs and many

Plants are the oldest source of pharmacologically active compounds, and have provided humankind with many medically useful compounds for centuries (Cordell, 1981). Plant cell produces two types of metabolites: primary metabolites involved directly in growth and metabolism (carbohydrates, lipids and proteins), and secondary metabolites considered as end products of primary metabolism and not involved in metabolic activity (alkaloids, phenolics, sterols, steroids, essential oils, lignins and tannins etc). They acts as defense chemicals. Their absence does not cause terrible effects in the plants. Today it is estimated that more than two thirds of the world's population relies on plant derived drugs; some 7000 medicinal compounds used in the Western pharmacopoeia are derived from plants (Caufield, 1991).

The world is fertile with natural and medicinal plants. Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmacology. The medicinal power of these plants lies in phytochemical constituents that cause definite pharmacological action on human body. Some of the

most significant bioactive phytochemicals are alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds and many more (Edeogo *et al.*, 2005).These natural compounds form the foundation of modern prescription drugs as we know today (Chopra *et al.*, 1986). Phytochemical is a natural compound occur in plants such as medicinal plants, vegetables and fruits, that work with nutrients and fibers to act against diseases or more specifically to protect against diseases. Phytochemicals are mainly divided into two groups, which are primary and secondary constituents according to their activity in plant metabolism. Primary constituents contain common sugars, amino acids, proteins and chlorophyll while secondary constituents comprise of alkaloids, flavonoids, saponins, tannins, phenolic compounds and many more (Krishnaiah *et al.*, 2007).

Herbal medicines are for superior than the synthetic drugs because they are naturally occurring, easily available without cost and have minimum side effects. Majority of plants have medicinal properties, i.e. most pharmaceutical drugs are originally derived from plants. The scientific study of indigenous medicines is called Ethno pharmacology, which is an interdisciplinary science practiced all over the world. Standardization herbal preparation is termed as phototherapeutic agents or pytho-medicine which contains active constituents, or complex mixture of plant materials in the raw or processed form. Phototherapeutic agents are usually not recommended to use in emergency treatment because of the fact that they normally do not possess an immediate or strong pharmacological action. The modern field of phytoscience comprise of the use of medicinal plants and their bioactive phyto-compounds. This science is developed from merging of vast range of disciplines that have never been linked before combining several different areas of economic, biochemistry, physiology, microbiology medicines and agriculture.

There is a promising future of medicinal plants as there are about half million plants around the world, and most of them are not investigated yet for their medical activities and their hidden potential of medical activities could be decisive in the treatment of present and future studies (Singh, 2015). There is a need for documentation of research work carried out on traditional medicinal plants.

Almost all the parts of *Tecoma stans* are of medicinal importance and used traditionally for the cure of various diseases. The *Tecoma stans* leaves, barks and roots have been used for a variety of purposes in the field of herbal medicine. Bark shows smooth muscle relaxant, mild cardio tonic and chlorotic activity. Applications include the experimental treatment of diabetes, digestive problems, control of yeast infections and other medicinal applications. It contains several compounds that are known for their catnip like effects on felines. The root of the plant is reported to be a powerful diuretic, vermifuge and tonic. A grinding of the root of *Tecoma stans* and lemon juice is reportedly used as an external application and also taken internally in small quantities as a remedy for snake and rat bites (Bhat, 2019). With this background, the current study was carried out to explore the plant *Tecoma stans* (L.) Juss. ex Kunth which is the rich source of sole active components with following objectives.

- Preliminary phytochemical screening of petroleum ether, acetone and ethanol extracts of stem, leaves and flowers of *Tecoma stans* (L.) Juss. ex Kunth.
- Estimation of free radical scavenging activity of petroleum ether, acetone, and ethanol extracts of stem, leaves and flowers of *T. stans* against DPPH radical.

REVIEW OF LITERATURE

The World Health Organization (WHO) estimates that 4 billion people (80%) of the world's population presently use herbal medicine for one form of primary health care or another. Because they show minimum or no side effects and are considered to be safe .Herbal medicines are promising choice over modern synthetic drugs (Kelechi and Nneamaka, 2011). Nowadays Synthetic drugs are becoming less effective due to the organism resistant activities. Plant based phytochemicals are becoming potentially more promising, there by replacing the synthetic drugs (Magaji *et al.*, 2019)

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Ahmad *et al.*, 2013). The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents (Nostro *et al.*, 2000). Phytochemicals are known as secondary plant metabolites and have biological properties such as antioxidant activity antimicrobial effect, modulation of detoxification enzymes, stimulation of immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases .

Tecoma stans

Tecoma stans has green foliage, inconspicuous yellow colour bell shaped flowers with an abundance of conspicuous brown seeds (Parrotta, 2001; Khare, 2007). It is an indigeneous medicinal plant which is gradually gaining popularity throughout the world. All the parts of

the plant have been in use traditionally as preventive and curative agents against various disorders and diseases. They have marked their use as efficient hypoglycemic agents, powerful diuretics and effective against infections (Raju *et al.*, 2011; Salem *et al.*, 2013). It was observed that the extracts of stem bark generally showed better antimicrobial activity against some organisms (Binuti and Lajubutu, 1994).

Phytochemical Screening

Phytochemicals are chemical compounds formed during the plants' normal metabolic processes. These chemicals are often referred to as *"Secondary metabolites"* of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, polysaccharides, phenols, tannins, terpenes and terpenoids (Okwu, 2004). In addition to these substances, plants contain other chemical compounds. These can act as agents to prevent unconsiderable side effects of the main active substances or to assist in the assimilation of the main substances. Plants have an almost limitless ability to synthesize aromatic substances, mainly secondary metabolites of which 12,000 have been isolated, a number estimated to be less than 10% of the total (Mallikharjuna *et al.*, 2007).

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants which provide health benefits for humans further than those attributed to macro nutrients (Hasler and Blumberg, 1999).

Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds (Costa *et al.*, 1999.).

There are more than thousand known and many unknown phytochemicals. It is well known that plants produce these to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases (Rao, 2003).

Hot aqueous and ethanol extracts of *Enicostemme littorale* showed the presence of alkaloids, saponins, steroids, tannins, proteins, reducing sugar, phenols and coumarins (Sanmugarajah *et al.*, 2013).

Oluwatoyin *et al.* (2011) screened the presence of alkaloids, saponins, tannins, glycosides and flavonoids from petroleum ether, chloroform, ethyl acetate and methanol extracts of aerial parts of *Heliotropium indicum.*

Mir *et al.* (2013) invertigate the qualitative analysis of the major bioactive constituents of medicinally important plant *Taraxacum officinale* in its aqueous and methanol extract of root, stem and flower. Saponins, flavonoids, alkaloids, phenols were highly concentrated in the stem, root and flower.

The preliminary phytochemical investigations of methanolic extract of *Tagetes erecta* flower was performed which shows the presence, steroids, terpenoids, flavonoids saponins and alkaloids (Vijay *et al.*, 2013).

According to the WHO (2002) estimations, 60-90% of the population in developing countries like India, Rwanda, Uganda, Tanzania and Ethiopia extensively use the traditional and alternative medicines for health care. Plants and plant based products are an integrated part of most of the traditional and alternative systems of medicines worldwide. In developed countries like Belgium, the USA, Australia, France and Canada 30-70% of the population use the traditional and alternative remedies at least once for health care. Some 7,000 medicinal compounds used in the western pharmacopoeia are derived from plants (Caufield, 1991). In USA, approximately 25% of all prescription drugs used contain one or more bioactive compounds derived from the vascular plants (Farnsworth, 1985). Farnsworth (1985) identified 119 secondary metabolites isolated from higher plants that were being used globally as drugs. These 119 useful drugs are obtained commercially and extracted from

about 90 species of plants. With more than 2, 50,000 species of higher plants, drugs that are more useful remain to be discovered (Farnsworth and Bingle, 1977).

The major categories of phytochemicals as the phytodrugs contain secondary metabolites like terpenoids, alkaloids, flavonoids, phenols and their derivatives, modified lipids and carbohydrates, alkanes like polyacetylene, sulphur compounds like isothiocyanates, thiophenes etc. (Williams, 2001).

Andrographis paniculata extracts is traditionally used as a medicine to treat different disease in India, China and Southeast Asia. The methanolic extracts of *A. paniculata* was fractionated into dichloromethane, petroleum ether and aqueous extracts and screened for bioactivity. The dichloromethane fraction of the methanolic extracts retains the active compounds contributing for both the anticancer and immuno stimulatory activity. On further fractionation of the dichloromethane extract, three diterpene compounds, ie. (1) andrographolide, (2) 14-deoxyandrographolide and (3) 14-deoxy-11,12-dihydroandrographolide were isolated (Kumar *et al.*, 2004).

Phytochemical investigation of the roots and aerial parts of *A. paniculata* yielded a new flavone, 5 – hydroxy 7, 2', 6' – trimethoxyflavone and an unusual 23 – carbon terpenoid, 14 – deoxy – 15 – isopyropylidene – 11, 12 – didehydroandrographolide together with five known flavonoids and four known diterpenoids. The structures of these compounds were determined on the basis of spectral and chemical studies (Reddy *et al.*, 2003).

Phytochemical screening of alkaloids, steroids or triterpenes and saponins was carried out by Din *et al.* (2002) in 103 leaf samples from 102 plant species representing 78 genera and 41 families of the Crocker range. From the samples screened, a total of 419 and53 samples were found to give positive results for alkaloids, steroids, terpenoids and saponins

respectively. Among the 55 plants investigated by Mojab *et al.* (2003) 39 were found to contain flavonoids, 20 were found to contain tannins and 44 were found to contain saponins.

Phytochemical investigation carried out by Ramesh *et al.* (2002) in the various extracts of the leaves of *Begonia malabarica* resulted in the isolation and identification of six known compounds viz., friedelin, epi-fridelinol, sitosterol, luteolin, quercetin and sitosterol-3, D-Glucopyranoside and also the presence of flavones ,sterols, triterpenes, phenols, quinones, saponins ,tannins and starch. *Erythrina* with over 110 species has shown to be good sources of C-prenylated flavones, isoflavones, isoflavonones and pterocarpans and of nitrogenous compounds (Barron and Ibrahim, 1996).

Faraz *et al.* (2003) carried out phytochemical screening in fifty five Iranian plants belonging to 21 families. Wang *et al.* (2003) isolated the active principles from selected Chinese herbs and used Gas Chromatography-Mass Spectrometric analysis for structure elucidation. Theeshan *et al.* (2005) studied the phytochemical constituents of *Cassia fistula.*

Falodun *et al.* (2006) reported the occurrence of flavonoids, saponins, diterpenes and phorbolestersin in the aqueous and methanol extracts of *Euphorbia heterophylla.* Two new homoisoflavonoids were isolated from *Caesalpinia pulcherrima* by Maheswara *et al.* (2006). Raghavendra *et al.* (2006) examined different solvent extracts of the powdered leaf of *Oxalis corniculata* and reported the presence of phenols, glycosides, carbohydrates, phytosterols and tannins. Rahaman *et al.* (2006) reported 3, 5, 7, 4-tetrahydroxy flavone from the leaves of *Cassia alata.*

Awoyinka *et al.* (2007) isolated eight bioactive compounds from the water and ethanol extracts of dried leaf of *Cnidoscolu saconitifolius.* Different extracts of *Semecarpus anacardium* were analysed by Mohanta *et al.* (2007) for their phytochemical properties.

Onwukaeme *et al.* (2007) detected reducing sugars, phenols, tannins and flavonoids in *Pycanthus angolensis*. Uma Devi *et al.* (2007) carried out the phytochemical analysis in *Achyranthes bidentata*. The methanol and acetone extracts of 14 plants belonging to different families were evaluated to detect the presence of various phytochemicals by Vaghasiya and Chanda (2007) and this study revealed the presence of tannins, cardiac glycosides, steroids and saponins.

Ravirajsinh *et al.* (2009) conducted both qualitative and quantitative studies for detecting the phytochemical constituents found in methanol extract of *Clerodendron glandulosum*. Sazada *et al.* (2009) studied preliminary phytochemicals found in some important medicinal and aromatic plants.

Sirohi *et al.* (2009) evaluated twenty one different herbal plants and their parts for total sugar, protein, tannin and saponin contents using aqueous, methanol and acetone extracts.

Swertiamarin found in 60% methanol extract of *Enicostemma littorale* was analysed by Alam *et al.* (2011) by high performance thin layer chromatographic densitometric method. Phytochemical and pharmacognostic analyses were carried out in *Dolichandrone arcuata* by Bojaxa and Henry Joseph (2011).

Jeeva *et al.*, (2011) identified the phytochemical constituents in methanol extract of the flower of *Albizia lebheck, Cordia sebestena, Thunbergia grandiflora* and *Antigonon leptopus*. Sukumaran *et al.* (2011) identified the pytochemical constituents of methanol extract of flower of *Peltophorum pterocarpum*.

Manohar *et al.* (2012) reported the phytochemicals present in the ethanol and methanol extract of fruit of *Terminalia belerica*. Bajaj *et al.* (2012) reported the phytochemicals present in the methanol extract of *Achyranthes aspera*. Narasimhan and Mohan (2012) studied the preliminary phytochemical screening of *Sesamum indicum* seed

Johnson *et al.* (2012) reported the phytochemical constituents of methanol flower extracts of *Helictresisora*, *Spathodea campunulata*, *Antigonon leptopus* and *Thunbergia grandiflora*. Kiruba *et al.* (2012) studied the phytochemical analysis of various solvents extracts of the flower of *Rhododendron arboretum* spp. *nilagiricum*.

Phytochemical screening and antioxidant activities in different solvent extracts of *Thymus satureioïdes* was carried out by Labiad *et al.* (2017). The extracts were subjected to various chemical test for phytochemical constituents, total phenolic contents were evaluated using Folin Ciocalteu method and their antioxidant activity was assayed through «in vitro» radical scavenging activity using DPPH· assay, FRAP and ABTS.

Antioxidant activity

Antioxidants are vital substances which possess the activity to protect the body from damage caused by free radicals including oxidative stress (Ozsoy *et al.*, 2008).

Antioxidant activities of 23 Iranian *Ocimum* accessions were studied by Javanmardi *et al.* (2003). Chen *et al.* (2004) used microplated ABTS, H_2O_2 and HRP system for evaluating total antioxidant activity of several popular vegetables and traditional Chinese herbals. An *in vitro* survey for the antioxidant potentials of three local Mediterranean food plant extracts (*Cichorium intybus*, *Sonchus oleraceus* and *Papaver rhoeas*) was made by Schaffer *et al.* (2005).

Pourmorad *et al.* (2006) carried out a comparative study on the antioxidant potentials of some selected Iranian medicinal plant extracts. The antioxidant properties of 25 edible tropical plants were studied by Wong *et al.* (2006). Badami and Channabasavaraj (2007) studied the *in vitro* antioxidant activities of thirteen medicinal plants collected from Western Ghats of India.

Ademiluyi and Oboh (2008) studied the antioxidant activity of methanol leaf extract of *Viscum album*. By using linolenic acid peroxidation and DPPH methods, Effat *et al.* (2008) screened thirteen medicinal plant extracts for antioxidant activity. Janat *et al.* (2008) prepared the crude extracts of stem and leaves of *Adenia lobata* and *Desmodium ascendens*, root and leaves of *Glyphea brevis* and *Palisota hirsuta* and analyzed antioxidant potentials using DPPH assay. Moni Rani *et al.* (2008) evaluated antioxidant activities of methanol extract of *Ixora coccinea* by DPPH free radical scavenging activity, reducing power and total antioxidant activity assays. Rahman *et al.* (2008) using DPPH assay, confirmed free radical scavenging activity of methanol leaf extract of *Cassia sophera*. Suresh Kumar *et al.* (2008) surveyed antioxidant activities of *Albizia amara, Achyranthes aspera, Cassia fistula, C. auriculata* and *Datura stramonium*.

Aliyu *et al.* (2009) evaluated antioxidant potentials of *Bauhinia rufescens* leaf extract by DPPH and reducing power assays. Amal Kumar *et al.* (2009) evaluated the antioxidant potentials of leaf and bark of *Azadirachta indica*. Bushra *et al.* (2009) prepared four solvent extracts of the leaves of *Terminalia arjuna* and *Aloe barbadensis* by adopting two extraction techniques and observed their antioxidant activities. Devi *et al.* (2009) evaluated the antioxidant activity of *Nephellium lappaccum*. Demiray *et al.* (2009) screened the leaves of *Tilia argentea* and *Crataegi folium* and roots of *Polygonum bistorta* for their antioxidant properties. Jaleel (2009) evaluated the antioxidant potentials of leaf and root tissues of *Withania somnifera*. Laetitia and Christian (2009) studied antioxidant activity of *Crithmum maritimum*. Ljiljana *et al.* (2009) studied the antioxidant activity of *Hieracium pilosella* extracts.

Using five *in vitro* assays, Patel *et al.* (2009) investigated the antioxidant activities of the methanol extract of *Grangea maderaspatana*. Total phenolic contents were also

determined by them to evaluate the relationship between the antioxidant activity and the phytochemical constituents. Preliminary phytochemical screening and *in vitro* antioxidant activity of *Gymnema sylvestre* leaf extract were investigated by Rachh *et al.*, 2009.

The antioxidant activity was studied in some *in vitro* antioxidant models like DPPH radical scavenging activity, Superoxide radical scavenging activity, Ferric reducing power and hydrogen peroxide scavenging activity. Vidyadhar *et al.* (2010) determined the in vitro antioxidant activity of chloroform extract of aerial parts of *Securinega leucopyrus*. The antioxidant capacity of leaf, stem and fruit extracts of *Andrographis paniculata* was investigated by Arash *et al.* (2010). The dried leaf aqueous and methanol extracts of *Vernonia amygdalina, Carica papaya, Persea americana* and *Cnidoscolus aconitifolius* were evaluated by Asaolu *et al.* (2010) for their antioxidant potentials.

Dheeraj *et al.* (2010) assessed the antioxidant potentials of different dried parts of *Cassia sophera*. Methanol extracts of *Carica papaya, Fagara zanthoxyloides, Cajanus cajan* and *Parquetina nigrescens* were evaluated for their antioxidant activities by Imaga *et al.* (2010).

Antioxidant potentials of methanol leaf extracts of *Caesalpinia coriaria, Flacourtia cataphracta, Hiptage benghalensis, Sesbania sesban, Persea macrophylla* and tubers of *Gloriosa superba* were analysed by Amutha and Shanthi (2011). Gokhan *et al.* (2011) examined *in vitro* antioxidant activity and fatty acid composition of *Centaurea urvillei*. Mishra *et al.* (2011) screened the extracts of ten Indian medicinal plant species for their antioxidant activities. Antioxidant potentials of *Gynura procumbens, Achyranthes aspera* and *Polygonum tomentosum* were studied by Mon *et al.* (2011). The chloroform and methanol leaf extracts of 124 Egyptian plant species belonging to 56 families were investigated and compared by Moussa *et al.*, (2011), for their antioxidant potentials.

Patel and Patil (2011) studied the *in vitro* antioxidant activity of seeds of blue and white flowered varieties of *Clitoria ternatea*. Panda *et al.* (2011) screened the *in vitro* antioxidant activity of aerial parts of *Cocculus hirsutus*.

Antioxidant compounds like phenolic acid, polyphenols and flvonoids scavenge free radicals such as peroxide, hydroxide of lipid hydroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Subramaniam *et al.*,2011)

The antioxidant potential of different solvent extracts of *Crataeva magnna* were evaluated using DPPH, ABTS, Superoxide radical, hydroxyl radical, nitric acid radical scavenging activities (Sridhar *et al.*, 2012). The antioxidant activities of methanol and aqueous extracts of 31 medicinal wetland plants in Taiwan were investigated by Ho *et al.* (2012). *In vitro* antioxidant activities of various parts of *Cinnamomum cassia* extracted with different extraction method were evaluated by Yang *et al.* (2012).

MATERIALSANDMETHODS

MATERIALS

Botanical Name : *Tecoma stans* (L.) Juss. ex Kunth (Plate I)

Family : Bignoniaceae

Common Name : Yellow Bells, Trumpet Flower

Tamil Name : Ponnarali, Thanga arali, Sonnapatti

Botanical Description : A large shrub or small tree, much branched, growing upto 1.5- 5m tall, but grows occasionally upto 10m in height. Twigs tan or reddish tan, smooth, scarcely 4-sided; leaves opposite, pinnately compound, leaflets 1-9, usually 3-7, ovate lanceolate, apex acuminate, base acute or obliquely acute, very shortly petiolate or all but sub sessile, slightly hirsute on midrib and in vein axils beneath, margins irregularly serrate, leaves quite variable, rachis and petiole slender, glabrous. Inflorescence an axillary or terminal raceme, pedicels short, irregularly curved or twisted, bracts reduced to minute scales, flowers rather few, calyx narrowly cylindric-campanulate, 5-7 cm long, with 5 sub-equal acuminate teeth, glabrous; stamens 4, attached at summit of tube, in 2 unequal pairs, included, filaments pilose at base, curved above, anthers versatile, linear, yellow, pilose, 6 mm long; sterile fifth stamen much reduced; pistil about equaling stamens, ovary narrowly cylindric, about equaling calyx, style filiform, glabrous, stigma flat, elliptic; capsule linear, compressed, 10-20 cm long, 7-8 mm wide, brown when ripe, with raised line or suture lengthwise on each flat side, tardily dehiscent along suture, septum parallel with flat sides, firm, seeds flat, oblong, 7-8 x 4 mm, with a membranous transparent wing on each end, ends of wing erose, seeds entire including wing about 20 x 6 mm" .

Plate I. *Tecoma stans* (L.) Juss. ex Kunth

METHODS

Collection and Processing

The stems, leaves and flowers of *Tecoma stans* were collected near Government Polytechnic College, Thoothukudi, Tamil Nadu. The collected fragments were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender and sieved to get uniformed particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant material.

Preparation of extracts of Phytochemical screening:

Hot maceration method:

The coarse powder of sample (100 g) was extracted successively with petroleum ether, acetone and ethanol using Soxhlet apparatus. All the extracts were filtered through Whatman No.41 filter paper. All the extracts (petroleum ether, acetone and ethanol) were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures (Brinda *et al.*, 1981; Annonymous, 1990 and Lala, 1993).

Qualitative phytochemical analysis of different extracts

The chemical test for various phytoconstituents in the extracts were carried out as described below:

Test for carbohydrates

To 2ml of plant extract, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish color indicates the presence of carbohydrates.

Test for tannins

To 1ml of plant extract, 2ml of 0.7M NaOH and few drops of Folin-Denis reagent was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for saponins

To 2ml of plant extract, 2ml of distilled water was added and shaken in a graduated cylinder for 15minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

Test for flavonoids

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

Test for alkaloids

To 2ml of plant extract, 2ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.

Test for quinones

To 1ml of extract, 1ml of concentrated sulphuric acid was added. Formation of red color indicates presence of quinones.

Test for terpenoids

To 0.5ml of extract, 2ml of chloroform was added and concentrated sulphuric acid was added carefully. Formation of red brown color at the interface indicates presence of terpenoids.

Test for fixed oil

The powder was gently scrubbed on the filter paper formation of grease spot indicates the presence of fixed oil.

Test for gums

1 ml of extract was mixed with water . Thickening of the extract shows the presence of gum.

Test for phenols

To 1ml of the extract, a few drops of Phenol-Denis reagent was added followed by 2ml of 15% Sodium carbonate solution. Formation of blue or green color indicates presence of phenols.

Test for coumarins

To 1 ml of extract, 1ml of 10% NaOH was added. Formation of yellow color indicates presence of coumarins.

Test for steroids

To 1ml of plant extract equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicates the presence of phytosteroids.

Test for protein

Ninhydrin test

To 1 ml of the sample add few drops of 0.2% ninhydrin solution . Appearance of pink or purple colour indicates the presence of protein.

Test for anthraquinones

To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate indicates the presence of anthraquinones.

Antioxidant Assays

DPPH free radical scavenging assay (Shen *et al.*, 2010)

The ability of the samples to annihilate the DPPH radical (1,1-diphenil-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 extract (100, 250 & 500 µg) of sample were added, at an equal volume to methanolic solution of DPPH (0.1mM). The reaction mixture is incubated for 30min in dark at room

temperature; the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid was used as standard control. Blank is pure methanol and control sample is methanol along with DPPH solution. The annihilation activity of free radicals was calculated in percentage inhibition according to the following formula

% of Inhibition = (A of control – A of Test)/A of control * 100

RESULT AND DISCUSSION

Medicinal plants keep the health and vitality of individuals and cure various diseases, including cancer without causing toxicity. In this view, the preliminary phytochemical screening and antioxidant activity of stem, leaves and flowers of *Tecoma stans* have been investigated and discussed.

Preliminary phytochemical analysis

Phytochemicals are non-nutritive plant chemicals that have either defensive or disease protective properties. They are non-essential nutrients and mainly produced by plants to provide them protection. These phytochemicals, either alone and or in combination, have tremendous therapeutic potential in curing various ailments and they offer protection against numerous diseases and disorders such as cancer, coronary heart disease, diabetes, high blood pressure, inflammation, microbial, viral and parasitic infection, psychotic disease, spasmodic condition, ulcers, osteoporosis and associated disorders (Prakash *et al.*, 2012).

Therefore the current study is attempted to find out the presence of preliminary phytochemicals in petroleum ether, acetone and ethanol extract of stem, leaves and flowers of *T. stans* and the results are depicted in Table 1-3.

The result of preliminary phytochemical screening reveals the presence of alkaloids, carbohydrate, coumarins, flavones, phenols, protein, quinones, starch and terpenoid in petroleum ether, acetone and ethanol extract of stem, leaves and flowers of *T. stans*. Anthroquinones present in the different extracts of leaf and stem of *T. stans* whereas saponin is present only in petroleum ether, acetone and ethanol extract of leaves of *T. stans*. Steroids are present in ethanol extract of stem and all the three extracts of flower. Tannin present in

Table 1. Preliminary phytochemical screening of *Tecoma stans* Stem

Bioactive Components	Different extracts		
	Petroleum Ether	Acetone	Ethanol
Alkaloids	+	+	+
Anthroquinones	-	-	-
Carbohydrates	+	+	+
Coumarin	+	+	+
Fixed oils	-	-	-
Flavones	+	+	+
Gum	-	-	-
Phenols	+	+	+
Proteins	+	+	-
Quinones	+	+	+
Saponin	-	-	-
Starch	+	+	+
Steroids	-	-	+
Tannins	-	-	+
Terpenoids	+	+	+

+ :Present

- : Absent

Table 2. Preliminary Phytochemical Screening of *Tecoma stans* Leaf

Bioactive Components	Different extracts		
	Petroleum Ether	Acetone	Ethanol
Alkaloids	+	+	+
Anthroquinones	+	+	+
Carbohydrates	+	+	+
Coumarin	+	+	+
Fixed oils	-	-	-
Flavones	+	+	+
Gum	-	-	-
Phenols	+	+	+
Proteins	+	+	+
Quinones	+	+	+
Saponin	+	+	+
Starch	+	+	+
Steroids	-	-	-
Tannins	-	-	-
Terpenoids	+	+	+

+ : Present.

- : Absent.

Table 3. Preliminary Phytochemical Screening of *Tecoma stans* Flower

Bioactive Components	Different extracts		
	Petroleum Ether	Acetone	Ethanol
Alkaloids	+	+	+
Anthroquinones	+	+	+
Carbohydrates	+	+	+
Coumarin	+	+	+
Fixed oils	-	-	-
Flavones	+	+	+
Gum	-	-	-
Phenols	+	+	+
Proteins	-	-	-
Quinones	+	+	+
Saponin	-	-	-
Starch	+	+	+
Steroids	+	+	+
Tannins	-	-	+
Terpenoids	+	+	+

+ : Present.

- : Absent.

ethanol extracts of stem and flowers of petroleum ether, acetone and ethanol extract of stem, leaves and flowers of *Tecoma stans*.

Phytochemical analysis is one of the important parameter to be evaluated in pharmacognostic study for standardization and authentication of medicinal plants with help of which adulteration and substitution can be prevented (Chandra, 2014). Presence or absence of certain important compounds in an extract is determined by colour reactions of the compounds with specific chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation. Various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds. Different chemical compounds were detected in whole plant of *T. stans* extracts which could make the plant useful for treating different ailments as having a potential of providing useful drugs of human use. This is because the pharmacological activity of any plant is usually traced to a particular compound.

Phenolic phytochemicals are the largest category of phytochemicals and the most widely distributed in the plant kingdom. Phenolic compounds are a large and complex group of chemical constituents found in plants (Walton *et al.*, 2003). They are plant secondary metabolites, and they have an important role as defense compounds. Phenolics exhibit several properties beneficial to human and it's antioxidant properties are important in determining their role as protecting agents against free radical mediated disease processes (Saxena *et al.*, 2013).

Phenols have been mainly studied for their properties against various degenerative diseases, such as cardiovascular diseases, inflammation and cancers (Mandal *et al.*, 2010). Many papers and reviews describe varied biological activities of phenols were reported. Increases bile secretion, reduces blood cholesterol and lipid levels and antimicrobial activity

against some strains of bacteria such as *Staphylococcus aureus* are some of biological activities of phenols (Gryglewski, 1987).

Flavonoids are polyphenolic compounds that are ubiquitous in nature more than 4,000 flavonoids have been recognized, many of which occur in vegetables, fruits and beverages like tea, coffee and fruit drinks (Pridham, 1960).The flavonoids appear to have played a major role in successful medical treatments of ancient time and their use has persisted upto now. More than 4,000 flavonoids have been described so far within the parts of plants normally consumed by humans approximately 650 flavones and 1030 flavonols are known (Harborne and Boxter, 1999). Flavonoids have been stated to possess many useful properties including anti-inflammatory activity, enzyme inhibition, antimicrobial activity, oestrogenic activity, anti-allergic activity, antioxidant activity, vascular activity and cytotoxic anti-tumour activity (Tapas *et al.*, 2008).

Tannins are a heterogenous group of high molecular weight polyphenolic compounds. Tannins are found commonly in fruits such as grapes, persimmon, blueberry, tea, choclate, legume forages, legume trees and in grasses (Ginge-chavez, 1996). Several health benefits have been recognized for the intake of tannins and some epidemiological associations with the decreased frequency of chronic diseases have been established. In medicine, especially in Asian natural healing, the tannin containing plant extracts are used as astringents against diarrhea, as diuretics, against stomach and duodenal tumours (De Buyne *et al.*, 1999), and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals (Dolara *et al.*,2005).

Alkaloids are natural products that contain heterocyclic nitrogen atoms, are basic in character. They are naturally synthesized by a large number of organisms, including animals, plants, bacteria and fungi (Saxena *et al.*, 2013). Alkaloids are significant for the protecting and

survival of plant because they ensure their survival against bacteria, fungi, insects and herbivores and also against other plants by means of allelopathically active chemicals (Molyneux *et al.*, 1996). Alkaloids have many pharmacological activities including antihypertensive effects, antiarrhythmic effect, antimalarial and anti- cancer activity (Wink *et al.*, 1998).

The terpenoids are a class of natural products which have been derived from five-carbon isoprene units. Terpenes are widespread in nature, mainly in plants as constituents of essential oils. Among plant secondary metabolites terpenoids are a structurally most diverse group; they play an important role as signal compounds and growth regulators of plants (McCaskill and Croteau, 1998). In addition, terpenoids have medicinal properties such as anti-carcinogenic, antiulcer, herpaticidal, antimicrobial and diuretic activity (Langenheim, 1994; Dudareva *et al.*, 2004).

Saponins are a group of secondary metabolites found widely distributed in the plant kingdom. They form stable foam in aqueous solutions, such as soap, hence the name 'saponin'. Saponins are glucosides with foaming characteristics. Saponins consist of a polycyclic aglycones attached to one or more sugar side chains. The aglycone part, which is also called sapogenin, is either steroid (C27) or a triterpene (C30). The foaming ability of saponins is caused by the combination of a hydrophobic (fat-soluble) sapogenin and a hydrophilic (water-soluble) sugar part. Saponins have a bitter taste. Some saponins are toxic and are known as sapotoxin. Saponins are phytochemicals which can be found in most vegetables, beans and herbs. The best known sources of saponins are peas, soybeans, and some herbs with names indicating foaming properties such as soapwort, soaproot, soapbark and soapberry. Commercial saponins are extracted mainly from *Yucca schidigera* and *Quillaja saponaria*. Saponins have many health benefits. Saponins bind with bile salt and cholesterol in

the intestinal tract. Bile salts form small micelles with cholesterol facilitating its absorption. Saponins cause a reduction of blood cholesterol by preventing its re-absorption. Saponins seem to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability. Plants produce saponins to fight infections by parasites. When ingested by humans, saponins also seem to help our immune system and to protect against viruses and bacteria. The non-sugar part of saponins have also a direct antioxidant activity, which may results in other benefits such as reduced risk of cancer and heart diseases (http://www.phytochemicals.info). Many saponins are known to be antimicrobial, to inhibit mould and to protect plants from insect attack. Saponins may be considered as a part of plants defense systems, and as such have been included in a large group of protective molecules found in plants named phytoanticipins or phytoprotectants (Dubois and Wagner, 2000).

Plants containing carbohydrates, glycosides and coumarin are known to exert a beneficial action on immune system by increasing body strength and hence valuable as dietary supplements. Coumarin is a phytochemical with a vanilla like flavour. Coumarin is an oxygen heterocycle. Coumarin can occur either free or combined with the sugar glucose (coumarin glycoside). Coumarin is found in several plants, including tonka beans, lavender, licorice, strawberries, apricots, cherries, cinnamon, and sweet clover. Coumarin has blood-thinning, anti-fungicidal and anti-tumor activities. Coumarin should not be taken while using anticoagulants. Coumarin increases the blood flow in the veins and decreases capillary permeability. Coumarin can be toxic when used at high doses for a long period (http://www.phytochemicals.info). Coumarins can be suggested to be beneficial for hyper proliferative skin diseases on the basis of their antimicrobial and anti-inflammatory effects (Setchell and Cassidy,1999).

Antioxidant Activity:

DPPH radical scavenging activity

DPPH radical scavenging activity of petroleum ether, acetone and ethanol extracts of stem, leaves and flowers of *Tecoma stans* are shown in figure 1-3. Among the solvent tested, ethanol extract exhibited highest DPPH radical scavenging activity. At 10% concentration, ethanol extract of whole plant of *T. stans* possessed 98.94% scavenging activity on DPPH which is higher than the standard ascorbic acid (96.3%). The result revealed that as the concentration of extracts increases the radical scavenging activity also increases.

· The 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical is widely used as a model system to investigate the free radical scavenging activities of several plant extracts. DPPH is stable, nitrogen centered free radical which produces violet color in ethanol solution. It was reduced to a yellow colored product, diphenylpicryl hydrazine, with the addition of the extracts. The reduction in the number of DPPH molecules can be calculated with the number of available hydroxyl groups (Abirami *et al.*, 2012). The result indicates that the different extract of *T. stans* showed concentration dependent scavenging effect against DPPH radical. This showed that the extract with their proton donating ability, could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants (Marxen *et al.*, 2007).

Phenolic compound, the principal antioxidant constitutes of natural plant products, are composed of phenolic acid and flavonoids. These compounds are potent radical terminations by donating a hydrogen atom to the radical and preventing lipid oxidation at the initial step. The high potential of polyphenols to scavenge free radical may be because of their many phenolic hydroxyl groups. In this respect, polyphenolic compounds commonly found in plants have been reported to have multiple biological effects like anticancer, antiproliferative,

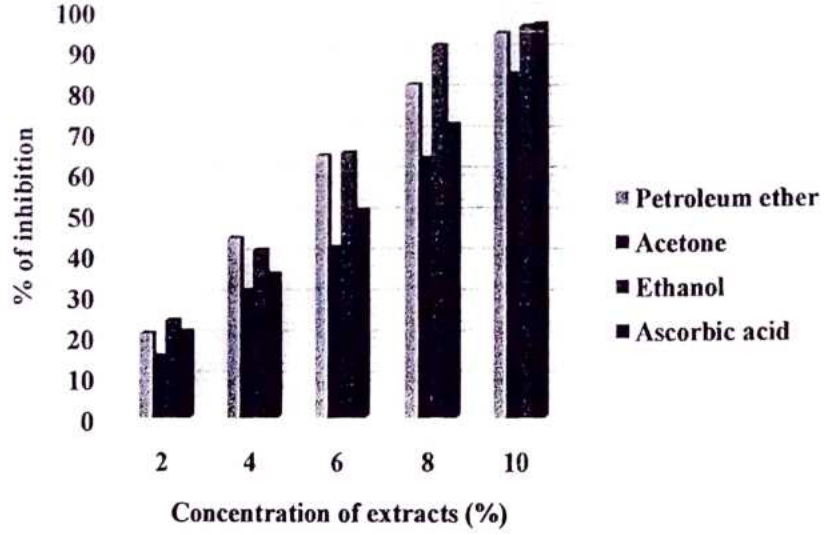

Fig 1: DPPH radical scavenging activity of different extracts of *Tecoma stans* stem

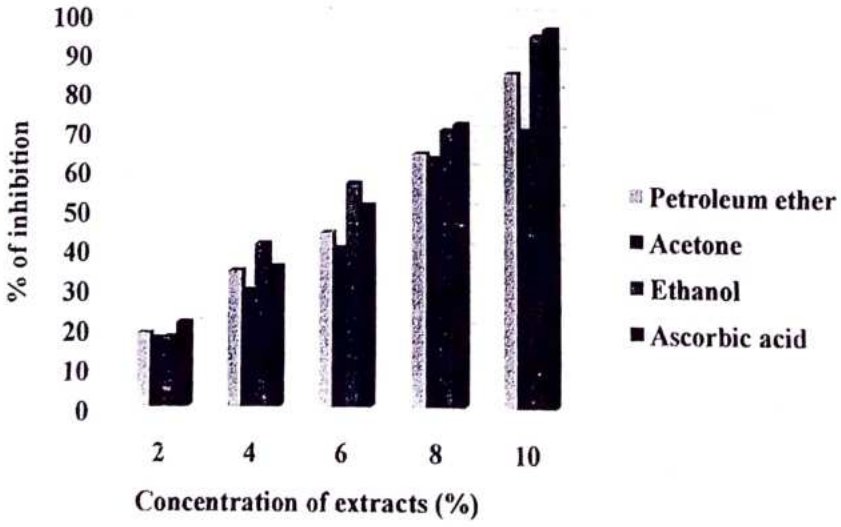

Fig 2: DPPH radical scavenging activity of different extracts of *Tecoma stans* leaf

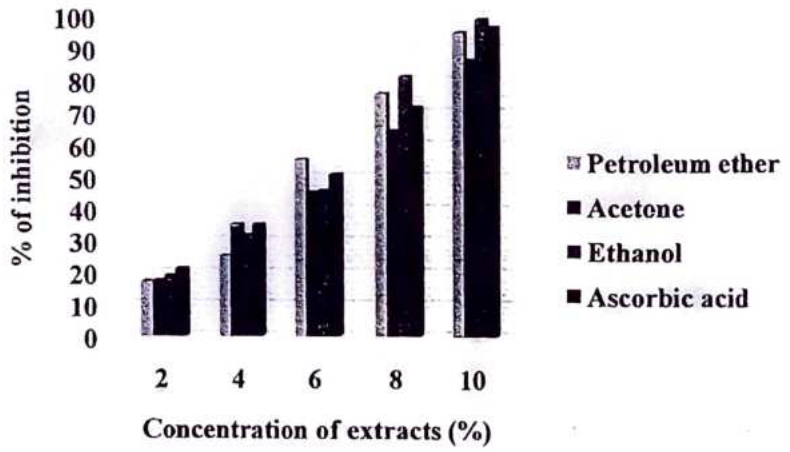

Fig 3: DPPH radical scavenging activity of different extracts of *Tecoma stans* flower

antimicrobial, wound healing and antibacterial activities including antioxidant activity. (Roopashree *et al.*, 2008; Senguttuvan *et al.*, 2014)

This study has confirmed the presence of phytochemicals and antioxidant activities of petroleum ether, acetone and ethanol extracts of stem, leaves and flowers of *Tecoma stans*. Ethanol was better solvent for extraction of antioxidant substances compared to the other solvents. Thus, the *T.stans* can be considered as an easily accessible source of natural antioxidants agents.

SUMMARY AND CONCLUSION

The stems, leaves and flowers of *Tecoma stans* were collected near Government Polytechnic College, Thoothukudi, Tamil Nadu for the current study. Preliminary phytochemicals like alkaloids, phenol, flavones, steroids, tannins, coumarin, quinones care qualitatively screened.

Alkaloids, carbohydrate, coumarins, flavones, phenols, protein, quinones, starch and terpenoid are present in petroleum ether, acetone and ethanol extract of stem, leaves and flowers of *T. stans*. Anthroquinones are present in the different extracts of leaf and stem of *T. stans* whereas saponin is present only in petroleum ether, acetone and ethanol extract of leaves of *T. stans*. Steroids are present in ethanol extract of stem and all the three extracts of flower. Tannin present in ethanol extracts of stem and flowers of petroleum ether, acetone and ethanol extract of stem, leaves and flowers of *T. stans*.

The result revealed that as the concentration of extracts increases the DPPH radical scavenging activity also increases. Among the solvent tested, ethanol extract exhibited highest DPPH radical scavenging activity. At 10% concentration, ethanol extract of whole plant of *T. stans* possessed 98.94% scavenging activity on DPPH which is higher than the standard ascorbic acid (96.3%).

The study revealed that the stem, leaves and flowers of *T. stans* contain a considerable quantity of phenolic and flavonoid compounds that were found to be the major contributor for their antioxidant activities. It may be considered in future to replace synthetic preservatives in food and pharmaceutical products due to its potent antioxidant activity. Further studies are needed to clarify the *in vivo* potential of this plant in the management of human diseases resulting from oxidative stress.

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L. Preethi

A STUDY ON THE EXTRACTION OF NATURAL DYE AND ITS APPLICATION ON SELECTED FABRICS

A Short Term Project Work Submitted to St. Mary's college (Autonomous) affiliated to Manonmaniam Sundaranar University in Partial Fulfillment for the Degree of

BACHELOR OF SCIENCE IN BOTANY

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2019 -2020

CERTIFICATE

It is certified that this short term project work entitled **"A STUDY ON THE EXTRACTION OF NATURAL DYE AND ITS APPLICATION ON SELECTED FABRICS"** submitted to **St. Mary's College (Autonomous)** affiliated to **Manonmaniam Sundaranar University** in partial fulfillment of the requirements for the degree of **Bachelor of Science in Botany** and is a record of work done in the Department of Botany, St. Mary's College (Autonomous), Thoothukudi during the year 2019-2020 by the following students.

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INTRODUCTION

INTRODUCTION

India boasts a diverse biodiversity and is not just one of the 12 mega diversity Countries in the world, but one of the eight principal centres of origin, domesticated taxa and diversification. It has approximately 4, 90,000 plant species of which about 17, 500 are Angiosperms; more than 400 are domesticated crop species and almost an equal number their wild relatives (Kumerasan and Kumar, 2011). Thus, India harbours a wealth of useful germplasm resources and there is no doubt that the plant kingdom is a treasure-house of diverse natural products. One such product from nature is the dye (Siva, 2007)

The art of dyeing was as old as human civilization. From the historical rewards it is learnt the natural colourants were only available to the people during Greco-Roman periods. Ancient Indians used natural dyes for dyeing of textile goods. The art of dyeing was known in India as early as the Indus valley period (3500 B.C.) (Gupta, 2001)

Dye is a substance used to imparts colour to textiles, paper, leather and materials such that the colouring is not reading altered by washing, heat, light or other factors to which the material is likely to be exposed. Dye differ from pigments, which are finely ground solids dispersed in a liquid, such as paint or ink or blended with other materials, most dyes are organic compounds (i.e., they contain carbon), whereas pigments may be inorganic compounds (i.e., they do not contain carbon) or organic compounds (Mahesh, 2011).

The textile industry worldwide produces and uses roughly1.3 million tonnes of precursors for colours, pigments and dyes that cost around 23 Billion Dollars. Textile processing is focused predominantly on synthetic dyeing materials. The textile

industries thus produce poisonous and hazardous chemical waste. That constitutes a threat to human health and the environment. (Adeel *et al.*, 2012). Since recent years the use of synthetic dye has increased exponentially in many important industries such as textile industry, pharmaceutical industry, food processing etc. The synthetic colouring is simple to use, and show superior properties of fastness over natural dyeing. Yet synthetic dye exhibits dominance fastness properties and it produces many side effects that cause allergic reaction on the human body (Meena *et al.*, 2013) For these reasons, the use of natural dyes is attracting more and more interests from dyeing industries again. Natural dyes are generally extracted from vegetable substances or fungal species or animals.

Dyes are one of the most important uses of the plants, as they are related with cultural practices, rituals, arts and crafts, fabrics and to satisfy personal embodiment, however, dye yielding plants have not received significant attention (Roy *et al.*, 2002). Recently, interest in the use of natural dyes has been growing rapidly due to the result of stringent environmental standards imposed by many countries in response to toxic and allergic reactions associated with synthetic dyes (Kamel, 2005).

Natural dyes, obtained from different source such plants, animals and minerals. The synthetic dye "mauve" by W. H Perkin 1856 and subsequent research, production and application of synthetic dyes, use of natural colourents declined sharply. Natural flora is full of exquisite colour fascinating and attracting human being towards a very partition of possibilities. A large number of plant and animals insects sources have been identified for extraction of colour and their diversified use in textile dyeing and other disciplines (Nilani, 2010).

Natural dyes are having following advantages (i) They are obtained from renewable resources (ii) No health hazards, some times they act as health care (iii) Practically no (or) mild chemical reactions are involved in their preparation (iv) No disposal problems (v) They are unsophisticated and harmonized with nature (vi) Lot of creativity is required to use these dyes judiciously (Saxena, 2013).

Besides they have limitations and drawbacks, like availability, colour yield, complexity of dyeing process, reproducibility of shade. Limited number of suitable dyes allowed only wool, natural silk, linen, and cotton to be dyed. Non-standardised, inadequate degree of fixation, inadequate fastness properties and water pollution by heavy metals and 3 large amounts of organic substances (Gupta, 2001).On the other hand, natural dyes are environment-friendly; for example, turmeric, the brightest of naturally occurring yellow dyes is a powerful antiseptic which revitalizes the skin, while indigo gives a cooling sensation.

Generally, three primary colours are required to get any given hue. This type of approach has been worked out for synthetic dyes. However in the case of natural dyes, the dyeing procedures are different for different dyes and they cannot be blended to get the required colour easily. In natural dye only limited number of dyes with good fastness properties is available. Natural dyes based on three colours blue, red and yellow are mainly available (Teliet al., 2013).

Mordant are considered as an integral part of the natural dyes. A close look at the chemical structures of natural dyes will show that these dyes like synthetic dyes also consist of vat, acid, basic, disperse, direct and mordant dyes (Prabhu and Bhute, 2012).

The continuing population use and the worsening environmental pollution make it necessary for researchers to find new ways of enhancing the health and hygiene qualities of consumer products. Antimicrobial finishes on fabrics can protect human beings against microbes. The application of antimicrobial textile finishes at present is confined to specialty products in the medical, technical, industrial, home furnishing and apparel categories Many of the plants used for dye extraction are classified as medicinal, and some of these have recently been shown to possess remarkable antimicrobial activity (Samanta *et al.*,2009)

Cotton is a very fine much elongated, single cell seed hair which grows on the outside of a cotton seed. It is a bundle of cellulose chain molecules. Cotton fabrics are affected by ordinary light and more particularly by U.V. light. They are attacked by strong acids like hydrochloric, sulphuric and nitric acid, volatile organic acids like acetic acid do not harm cotton fabric. Dilute alkalies at room temperature do not injure cotton, if boiled with alkali, cotton turns yellow and loses strength. Cellulose structure consists of two regions, a) amorphous region b) crystalline region (ShenaiSamanta and DeepaliSinghee, 2003).

The fibroin is surrounded by a gummy substance called serum. The serum gives a coarse feeling. The smooth silk is obtained by removing the serum using alkali and this process is called degumming. On heating it liberates dirty odour, it is unaffected by acid and alkali. Dyeing of silk with the natural dyes is a traditional craft in India (Cook Gordon, 1993).

SCOPE AND OBJECTIVES

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Colours are one of nature's greatest gifts. They are present everywhere in the world, all around us. Of course textile fabrics are colourless or off-white / creamy. Colouring textiles therefore proves appropriate in order to make them elegant and appealing. Coloring is the materials with unique colours (colour-bearing functional groups) that can impart colour on other substrates such as food, textiles (fibres), cloth , paper, etc. Mankind has been using natural dyes originating from different sources since time immemorial. The production and use of natural textile dyes is both an art and science synonymous with human culture. To achieve a perfect performance the process requires both skills and experience. In addition to dyeing textiles, natural dyes are used in the form of pigments in paintings.

By keeping the above scope in mind the flower of *Zinnia elegans* Jacq. was selected for the present study to carry out the following objectives.

- Extraction of dyes from *Zinnia elegans* flower using Aqueous, Acidic, Alkaline and Ethanol medium
- Dyeing of (i) cotton (ii) Polyester and (iii) silk using above dye extracts
- Dyeing of extracted dye with different mordants.
- Assessment of light fastness and washing fastness for each dyed sample.
- Antimicrobial activities of each extracted dyes.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Red rose is a one of the most important ornamental plant mainly growing in garden and rich in red and pink pigments. The dyeing pigments present in flowers of red rose were extracted by using four different solvent extraction methods .The three different mordant were used. The result revealed that different shades of pink an yellow colour were obtained from the dye to mordant .The colour dye extracted from red rose flower can be used for coloration of cotton ,silk and wool fabrics (Gopika *et al.*, 2018).

The dye potential colourants obtained from the marigold evaluated by colorin pure cotton fabrics ,yarns of the pure cotton and wool various metal salts were used as mordant extract dye on the fabrics and yarns fastness tests of dyed clothes was undertaken .The colour shades differences .The maximum strength of the dye was found in the ethanol-water-mixture solvent .The surface colour of the dyed fabrics and yarns was not affected by washing .The different colour shades were obtained for mordant used .Good light fastness ,wash fastness and rubbing fastness were observed of fabrics mordanted ferrous sulphate. These finding reveal that *tagetes erecta* can be used in textile industry for dyeing purpose (Selvam *et.al.*, 2015).

Merina *et al.,(*2016) made an attempt has been made to utilize petal parts of chrysanthemum flower and peel of Badamfraie to extract dye for application on the fabrics .The natural dye was extracted from the flower of chrysanthemum with two different colours. To the experimental results the performance was better with that all the natural dye which can be the better alternative of synthetic dyes.

According to Thilagavathi and Rajendrakumar (2005) *Celosia argenteal* is a species of the genus celosia belonging to the family Amarantheceae .The present work highlights the use of *Celosia argenteal* aqueous flower extract as acid base indicator in acid base titrations .The equivalence point obtained by the flower extract .The natural indicator was found be a very useful ,economical simple and accurate for the acid.

Singh *et al.*, 2006 founded that natural dyes are obtained from natural sources such as plants ,insects and minerals .All the plant based dye sources are more important for textile dyeing as it provides both dye as well as frequence. This paper review the available for floral dye sources application and extraction of colourant from flower and effect of different mordant.

Rupali Respande and Alka Chaturauedi (2014) made the dye extracted from pink flower of *Plumeria rubrain* pure aqueous medium and producing various green, ivory, brown shades on silk cloth. The antibacterial assay showed that both the extract inhibit growth of bacterial strains. The distilled water extract of the flower was more active against all bacterial strains. Anthocyanins from coloured petals of *Sesbania sesban* flower were extracted by using solvents such as methanol and acidified methanol. From the extracts of *Sesbania sesban* flower petals the antioxidant and antimicrobial properties of anthocyanins were analysed. These merits can put the plant in a wider and more significant perspective with respect to national and global interest in medicinal uses and food industries.

Samanta and Agarwal (2007) studied the dyeing of bleached jute and cotton fabrics with mordants using Jack fruit wood extract. It is observed that the application of 10-20 per cent myrobolan followed by 10- 20 per cent of Al2(S04)3 or FeS04 in

sequence have been identified as two most prospective mordanting systems. The study on the effect of dyeing process variables on surface colour strength indicates that the 90 min dye 33 concentration, and 15 gpl common salt are the optimum values with minor differences among the different fibre mordant systems studied. Colour fastness to washing, rubbing and exposure to sunlight, in general, and dyeing-pH sensitivity, in particular, for selective fibre-mordants-dye systems have also been assessed and compared. Dyeing at pH 11.0 for both the double pre-mordanting systems offers overall good colour fastness properties.

Vasundhara *et al.*, (2016) extracted organic colour from kokum (*Gracinia indica*) fruit rind to dye textiles such as cotton, jute, silk and polystyrene. The fruit rind is used in three different forms for dye extraction: 1. fresh rind, 2. dry rind (the fresh rinds dried in hot air oven at 50°C for 5 days), 3. sugar rind (the rinds are soaked in sugar for a week and kokum juice was extracted and dried in hot air oven at 50°C for 5 days. The extracts are found to be rich in anthocyanins and were quantified as 79.93, 85.03 and 7.83 mg/kg, respectively. One set of fabrics were dyed without mordant and the other set was mordanted 20 with 2% ferrous sulphate for 30 minutes; then all the fabrics were dyed with the dye extracts for 60 minutes. Mordanted fabrics produced better shades than un-mordanted samples. Jute exhibited best shades than other fabrics dyed.

Mamta *et al.*, (2017) defines "Cosmetotextiles" as a concept that (fabric) releases cosmetic ingredients to the skin of the wearer. The functions of cosmetotextiles include moisturizing, UV protection, skin whitening, anti-wrinkle treatment, aromatic, refreshing and relaxing. The methods followed to impart cosmetic effects on textiles are dyeing and finishing (microencapsulation). The cosmetic ingredients include bio-active materials like aloe vera, ginseng, chitosan, squalene, fruits, flowers, extracted

essential oils, vitamin E, etc. The cosmetotextiles might have future scope if developed through research.

The oldest natural dye for textile, used by humans is indigo dye obtained from the plant *Indigofera tinctoria* opines Samanta *et al.*, (2009). It is the most popular dye that ruled the world from the middle of seventeenth century to the end of nineteenth century. The Indian subcontinent has played a major role in the history of indigo. The indigo plant grows favorably in tropical climate rather than temperate climates. The emergence of the indigo plantations in the seventeenth century has been a marked phase in the history of indigo states Kumar (2012). Indigo plantations were introduced in India during eighteenth century and in the beginning of nineteenth century. Bengal became a major supplier of indigo to the entire world. Then the indigo plant was cultivated in different parts of India. Indigo dye became more popular with the denim fabrics manufacture.

Sonia John *et al.*,(2018)stated that the extracts of *Ixoralutea* (Rubiaceae) as an effective antimicrobial agent and a natural dye. The preliminary phytochemical analysis of the stem and leaf extracts in various solvents (polar and non-polar) revealed the presence of alkaloids, carbohydrates, phenols, tannins, saponins, reducing sugar, triterpenoids and steroids. The flower showed great antibacterial and antifungal properties.the extraction of natural dye from the flower and their application on textiles. The extracted dye along with the mordants gave varying shades of colors on the fabric.

SandeepBains *et al.*, (2003) studied the dyeing of cotton with peach leaves using different mordants. The dyeing was carried out at optimized dyeing conditions namely, dye material, extraction time, dye material concentration and dyeing time and

using combinations of mordants such as alum: chrome, alum: copper sulphate, alum: ferrous sulphate, chrome: copper sulphate, chrome: ferrous sulphate, copper sulphate: ferrous sulphate in the ratio of 50:50, 25:75 and 75: 25 respectively. The dyed samples were evaluated for colour fastness to washing, rubbing, perspiration and light. The dyeing of cotton at optimized conditions resulted in good to very good colour fastness to light (rating range 5-6), fair to excellent colour fastness to washing (rating range 3-4/5), good to excellent colour fastness to rubbing (rating range 4-5) and poor to fair colour fastness to perspiration (rating range 2-3) as found by evaluation of the colour fastness of the dyed samples by prescribed methods. The shades obtained were khaki, greenish khaki, bamboo light, platinum blonde, beige, shallow to dark shallow, greyish military green, mouse grey and brownish grey to dark brownish grey.

Sudhakar and NingeGowda (2005) analysed the degummed silk fabric dyed with the flower extract of *Spathodea companulata* along with varying concentrations of different mordants. Colour values with respect to K/S and CIE L* a* b* were influenced by the mordants and the mordanting techniques. Pre-mordanting was found to be better in the case of stannous chloride whereas meta-mordanting was found better in case of potassium aluminiumsulphate and tannic acid with respect to colour values. The unmordanted dyed samples exhibited good fastness to washing, rubbing and perspiration, barring light. A very slight improvement in fastness to light was recorded with the use of tannic acid as mordant.

Susan *et al.*, (2006) studied the effect of mordants alum, chrome ferrous sulphate and copper sulphate on colour fastness properties of cotton dyed with Kilmora dye. It was found that different mordants improved the colour fastness of Kilmora dye on cotton.

Silk fabric was dyed with natural dye extracted from eucalyptus leaves by Mongkholrattanasit *et al.*, (2011). The fabric was dyed with mordants such as alum, ferrous sulphate, copper sulphate and stannous chloride and also without any mordant. The optimum results were obtained with dyeing at 90°C for 60 minutes at pH 4. The samples dyed with ferrous sulphate mordant produced dark greyish-brown shade while with other mordants yellowish-brown shades were obtained. The dyed samples exhibited fair to good fastness properties and good to excellent UV protection properties. Sharma and Jahan (2003) used the natural dye obtained from barks of peepal (*Ficus religiosa*) tree to dye silk fabrics with myrobalan and cow dung as natural mordants and metal mordants namely, alum, ferrous sulphate, potassium dichromate, stannous chloride and nickel sulphate. The dye produced different shades with each of the mordants mentioned above and the fastness properties were found to be good. The dye is free from heavy metals and hence free from skin problems. Punrattanasin *et al,.* (2013) dyed silk fabrics with natural dye extracted from mangrove bark with mordants like alum, ferrous sulphate, copper sulphate and stannous chloride. The dye produced different shades with different mordants and the optimum conditions for dyeing are 90°C for 60 minutes at pH 3.

Cotton yarn was dyed by Cristea *et al.* (2006) with natural dyes such as weld (*Reseda luteola*), woad (*Isatis tinctoria*) and madder roots (*Rubia tinctoria*) that produce yellow, blue and red colours respectively. In addition to the dyes, antioxidants and UV absorbers like cafeic acid, gallic acid, vitamin C, phenyl salicylate, benzophenone and vitamin E to enhance the light fastness of the dyed cotton yarn. Since the UV light from the sun is the major cause of fading of dyes, the UV absorbers and antioxidants act as neutralizers for the destructive effect of UV light. Kamel *et al.* (2011) dyed the cationized cotton fabric with natural dye,

cochineal, by conventional heating and ultrasonic methods. The cotton fabrics were cationized by padding the fabric with fresh solution of 3-chloro-2-hydroxypropyltrimethyl ammonium chloride. The cationization and ultrasound technique improved the colour strength of the fabric. Ticha *et al.* (2016) used natural dyes derived from red cabbage to dye modified cotton fabrics treated with cationic agent at 50°C for 60 minutes, where cationization improved the dyeability of cotton further.

Lin (2005) used natural dye turmeric to dye polyester fabric by five different methods and achieved excellent washing and rubbing fastness. Hence turmeric dye could be one of the best alternatives to hazardous disperse dyes used for polyester dyeing. Singh *et al.*, (2006) dyed polyester fabric directly with natural henna dye powder, without dye extraction. The dyeing was carried out at high temperature with material to liquor ratio (MLR) 1:20 and 4.5 pH. The shades obtained varied from pale yellow to dull orange. Use of solvents resulted in brighter dyeing than aqueous dyeing.

Shahin *et al,*. (2014) extracted natural dye from Chinese Rhubarb (*Rheum officinale*) to dye polyester fabric. Dyeing was carried out without the aid of mordants. Dyeing at 90°C produced highest colour strength (K/S) of 6.84. The dye gave a yellow shade on polyester fabric with good fastness ratings.

The bark of *T. arjuna* is rich in polyphenols, about 60 to 70%, mainly flavonoids, tannins and triterpenoids. Fruits of *T. arjuna* are drupe, ovoid, fibrous-woody and smoothskinned with five hard wings or angles which are oblique and curved upwards. Traditional healers in India apply the fruit paste on wounds topically. The fruits contain mainly glycosides and flavonoids (luteolin) states Dwivedi and Chopra (2014). The fruit extract contains saponins, flavonoids, glycosides and polyphenolic

compounds. Qualitative and quantitative analysis of the fruit extract of Terminaliaarjuna confirmed the presence of carbohydrate, saponin, tannins, terpenoids, glycosides, flavonoid and phenol, reports Guesmi *et al.*, (2013).

The aqueous extracts of the fruit contains carbohydrates, alkaloids, flavonoids, tannins and polyphenols, states Shirwaikar *et al.*, (2011). The anti-inflammatory activity of the fruit extracts aids faster wound healing. The poultice prepared from the fruits, leaves and flowers is used for the treatment of rheumatoid arthritis. In Ayurvedic medicine, the fruits are used to control diabetes. Nagappa *et al.*, (2001) extracted active compounds from the dried fruits of *T. populnea* with water and found to heal excision and incision wound models in rats by topical and oral administration respectively. The Phytochemical screening confirmed the presence of alkaloids and flavonoids. Hence the fruit extract is a good wound healing medicine.

Lee (2007) extracted natural dye from coffee, Coffeaarabica, and dyed cotton, silk and wool fabrics with mordants such as ferrous sulphate, copper sulphate, aluminiumsulphate, manganoussulphate, stannous sulphate and zinc sulphate. The dyed samples exhibited average to good colour fastness and good deodorizing property.

Helmy *et al.* (2017) extracted dye from peanut red skin to dye wool with alum and ferrous sulphatemordants. 18 Dyeing was carried out by three methods: infra-red, ultrasonic and microwave. The dyed fabric possesses good antibacterial activity against pathogens like *Staphylococcus aureus, Klebsiella pneumonia* and a fungi, *Candida albicans.*

The natural dye isolated from *T. populnea* bark dyeing fabrics show good fastness properties .The dyeing property of fabrics after washing water ,soap ,sunlight

does not get affected cupric sulphate is slightly colour .It was concluded that the ethanolic extract having good dyeing property (Shirwaikar *et al.*, (2011).).

Samy.D *et al.*,(1998) stated that the extract natural dye from *Callistemon eitrinus* plant the dyes was extract by boiling method. A part of the extract autoclaved .*Callistemon citrinus* flower dye was used for dyeing the scouled cotton cloth using two mordant .copper sulphate and ferrous sulphate. Fastness tests dyed clothes undertaken .Good light fastness, such fastness ,wash fastness in fabrics mordant with ferrous sulphate.

Monali *et al.*, (2016) identified natural Dye from *Ixora coccinea* the dye potential of the extract was evaluated by dyeing on cotton fabrics under the normal dyeing conditions and tested for their colour fastness to washing properties..Secondly, mordanting with the different metal salts exhibited variation in color hue because of their ability to form coordination complexes with the dye molecules, which resulted in different shades to cotton fabrics. These findings revealed that the extract of floral petals of *Ixora coccinea* (Linn). can be used for cotton fabric coloration.

Gopika *et.al* ., (2018) stated that Water hyacinth is reported to be used for several purposes. In this study, the extracts of water hyacinth flowers were tested as a potential source of natural dyes. Three different methods were followed for the extraction of the dye. Potassium dichromate (K_2Cr_2O7), copper sulphate ($CuSO_4$), oxalic acid ($C_2H_2O_4$), stannous chloride ($SnCl_2$) and ferrous sulphate (FeSO4), each at a concentration of 6 % of the dye were used as mordants. The dyed clothes were washed and checked for the fastness. This study proves that the flowers of water hyacinth could be used as a source of natural and eco-friendly dye with potential for a range of applications.

The isolation of natural dyes from *Hibiscus rosasinensis* are analysed from Gc-Ms-UV visible and IR spectroscopy instrumental analysis. The mordant are important chemical component. In this project use the alum, potassium dichromate, copper chloride, and stannous chloride are used. The colour strength analysed with DATA colour spectrophotometer .wash fastness, xenon fastness also carried out. Calculate the strength of the colour is increased by means of the stannous chloride mordant used componed to other mordants applied to the cotton dyed with the natural dyes extracted from *Hibiscus rosasinensis* (Rahman *et al* , 2013).

Five flower plants namely *Portulaca grandiflora* (Time flower), *Rosa ardsrovar* (red rose), *celosia argenteavarcristia* (plumed cocks comb), *pereskiableo* (Deserteose), *Alternanthera ficoida* (Border plant) common dye sources for the present research work. Differnet solvents ethanol and water as used to extract the natural dyes .The analytical studies UV spectroscopy, coloumn chromatography ,vacuum evaporation for isolating dye from their solution x-ray performed on the dye extract . Dye extraction yield rate extended from 1.08% to 6.7%. Determined the plant removed and solubility of pigments extracts two solvents. Coloumn chromatography was utilized as a dye purification method and also used for the study of the aging impact for 60 days at room temperature (25°C) and at 60°C Arup *et al*., 2003).

Selvam *et al*., (2015) made the extraction of natural dyes from *Curcuma longa*, *Trigonella foenumgraecum* and *Nerium oleander*. Dyes were prepared using aqueous, acidic, alcoholic and alkaline extraction techniques. The dyes prepared from turmeric using aqueous extraction technique and from fenugreek using alkaline extraction showed good antibacterial activity. The aqueous and alcoholic extraction of *Nerium oleander* was able to inhibit the growth of many fungal strains including

Tricoderma spp., *Tricophyton rubrum*, *Candida albicans*, *Aspergillusniger*, *Cladosporium* spp. etc. The antimicrobial property of the dyes was used in developing antimicrobial fabric.

Yadav *et al.*, (2008) selected the three plants and analysed their ash content, acid insoluble ash, moisture content, water extractive value, UV/vsible spectra, pH of 1 per cent solution and tannin content as per the standard methods. The study revealed that moisture content (9.60%) ash content (19.2%) acid insoluble (15.5%) and pH of 1 per cent solution (7.4) were maximum in Bhringraj leaves. Rein wardtia flowers had maximum water extractive value (44.93%) and Kachnar bark had highest tannin content (24.35%).

Ali *et.al.*, (2009) antimicrobial activities of some natural dyes extracted from different plants such as onion, saffron ,madder against some species of pathogenic bacteria and fungi as *Eschericia coli* and *Staphylococcus aures* and fungal strains *Aspergillus* spp and *Penicillium.* The antimicrobial activity of wool fibres pretreated with cihitosan dye with the tested natural dye were evaluated. The all tested natural dyed showed considerable inhalation against all tested microbes. The result indicated the sample pretreated with chitosan dyed with there natural dyes exhibit higher inhibition precent against all tested pathogens than the untreated samples.

Antibacterial activity of *Hibiscus rosasinensis* flower extract and *E.Coli* against *Calendula officinalis* among these organisms. Simultaneously standard antibiotic solution of ampicillin was used .This experiment was done by agar gell diffusion method .The flower materials can be taken as an alternative source of antibacterial activity against the human pathogens (Madhusudhana, 2013).

Madhu *et.al* .,(2015) stated the antibacterial activity of some natural dyes *Acacia catechu*, *Kerrialacca, Rubiacordifolia* and *Rumex maritenius* were tested against common pathogens *Escherichia coli*, *Bcillussubtiles*, *proteusvulgaries*,*pseudomonas aeuginosa*. Quercus infectoria dye was most effective and showed maximum zone of inhibition there by indicating best antimicrobial activity against all the microbes tested. Minimum inhibitory concentration was found to be varying from 5 to 40 mg . The textile material impregnated with less natural dyes, should less antimicrobial activity.

Mangathayaru *et al.*, (2005) found that the antimicrobial activity of silk fabric pretreated with *Leucas aspera.* The antimicrobial activity with some units of bacteria and fungi were tested and the result indicated that the samples pretreated exhibit higher inhibition present than the untreated samples.

Popoola, (2000) stated that the natural dye extracted from *African wood rose* are used to dye wool that is pretreated with chitosan by using tannic acid as a mordant .The antibacterial activity of chitosan treated wool fabric is tested in accordance to diffusion agents. Test organisms such as *Escherchia coli, Bacillus subitilus*, *Pseudomones aeruginana* and *Staphylococcus* are used and the indicate that the samples treated with a lower concentration of chitosan exhibit a smaller inhibition zone.

Yash Punjabi *et al.*,(2014) founded that the flowers of *N. nouchali* extracted with Pet ether, toluene, chloroform, acetone, ethyl acetate and methanol. These crude extracts were tested for antibacterial activity by agar streak method. All the extracts showed antibacterial activity against the tested strains. Methanolic extract was the most active. The highest activity was seen against *Salmonella paratyphi*

A and the lowest activity was seen against *Salmonella paratyphi* B. The present study showed the effectiveness of the crude plant extract against the tested bacterial strains and indicates the potential use of the extract as antimicrobial agent for the control of infectious diseases.

MATERIALS AND METHODS

MATERIALS AND METHODS

Materials:

Selection of Dyeing Material:

Natural dyes are extracted from different parts of the plant. For the present study the flower of *Zinnia elegans* Jacq. was selected. That is because it was easily available in the market also it comes in different colours. The yellow variant of *Zinnia elegans* is chosen and brought from the Flower market of Thoothuthukudi. The flowers are carefully bought in the fresh and disease free condition.

Selection of fabrics:

Three different fabrics like cotton, polyester and silk are chosen for the present study. The fabrics were bought from the local shop Kanna Silks, Thoothukudi. Since the clothes contains impurity and possible chemicals, they are later washed and stored for further studies. This is shown in plate 1.

Methods:

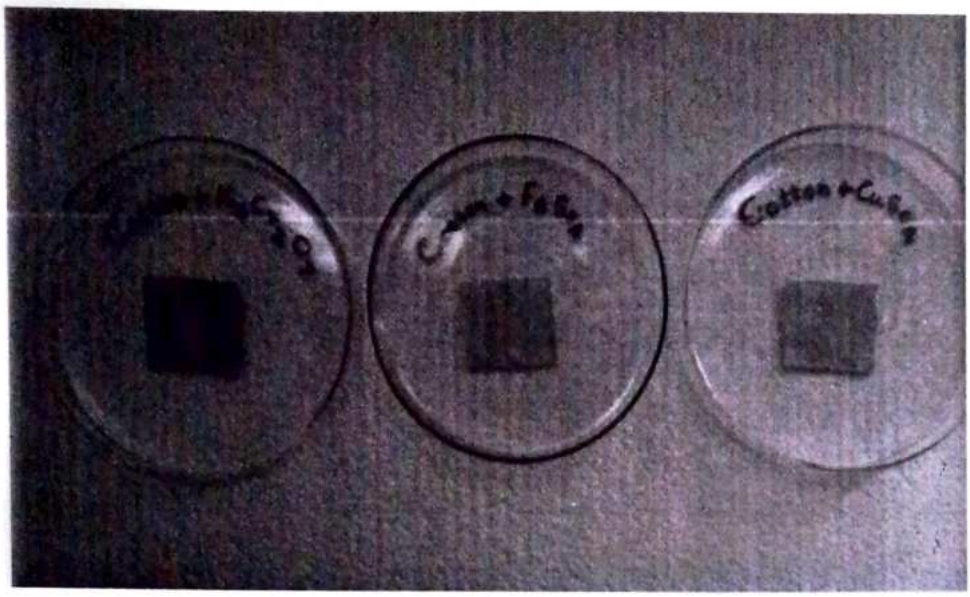
Scouring:

Scouring is meant to remove all impurities in the fabric other than the resinous impurities and the colouring matter, which can only be destroyed with oxidizing agents (Kulkarni *et al.*, 2011). Wingate and Jane described scouring as the treatment aimed to remove any sizing, dirty, oil or other substances that have adhered to the yarn.

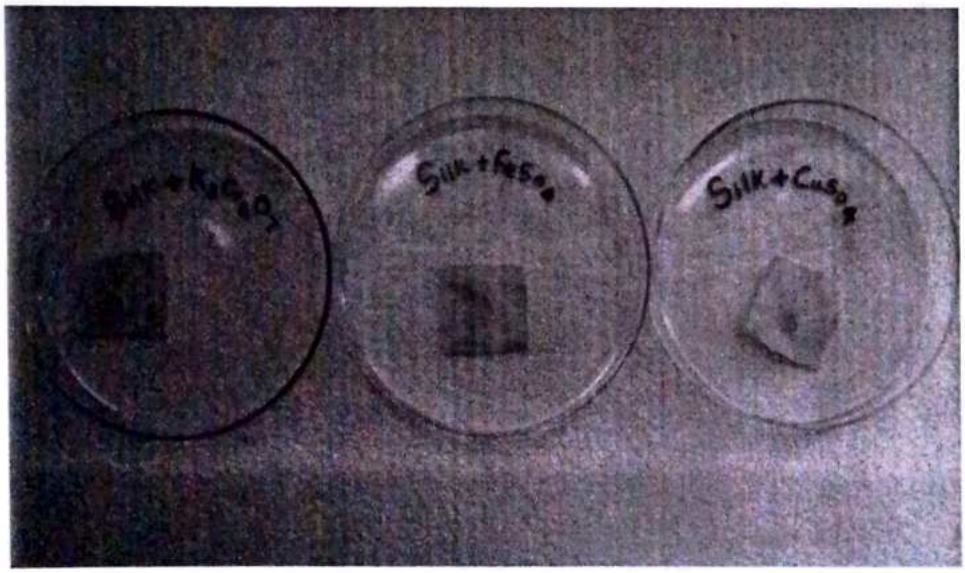
For scouring 2 g/l of sodium carbonate and 1 g/l non ionic (synthetic) detergent were dissolved in soft water and the material to liquor ratio (MLR) of 1:40.

Plate 1

Fabrics treated with mordant



Cotton cloth with different mordant



Silk cloth with different mordant

The samples were kept in the scouring solution at 100°C for 30 minutes. Then the samples were taken out, rinsed thoroughly in soft water and dried (Ansari, 2000).

Selection of mordants:

Mordants form the link between dyestuff and fibre that allows the dye with no affinity for the fibre to be fixed. Among the mordant used for fixing natural dyes, metallic mordants are most common therefore copper sulphate and potassium dichromate and ferrous sulphate were selected for the present work and is shown in Plate 1

The scoured cloth and thread samples were treated with different metal salt, only premordanting with metal salts was carried out before dyeing. The mordant such as potassium dichromate, copper sulphate and ferrous sulphate 2%, 4% and 6%, were dissolved in 100ml distilled water in different 250ml beaker. Then wetted samples were dipped into the mordant solution and then brought to heating temperature of the dye bath was raised up to 80°C for 30minutes. Then cooled for 15minutes and washed with water and air dried. Further the dyed samples were used for dyeing process.

Methods of Dye extraction:

Aqueous method

2 g fresh flower petals were mixed in 100 ml of water and heated in 80° to 90°C for different time periods. The extract was then filtered through sieve. The extraction time was determined based on maximum optical density of the extract obtained after particular period of extraction.

Acidic method

Acidic solution was prepared by adding of 1 ml of cone. HCl solution in 100 ml of water. 2 g flower petals was then added and heated at 80° to 90°C for different periods. Dye solution was filtered after it got cooled.

Alkaline method

1 per cent solution was prepared with the addition of 1 g sodium hydroxide in 100 ml of water. 2 g flower petals was added and heated at 80° to 90°C for different periods. The cooled dye solution was then filtered. Extraction time was optimized depending upon the maximum optical density of the extracted dye solution.

Ethanol method

50 per cent solution was prepared with the addition of 50 ml of ethanol in 50 ml of distilled water. 2 g flower petals was added and heated at 80° to 90°C for different periods. The cooled dye solution was then filtered. Extraction time was optimized depending upon the maximum optical density of the extracted dye solution.

Dyeing:

The cloth and thread samples were dyed with extracted natural dye from various extracts of Zinnia flower keeping M:L ratio as 1:20; however for cloth dyeing it was used directly while in the case of thread dyeing the pH was maintained by adding a buffer solution (sodium acetate and acetic acid) for plant extract. The dye extract was prepared by adding 10ml of natural dye extract in 20ml water (M:L - 1:2). The samples are kept in the boiling water bath at 75°C for 45 mins as shown in plate

Plate 2

Extraction of dyes using different medium

2. Dyeing was done by the conventional dyeing method. After dyeing, the dyed material was washed with cold water and dried at room temperature.

Determination of absorption (%)

A fixed concentration of dye solutions was maintained. The absorbance of the solution was recorded before and after dyeing of the samples at particular wavelength in each case. An average of three measurements at each concentration was recorded. The dye absorbance was calculated as given below.

$$\% \text{ dye absorption} = \frac{\text{OD of the dye liquor before dyeing} - \text{OD of the dye liquor after dyeing}}{\text{OD of the dye liquor before dyeing}} \times 100$$

Solid dye content (%) of extracted dye solution:

For determining the solid content of the dye extracts, three extraction media, i.e .aqueous, acidic and alkaline and ethanol solutions were used for extraction of the dye. A measured quantity of extracts was taken in a pre-weighted petridish and the contents were dried in an oven at 100±5°C till completely dried residue was obtained. The material was kept in a desiccator to cool down and then weighted

The solid content of the extracted dye solution was obtained as follows ;

$$\% \text{ of solid content} = \frac{W2 - W1}{\text{wt of the solution}} \times 100 \text{ wt of the solution}$$

W1 = Weight of the petridish

W2 = Weight of the petridish + solid

Wavelength scan for the selected dyes

Diluted extracts of dye samples were used for wavelength scanning for each dye, as it is difficult to get maximum optical density value of dye solution obtained by optimized extraction procedure. Dye solution obtained by optimized extraction procedure was diluted 10 times to get an optical density value of around 0.3 (Gohl and Vilensky, 1983). The diluted solution was then subjected to wavelength scanning and the wavelength at which maximum optical density obtained was noted for each dye.

Determination of fastness properties of dyed fabrics:

All the dyed samples were evaluated for colour fastness to washing and exposing to sunlight, by the standard procedures.

Colour fastness to light (ASTM Standard, 1968)

For assessing the resistance of the colour of textiles to the fading action of sunlight, the ASTM method (AATCC Method 16B-1964) was followed.

A specimen from the textile to be tested and a standard dyeing or dyeings were exposed simultaneously under specified conditions for sufficient time to produce "just appreciable fading" of the test specimen or the standard. Colour fastness was then rated in terms of the relative fastness of the specimen and the standard.

The dyed samples were cut into 6 cm long pieces. A set of standards and the specimens to be tested on the card board with an opaque cover made of the same material across one half of each of the standards and specimens were mounted on the exposure rack. The specimens and standards were then exposed simultaneously to

light from 9 am to 5 pm. Samples were evaluated for colour change after 48 h of exposure; the intervals of exposure being 8, 16, 24, 32, 40 and 48 h.

Interpretation of results

The effect on the colour of the test specimens were expressed in the International Gray Scale by grading the samples 1 to 6.

1 - Very poor

2 - Poor

3 - Fair

4 - Very fair

5 - Good

6 - Very good

Colour fastness to washing (ASTM Standard - 1968)

This test was developed to differentiate between the types of colours applied to textiles by subjecting the dyed or printed fabrics to washing under four sets of washing formulas graduated in temperature and alkalinity conditions (AATCC Method 36-1965). Coloured specimens were laundered in a soap solution in 3 x 5 inch glass or metal cylinder containing magnetic stirrer. Laundering conditions were varied from mild to severe by control of time, temperature, alkalinity and chlorine content in four test procedures.

The test specimens were placed in magnetic stirrer 100 ml of 0.5% soap solution at required temperature and washed for 20 mins. The container was emptied

and the specimens were rinsed twice with distilled water at 40°C by vigorously shaking for 1 min. The specimens were then poured in 100 ml of 0.015% solution of acetic acid (0.05 ml of 28% acetic acid per 100 ml of water) for one minute at room temperature and were again rinsed in 100 ml of water for 1 min. The cloth samples were then squeezed, dried and ironed.

Interpretation of results

The effect on the colour of the test specimens was expressed in the International Gray Scale by grading the samples 1 to 6.

1 - Very poor

2 - Poor

3 - Fair

4 - Very fair

5 - Good

6 - Very good

Antibacterial assay

Escherchia coli, Bacilulssubtilis, Proteus vulgaris and *Klebsiella pneumonia* obtained from our department, were used for evaluating antibacterial activity. The bacteria were maintained on nutrient broth on 37°C.

Preparation of Inoculums:

The gram positive bacteria (*Baciluls subtilis)* and gram negative bacteria (*Escherchia coli, Proteus vulgaris, Klebsiella pneumonia)* were pre-cultured in nutrient broth overnight and incubated at 37°C.

Disc Diffusion Method:

The antibacterial assays were done on human pathogenic bacteria such as *Escherchia coli, Proteus vulgaris, Klebsiella pneumonia and Baciluls subtilis* by standard disc diffusion method. Nutrient agar/broth medium was used to cultivate the bacteria. Cultures were spread on the agar plates using sterile cotton swabs. Sterile paper disc of 5mm diameter with standard antibiotic (Ofloxacin 100µgm/ml) and dye extracts were placed over the inoculated plates followed by overnight incubation at 37°C. The antibacterial activity was assigned by measuring the diameter of the zone of inhibition around the disc.

RESULT AND DISCUSSION

RESULT AND DISCUSSION

The results of the experimental works carried out along with discussion and the results achieved were included in this chapter keeping in view the objectives of the study and the data generated were recorded systematically.

Botanical Description of *Zinnia elegans*

The flower was collected from the market, described and identified with the help of Flora of Presidency of Madras by Gamble. The flower belongs to the family Compositae. It was named for Johann Gottfried Zinn (1727-1759), a professor of medicine at Goettingen, Germany. The specific epithet name "elegans" means elegant. The image was given in Plate 3

Description:

Botanical Name : *Zinnia elegans* Jacq.

Common Name : Youth and Old Age, Zhiniya

Plant Family : Asteraceae (Compositae)

Habit : A small herb.

Leaves : Ovate opposite, entire, wavy.

Flowers :Flowers in large, terminal heads of various beautiful colours.

Involucral bracts ovate, in 3-series.

Ray florets, female, ligulate, fertile.

Disk florets bisexual, fertile corolla tubular.

Flowering and Fruiting Time : September - October

Significance : Cultivated in the gardens in rains for the beautiful flowers.

Plate 1

Zinnia elegans Jacq

An Individual Flower

Colour of the dye exacts using different solvents.

The extract of the *Zinnia elegans* flower dye was in different colours based on the extraction medium. The colour of *Zinnia elegans* flower dye in alkaline medium is light orange, in alcoholic medium the dye was pale yellow colour, in acidic condition the dye was red colour and aqueous medium it was golden yellow colour. It is given in plate 4.

Similar results were observed in *Syzygium cumini* seed endosperm dye when extracted with different extracts by Mariselvam *et al.*, 2017.

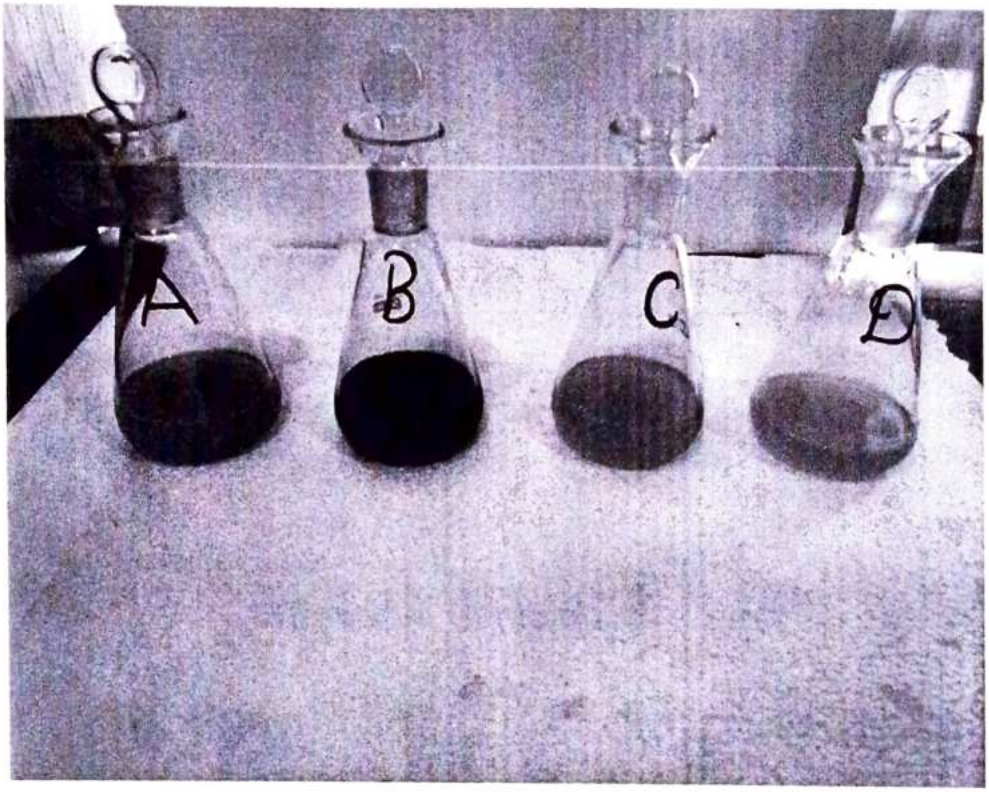
Determination of absorption percentage.

The absorption percentage of various dye extracts of *Zinnia elegans* flower is given in Table 1 and Figure 1. From the table it is observed that the maximum absorption of cotton fabric is shown in the Aqueous extract followed by ethanol extract, alkaline and acidic extracts. In the same way, the maximum absorption by Polyester fabric is seen in acidic extract followed by aqueous, alkaline and ethanol extracts. Maximum absorption of Silk fabric is seen in ethanol extract followed by aqueous, alkaline and acidic dye extracts.

This helps to understand the required method of extraction for the dyeing process of specific fibres. Similar results were observed in the textile coloration of cotton using marigold flower by Jothi, 2008. Meena *et al.*, 2013 also observed the similar results.

Plate 4

Extracted Dyes



A – Aqueous dye extract

B – Acidic dye extract

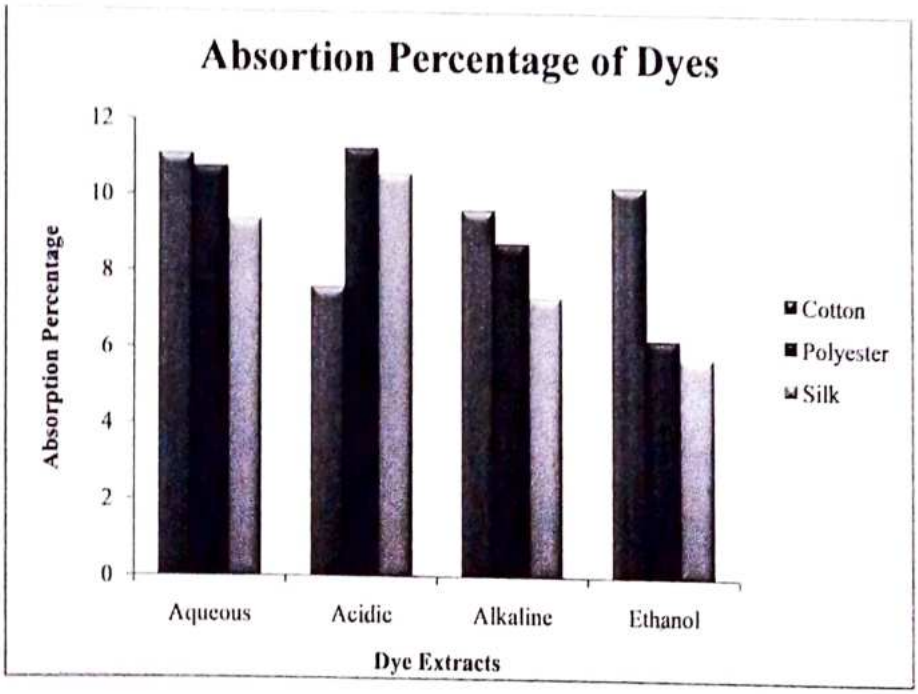
C – Alkaline dye extract

D – Ethanol dye extract

Table 1: Absorption percentage of Dye extracts on various fabrics

S. No	Extracts	Absorption Percentage		
		Cotton	Polyester	Silk
1	Aqueous	11.08	10.75	9.35
2	Acidic	7.56	11.25	10.58
3	Alkaline	9.62	8.75	7.32
4	Ethanol	10.26	6.23	5.75

Figure 1: Absorption percentage of Dye extracts on various fabrics



Determination of solid dye content:

Aqueous, acid and alkali extraction media were tried to obtain solid dye content (%) of extracted dye solution and it is shown in Table 2 and Figure 2. It is interesting to note that the dyes showed the maximum per cent of solid dye content in alkali extraction media and the minimum in aqueous extraction. It is shown that maximum solid content in the alkaline extract is 26.2% followed by acidic solution with 11.4%, Ethanol extract with 10.8% and aqueous solution with 9.5%

This was in agreement with the findings of Dayal and Dobhal (2001) on "Natural dyes from some Indian plants". He has also reported that the highest solid dye content (%) was possible with alkaline media (29.0%) and the lowest amount of solid dye content i.e. 10.6% was reported with water extraction followed by acid (12.5%).

Effect of Mordant

Effect of $CuSo_4$

The comparative effect cotton, polyester and silk fabrics with Copper Sulphate were studied. Treated fabrics are first treated with extracts and then subjected to treatment with Copper Sulphate. It was observed that treated fabrics showing good staining property and upon treatment with Copper Sulphate. It is shown in plate 5 – 8.

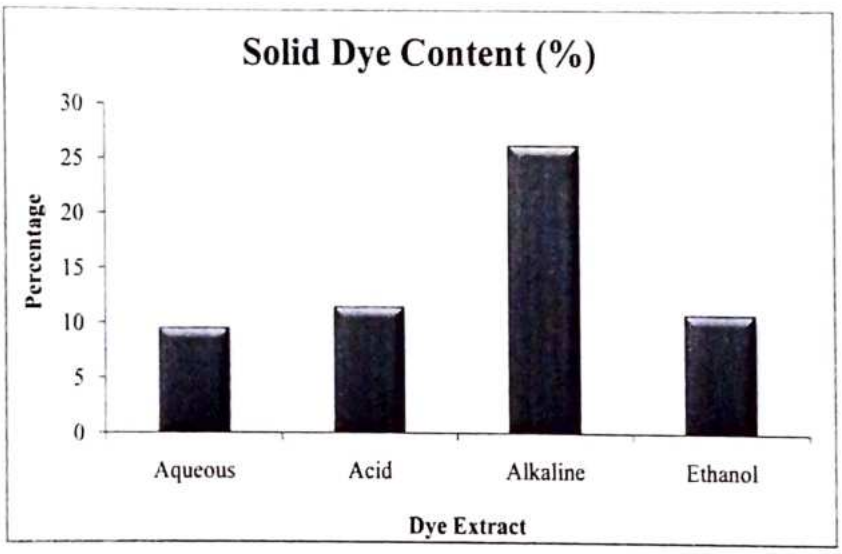
Effect of $FeSO_4$

The comparative effect cotton, polyester and silk fabrics with Ferrous sulphate were studied. Treated fabrics are first treated with extracts and then subjected to

Table 2: Solid Dye content (%) of extracted dye solution

S.No	Dye Extraction	Solid Dye Content (%)
1	Aqueous	9.5
2	Acid	11.4
3	Alkaline	26.2
4	Ethanol	10.8

Figure 2: Solid Dye content (%) of extracted dye solution



treatment with Ferrous sulphate. It was observed that treated fabrics showing good staining property and upon treatment with Ferrous sulphate. It is shown in plate 5 – 8 respectively.

Effect of $K_2Cr_2O_7$

The comparative effect cotton, polyester and silk fabrics with Potassium dichromate were studied. Treated fabrics are first treated with extracts and then subjected to treatment with Potassium dichromate. It was observed that treated fabrics showing good staining property and upon treatment with Potassium dichromate. It is shown in plate 5 – 8 respectively.

UV Visible Spectral Analysis

The absorbance pattern of the *Zinnia elegans* dye extracted with different solvents are presented in Table 4 and Fig. 4. From the extracted dye liquor, it was observed that all the dyes exhibited the visible colour as yellow. From table it is clear that alkaline and ethanol dyes showed peak in the 300nm region and the acidic and distilled water dyes showed in the 350 region. The yellow dye obtained from annatto seeds has also been studied at 350 nm wavelength (Shirwaikar *et al.*, 2011).

Colour fastness grade

Colour fastness grade of Aqueous extracted dye treated cotton at optimum dyeing conditions with different mordants, mordanting methods was shown in Table 4 and in plate 9 – 12. These were evaluated in terms of sunlight and washingby using international standard Gray Scale.

From that it was inferred that for the cotton fabric the alkaline and ethanol dye extract with potassium dichromate mordant show very fair results. In the polyester

Table 3:Absorbance pattern of the dyes extracted with four different solvents using Zinnia

S.No	Wave length (nm)	Zinnia Dye			
		Distilled water	Acidic	Alkaline	Ethanol
1	200	1.612	1.987	0.983	1.025
2	250	0.789	0.987	0.846	0.068
3	300	0.538	0.715	0.829	0.816
4	350	0.809	0.711	0.765	0.863
5	400	0.520	0.347	0.685	0.881
6	450	0.336	0.168	0.561	0.742
7	500	0.120	0.051	0.568	0.645
8	550	0.108	0.056	0.487	0.549
9	600	0.010	0.049	0.490	0.458

Figure 3:Absorbance pattern of the dyes extracted with four different solvents using Zinnia

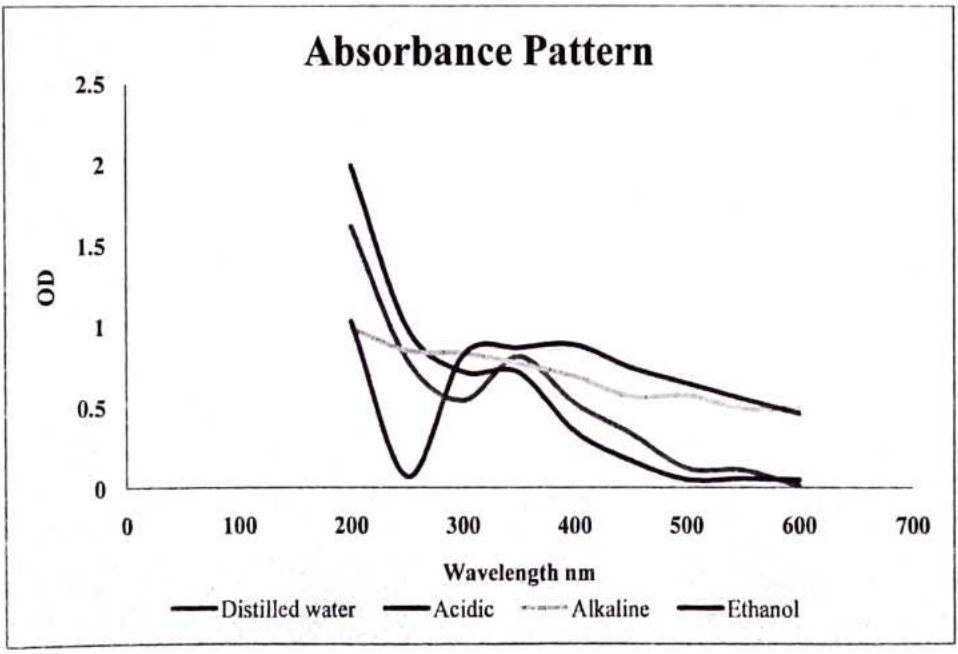
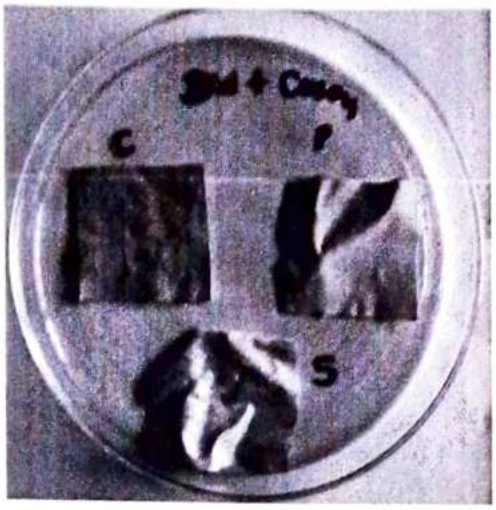
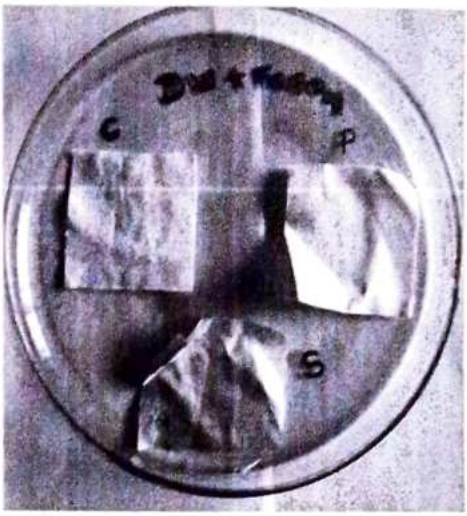
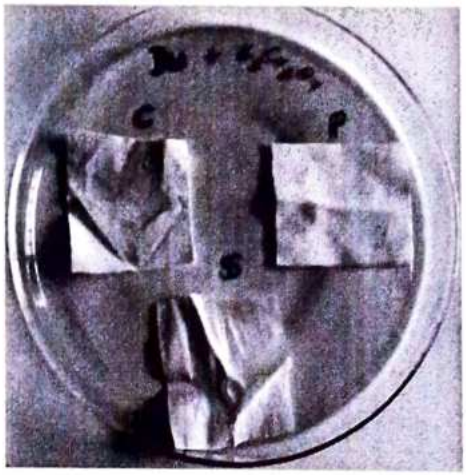


Plate 5

Mordant treated fabrics in aqueous dye extracts







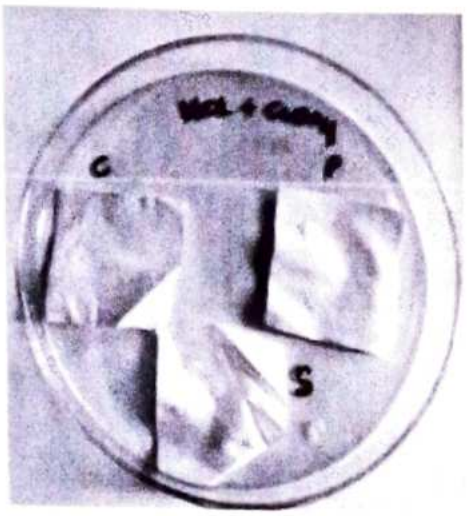
C – Cotton

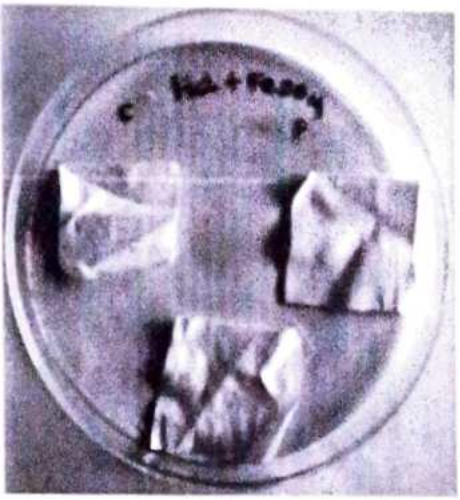
P – Polyester

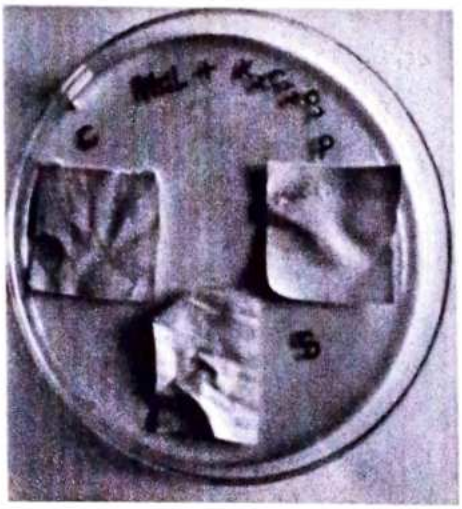
S – Silk

Plate 6

Mordant treated fabrics in Acid dye extracts







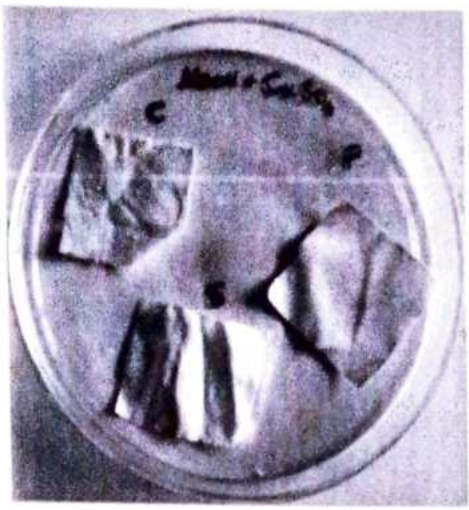
C – Cotton

P – Polyester

S – Silk

Plate 7

Mordant treated fabrics in Alkaline dye extracts



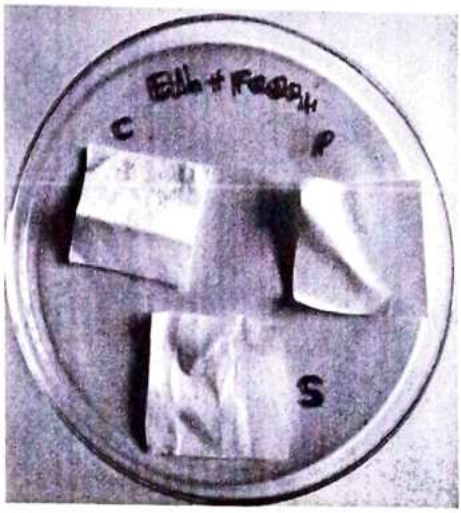
C – Cotton

P – Polyester

S – Silk

Plate 8

Mordant treated fabrics in Ethanol dye extracts



C – Cotton

P – Polyester

S – Silk

Plate 9

Colour Fastness of Different fabrics in Aqueous dye

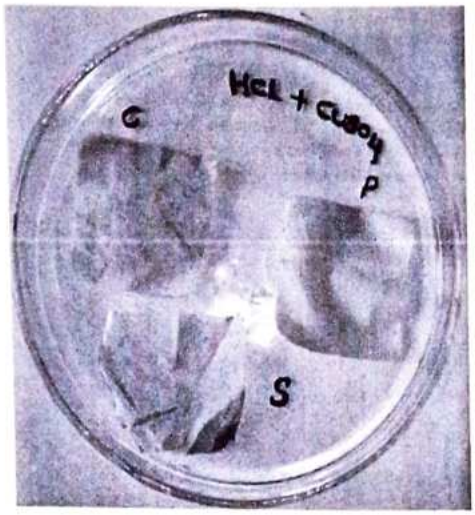
C – Cotton

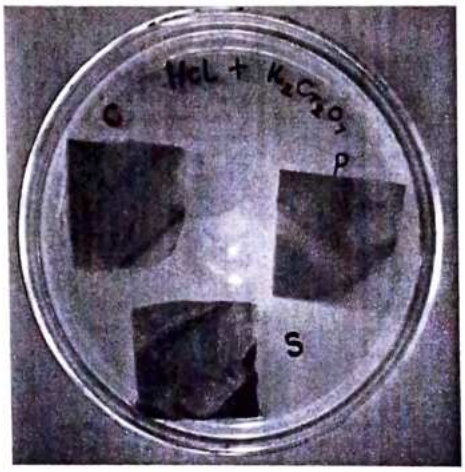
P – Polyester

S – Silk

Plate 10

Colour Fastness of Different fabrics in Acidic dye





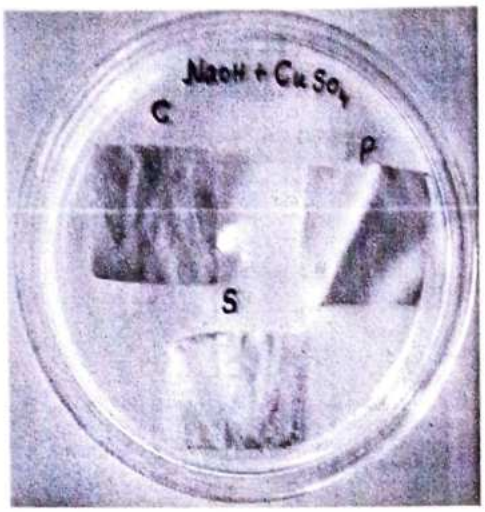
C – Cotton

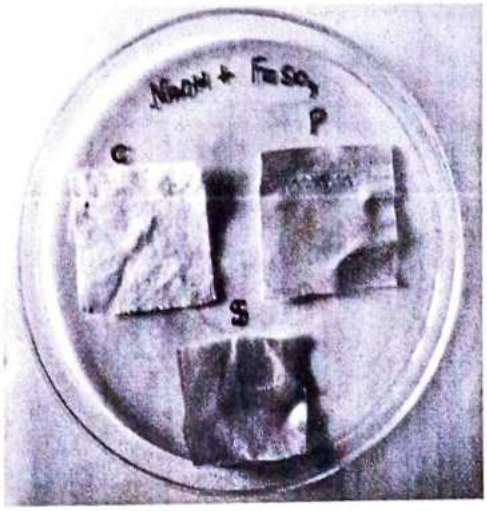
P – Polyester

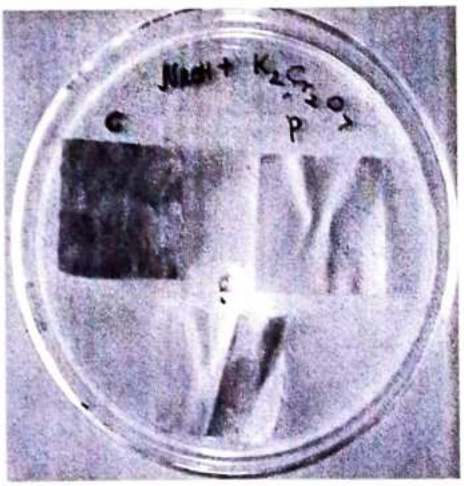
S – Silk

Plate 11

Colour Fastness of Different fabrics in Alkaline dye







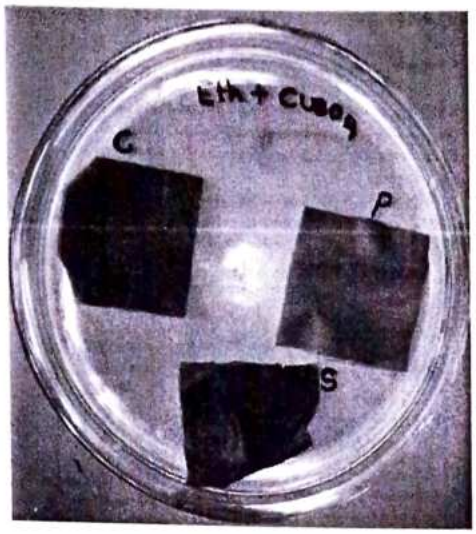
C – Cotton

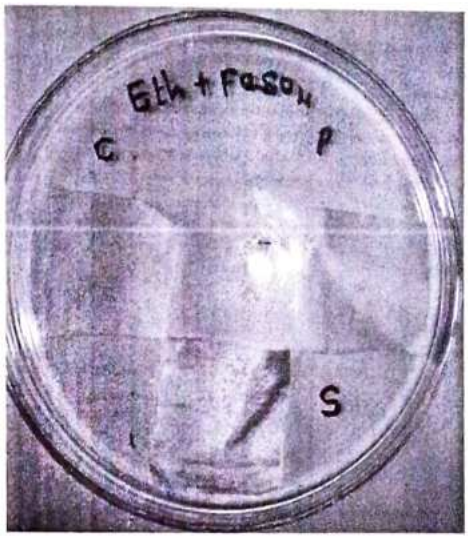
P – Polyester

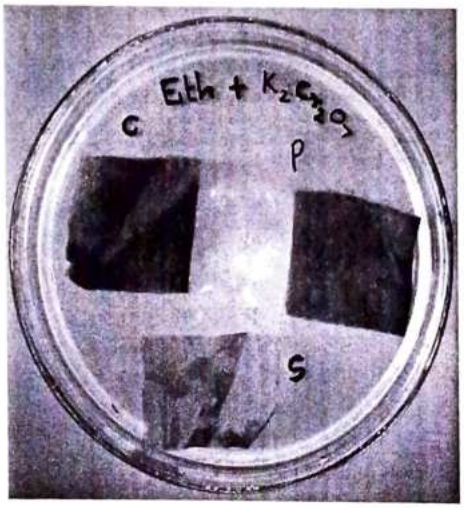
S – Silk

Plate 12

Colour Fastness of Different fabrics in Ethanol dye







C – Cotton

P – Polyester

S – Silk

Table 4: Colour fastness of different dye treated fabrics

S.No	Dye Extract	Mordant	Colour Fastness Grade			
			Aqueous	Acidic	Alkaline	Ethanol
1	Cotton	$CuSO_4$	3	3	3	3
		$FeSO_4$	2	2	1	1
		$K_2Cr_2O_7$	1	3	4	4
2	Polyester	$CuSO_4$	2	2	1	4
		$FeSO_4$	2	2	4	1
		$K_2Cr_2O_7$	1	3	1	4
3	Silk	$CuSO_4$	4	2	1	4
		$FeSO_4$	2	4	4	1
		$K_2Cr_2O_7$	2	4	1	4

Fastness grade (1 - Very poor, 2 - Poor, 3 - Fair, 4 - Very fair, 5 - Good; 6 - Very good)

fabric the alkaline and ethanol extracts with ferrous sulphate and potassium dichromate mordants show fair results respectively. In silk all the mordants show relatively fair results. Similar results were observed by Zhang *et al.*, 2016 in their work with urea-free printing of cotton fabrics

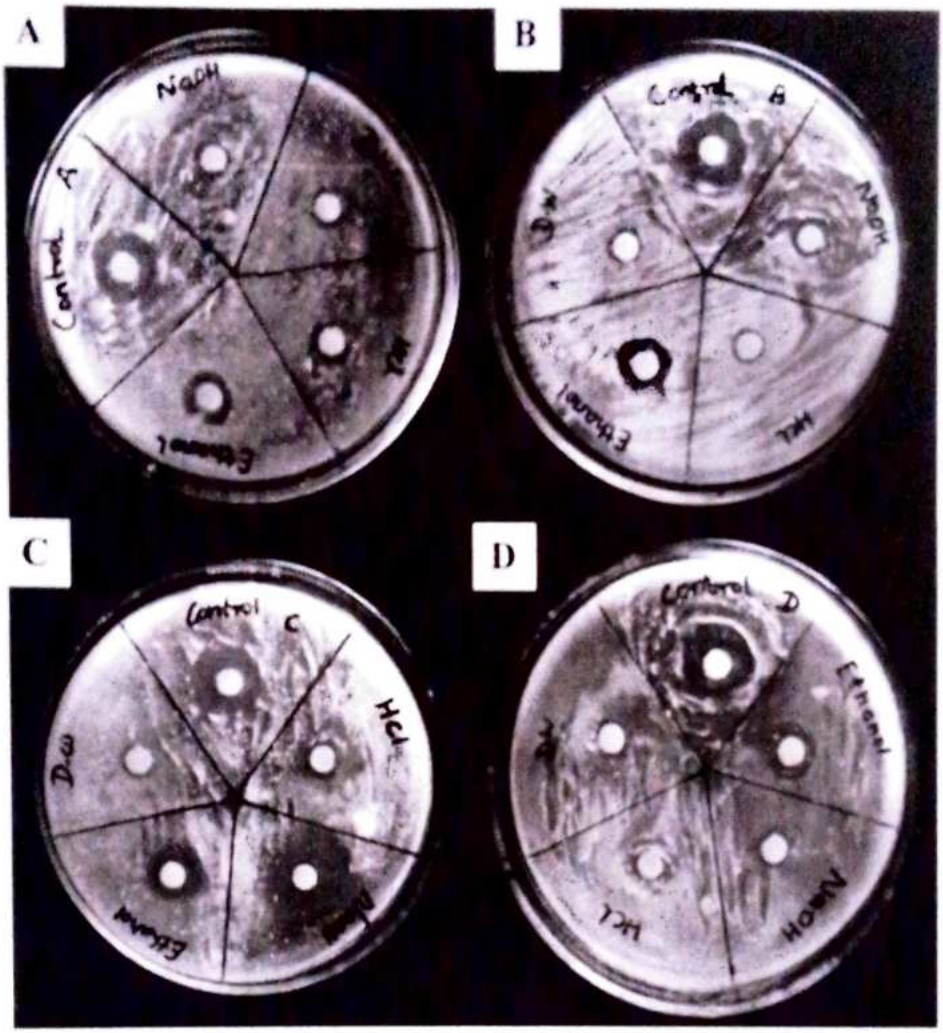
Antibacterial assay:

The results of the antibacterial study was shown in Table 5 and Plate 13. The inhibition zone developed stated the activity of the dyes against the selected bacterial strains. Though this result was not very standard than the control used, they showed little changes. The ethanol extracts showed maximum effect on all the strains used. This may be due to the alcohol used for the extraction.

Anti microbial activity of *Terminalia arjuna* had great impact on these selected pathogens and were reported by Aneja *et al.*, in 2012.

Plate 13

Antibacterial activity of different dye extracts against selected Pathogens



A - *Escherchia coli*

B - *Baciluds subtilis*

C - *Proteus vulgaris*

D - *Klebsiella pneumonia*

1

Table 5: Antibacterial activity of dye extracts

S. No	Bacteria	Zone of Inhibition (mm)				
		Control	Aqueous	Acidic	Alkaline	Ethanol
1	*Escherchia coli*	4.5	2	1	2	3
2	*Bacillus subtilis*	5	1	1.5	2.5	4
3	*Proteus vulgaris*	5	1	1	1	3.5
4	*Klebsiella pneumonia*	5.5	1.5	1.5	2	3

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

A natural dye satisfies the world's present demand for eco-friendliness. The natural colourants are unsophisticated and harmonized with nature. They are obtained from renewable sources and their preparation involves a possibility of very little chemical reaction. Furthermore, the use of natural dyes offers no disposal problem.

From the present work, it is shown that the dye extracted from *Zinnia elegans* Jacq. With different medium showed different coloured dyes. They are mostly in the shades of orange to reddish brown. The acidic dye produced the darkest colour.

The different fabrics showed various dye absorbing properties too. When the clothes were directly dyed, the dyeing was not shown properly or it was shown less absorption. When they were treated with various mordants like Copper sulphate, Ferrous sulphate and Potassium dichromate, it showed significant changes in the absorption. The maximum absorption was shown in the silk material with mordant potassium dichromate with ethanol and alkaline extract. For the cotton cloth, the maximum dyeing effect was shown in the aqueous and ethanol treated with copper sulphate and potassium dichromate mordant.

The colour fastness of the fabrics also showed varied changes. When the dyed clothes were washed and exposed to sunlight, they showed very fair amount of dye retaining capacity. The maximum colour was retained in the silk cloth treated with copper sulphate and potassium dichromate mordant.

The antimicrobial activity of the dyes extracted from *Zinnia elegans* Jacq. had not shown any significant changes. The inhibition capacity was very limited when the

control is concerned. Only in the ethanol extract the inhibition activity was considerable.

From the study, it was concluded that though *Zinnia elegans* Jacq. could be used to extract natural dyes, it needs certain extra care and methodology in the extraction process. Since we have conducted only preliminary level tests here, more advanced method of dye extraction and determination of fabric nature and microscopic studies are recommended for the future works.

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COMPARATIVE STUDY OF EFFECT OF DIFFERENT SUBSTRATES ON YIELD OF OYSTER MUSHROOM AND ITS NUTRITIONAL VALUE

A Short-Term Project Work Submitted to St. Mary's college (Autonomous) Affiliated to Manonmaniam Sundaranar University in Partial Fulfillment for the Degree of

BACHELOR OF SCIENCE IN BOTANY

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CERTIFICATE

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INTRODUCTION

INTRODUCTION

Mushrooms have been recognized as an important food item and their usage is being increased day by day for their significant role in human health, nutritional and medicinal properties **(Mshandete, 2011).** Oyster mushroom (*Pleurotus* species) is an edible fleshy fungus which is the third largest commercially produced mushroom in the world. It is popularly called as Dhingri in India which grows as saprophytes on dead branches of trees. It belongs to the class Basidiomycetes. *Pleurotus* species are also rich in medicinal values and they are effective against certain life-threatening diseases. Major medicinal properties attributed to oyster mushrooms include anti-cancer, anti-biotic, anti-inflammatory anti-viral activities, immune - modulator effect and blood lipid lowering effects **(Lavi *et al.*, 2010)**. An attractive feature of oyster mushrooms is that they can utilize a large variety of agricultural waste products and transform the lignocelluloses biomass into high quality food, flavor and nutritive value.

In India, huge amount of agricultural wastes is produced annually and are of no uses. These wastes could be used as source of food i.e. substrate for mushroom cultivation. *Pleurotus* species can be cultivated on different substrates containing cellulose, hemicellulose and lignin such as soybean straw, paddy straw, coffee pulp, cotton wastes, corn cobs waste **(Poppe, 2004),** bean straw, crushed bagasse, molasses wastes **(Ahmed *et al.*, 2009),** cardboard and paper wastes **(Kulshreshta *et al.*, 2013; Owaid *et al.*, 2015)** and sawdust **(Pathmashini *et al.*, 2008; Owaid *et al.*, 2015)**. Presently, *P. ostreatus* is cultivating on date palm residues such as empty palm fruit bunch **(Tabi *et al.*, 2008; Mohamad *et al.*, 2008),** date palm leaves

(Daneshvar and Heidari, 2008; Kabirifard *et al.*, 2012), stalk and base stalk of date palm **(Hassan, 2011)** and date palm fibers **(Alheeti, 2013; Owaid *et al.*, 2014)** mixed with other cellulosic wastes.

Cultivation of edible mushrooms is a biotechnological process for lignocellulosic organic waste recycling. It might be the only current process that combines the production of protein rich food with the reduction of environmental pollution **(Beetz and Kustudia, 2004).** The production of mushrooms is regarded as the second most important commercial microbial technology next to yeast **(Pathak *et al.*, 2009).** Mushrooms have been eaten and appreciated for their flavor, economic and ecological values and medicinal properties for many years. In general, mushrooms contain 90% water and 10% dry matter **(Morais *et al.*, 2000; Sánchez, 2004).** They have chemical compositions which are attractive from the nutritional point of view **(Gbolagade *et al.*, 2006; Dundar *et al.*, 2008).** Their nutritional value can be compared to those of eggs, milk and meat **(Oei, 2003).** Mushrooms also contain vitamins and an abundance of essential amino acids **(Sánchez, 2004).** The total energetic value of mushroom caps is between 250 and 350 cal/kg of fresh mushroom **(Oliver and Delmas, 1987; Laborde, 1995).** Some mushrooms can be cultivated easily and have significant worldwide markets. Over 200 species have been collected from the wild and used for various traditional medical purposes **(Sánchez, 2004).** Roughly 300 mushrooms species are edible but only 30 have been domesticated and ten grown commercially **(Barny, 2009).** The principal cultivated mushroom worldwide is *Agaricus bisporus* followed by *Pleurotus* sp. **(Rühl *et al.*, 2008).**

Edible mushrooms are able to colonize and degrade a large variety of lignocellulosic substrates and other wastes which are produced primarily through the activities of the agricultural, forest and food processing industries. Particularly, *P. ostreatus* requires a shorter growth time in comparison to other edible mushrooms. Growing oyster mushrooms convert a high percentage of the substrate to fruiting bodies, increasing profitability. *P. ostreatus* demands few environmental controls and their fruiting bodies are not often attacked by diseases and pests and they can be cultivated in a simple and cheap way. They are easily dried to provide for a longer shelf life and export possibilities. All this makes *P. ostreatus* cultivation an excellent alternative for production of mushrooms when compared to other mushrooms.

In India, mushroom cultivation has never been the priority of farmers despite of its delicious taste, highly nutritious and gives high production with little input. Mushroom growth may be coincided with type of substrates (waste material) used that leads to tremendous utilization of nutrients in the agro-industrial bio-wastes. Therefore, the present study was planned to investigate the effect of various substrates like paddy straw, sugarcane bagasse, banana leaves and their combinations on the growth yield, biological efficiency and nutritive values of *Pleurotus ostreatus*.

SCOPE AND OBJECTIVES

SCOPE AND OBJECTIVES

Oyster mushroom can be grown on different substrates containing lignin and cellulose. Demand of mushroom for consumers has been increasing day by day. As substrate plays an important role in determining yield of mushroom and it is necessary to evaluate different substrates for mushroom yield and also to find the best suitable substrate for its cultivation. The present research work was planned to protect the environment by utilizing the agro waste for mushroom cultivation because it is not only capable of bioremediation of waste but also provides a highly proteinaceous food. Therefore, the present study was carried out with the following objectives:

- Preparation of substrate for spawn production
- Cultivation of *Pleurotus ostreatus*
- Study the effect of different substrates on the growth and morphological parameters of *Pleurotus ostreatus*
- Qualitative analysis of the fruit body of *Pleurotus ostreatus*
- Estimation of carbohydrate, protein, amino acid and lipid content of *Pleurotus ostreatus*

REVIEW OF LITERATURE

Mushroom is a fruiting body of saprophytic fungi which is commonly found on decaying natural materials. Edible mushrooms have been a part of human diet since time immemorial. It serves as a good source of many macro and micro nutrients which helps to maintain healthy immune system and reduce the risk of nutrient deficiency. Mushroom cultivation also plays a key role in managing agricultural wastes. Mushrooms represent one of the world's greatest untapped resources of nutritious food. Cultivation of saprophytic edible mushrooms may be the only currently economical biotechnology for lignocelluloses organic waste recycling that combines the production of protein rich food with the reduction of environmental pollution **(Obodai, 2003).**

Thousands of years ago, fructifications of higher fungi have been used as a source of food **(Mattila *et al.*, 2001)** due to their chemical composition which is attractive from the nutrition point of view. During the early days of civilization, mushrooms were consumed mainly for their palatability and unique flavors. Edible mushrooms are regarded as a curative food having anti-carcinogenic, anti-cholesteromic, anti-viral properties and prophylactic properties with regard to hypertension and heart disease (**Mattila *et al.*, 2000**).Some Asian communities use mushrooms for food and medicine traditionally. Generally, mushrooms have higher protein content than most vegetables. They are rich in minerals and vitamins. Fat content is low. The fat fraction is mainly composed of unsaturated fatty acids **(Manzi *et al.*, 2001; Mattila *et al.*, 2001).**

The nutritional and medicinal values of mushrooms have long been recognized. In recent times, mushrooms have assumed greater importance in the diets of both rural and

urban dwellers. For example, they are being marketed along major highways and urban centers where the trade now booms. It is conceivable that the increased demand for mushrooms is contingent upon the phenomenal rise in the unit costs of the conventional sources of meat (e.g. beef, pork, chicken, etc.) **(Abulude, 2017).**

According to **Sánchez (2004),** over 200 species have been collected from the wild and used for various traditional medical purposes, mostly in East. About 35 species have been cultivated commercially and 20 species are cultivated in an industrial scale. The most cultivated mushroom worldwide is *Agaricus bisporus* (Button mushroom), followed by *Lentinus edodes* (Shiitake), *Pleurotus* species (Oyster mushrooms), *Auricula auricula* (Wood ear mushroom), *Flamulina velutipes* (Winter mushroom) and *Volvariella volvacea* (Straw mushroom). In India, cultivation of *Pleurotus ostreatus* is very popular next to *A. bisporus* mushroom in popularity and consumption. This together form bulk of edible mushrooms produced in country (about 100000 tons per annum, 2008-09) **(Dhar and Sharma, 2009).**

Use of costly substrate for growing oyster mushroom increases their cost of production. So, there was need to search for certain alternative materials which should be available in sufficient quantity at relatively cheaper price (**Arya and Arya, 2003**). Rice straw, wheat straw, ragi straw, hulled maize cab, waste paper were tried in different studies. *Pleurotus* has been reported to grow readily on a number of non-conventional substrates **(Das *et al.*, 2000; Mukherjee and Nandi, 2002; Nageswaran *et al.*, 2003). Bandopadhyay and Chatterjee (2009)** reported that highest yield of *P. florida* was obtained after three flushes in beds of combined substrates (paddy straw and water hyacinth) followed by paddy straw alone and water hyacinth alone.

Jeznabadi *et al.*, (2016) evaluated the effect of different sources of Iranian agricultural wastes on the production parameters and protein content in the cultivation of *P. eryngii*. Wheat straw, wood chips, sawdust, sugar beet pulp, barley straw and maize stem residues were used as basal substrates, whereas wheat bran, rice bran, soybean powder and their combinations were used as supplement. Barley straw supplemented with rice bran gave the highest mushroom yield, followed by sugar beet pulp supplemented with rice bran, whereas the substrate with the worst performance was sawdust supplemented with rice bran. Protein content was differently affected by the various substrates, ranging between 4.64% (barley straw +wheat bran and wood chips + soybean powder + rice bran treatments) and 13.66% (wheat straw + wheat bran+ soybean powder treatment).

Yildiz *et al.* (2002) used different lignocellulosic wastes as raw materials for the cultivation of *P. ostreatus* and they reported that a mixture of sawdust with hazelnut leaves (50:50) was one of the substrates with the major biological efficiency. However, when the percentage of hazelnut leaves was increased over 50%, the mushrooms yields was decreased, so the authors concluded that leaf of hazelnut is not very appropriate, as the growth substrate in percentages higher than 50%. **Peksen and Kucukomuzlu (2004)** evaluated the effect of different substrates on the cultivation of different *Pleurotus* species (*P. ostreatus, P. sajor-caju,* and *P. sapidus*). The authors observed that total yield, biological efficiency and morphological parameters were statistically different in different species. They concluded that the optimum substrate for *Pleurotus* growth was the combination of hazelnut husk, wheat straw and wheat bran in the ratio 1.5:2:0.5. However, the biological efficiencies of the substrates

containing hazelnut husks were lower compared to the control composed of wheat straw + 5% wheat bran.

Edible mushroom has been recognized for a long time not only as a delicacy, but also for their use as food in man's diets. Mushrooms have been found to be rich sources of protein, lipids, amino acids, glycogen, vitamins and mineral elements **(Okhuoya *et al.*, 2010).** Mushrooms are used as possible treatments for diseases. *Lentinula edodes* (Shiitake), *Grifola frondosa* (Maitake) and *Ganoderma lucidum* (Reishi), have a history of medicinal use spanning millennia in parts of Asia. Medicinal mushroom possesses cardiovascular, anti-cancer, anti-viral, anti-bacterial, anti-parasitic, anti-inflammatory, hepato protective, and anti-diabetic activities **(Lentinan, 2009).**

The species that have been properly analyzed for medicinal value are: *Ganoderma lucidum* (Reishi), *Lentinus edodes* (Shiitake), *Grifola frondosa* (Maitake), *Agaricus blazei* (Hime-matsutake), *Cordyceps militaris* (Caterpillar fungus), *Pleurotus ostreatus* (Oyster mushroom) and *Hericium erinaceous* (Lions mane). There are many more species of cultivated and wild edible and non-edible mushrooms that have been analyzed for both their nutritional and nutraceutical components **(Lakhanpal and Rana, 2005).** The active constituents found in mushrooms are polysaccharides, dietary fibres, oligosaccharides, triterpenoids, peptides and proteins, alcohols and phenols and mineral elements **(Pardeshi and Pardeshi, 2009)**

Bioactive proteins are an important part of functional components in mushrooms and also have great value for their pharmaceutical potential. Mushrooms produce a large number of proteins and peptides with interesting biological activities such as lectins,

fungal immune modulatory proteins, ribosome inactivating proteins, antimicrobial proteins, ribonucleases and laccases (**Xu, *et al.*, 2011**). Polyunsaturated fatty acids are mostly present in edible mushrooms; thus, they may contribute to the reduction of serum cholesterol. It is noteworthy that trans isomers of unsaturated fatty acids have not been detected in mushrooms (**Guillamon *et al.*, 2010; Barros *et al.*, 2007**).

The major sterol produced by edible mushrooms is ergosterol, which shows antioxidant properties **(Guillamon *et. al.*, 2010).** It has been observed that a diet rich in sterols is important in the prevention of cardiovascular diseases **(Kalac, 2013). Singh and Singh (2002)** reported that mushroom contain raffinose, sucrose, glucose, fructose and xylose form of sugars in it. *Pleurotus ostreatus* is the second most cultivated edible mushroom worldwide after *Agaricus bisporus*. It has economic and ecological values and medicinal properties. Mushroom culture has moved toward diversification with the production of other mushrooms. Edible mushrooms are able to colonize and degrade a large variety of lignocellulosic substrates and other wastes which are produced primarily through the activities of the agricultural, forest and food-processing industries **(Carmen, 2010).**

MATERIALS AND METHODS

MATERIALS

Collection of Agricultural Waste

Agro waste materials such as paddy straw, sugarcane bagasse and banana leaves were used as the substrates for the present study. Paddy straw and banana leaves were collected from farmers and sugarcane bagasse was purchased from sugarcane vendors. These substrates were dried and stored. They were used for the experiments.

Spawn

Sorghum grain-based spawn of *Pleurotus ostreatus* was procured for the present study from certified cultivation centre, MSM Mushroom Corner, Mushroom Cultivation Training and Seed Sale, Rediyarpatti, Tirunelveli.

METHODS

CULTIVATION OF MUSHROOM:

In the present study, the edible oyster mushroom *Pleurotus ostreatus* was cultivated using the standard procedure given by Tamil Nadu Agricultural University.

Experimental Design:

In the present study, *Pleurotus ostreatus* was cultivated by bag method using three different agro waste materials as substrates. The composition of the treatments was given below. Each bag consists of 750 g of dry substrate.

- **Treatment 1:** Paddy straw
- **Treatment 2:** Sugarcane bagasse
- **Treatment 3:** Banana leaf
- **Treatment 4:** 1:1:1 ratio of Paddy straw, Sugarcane bagasse, Banana leaf

Substrate Preparation:

Soaking:

The selected agro wastes (paddy straw, sugarcane bagasse and banana leaves) were cut into small pieces (6 -10cm) and soaked in water for 12 – 14 hrs **(Plate 1 & 2)**.

Sterilization:

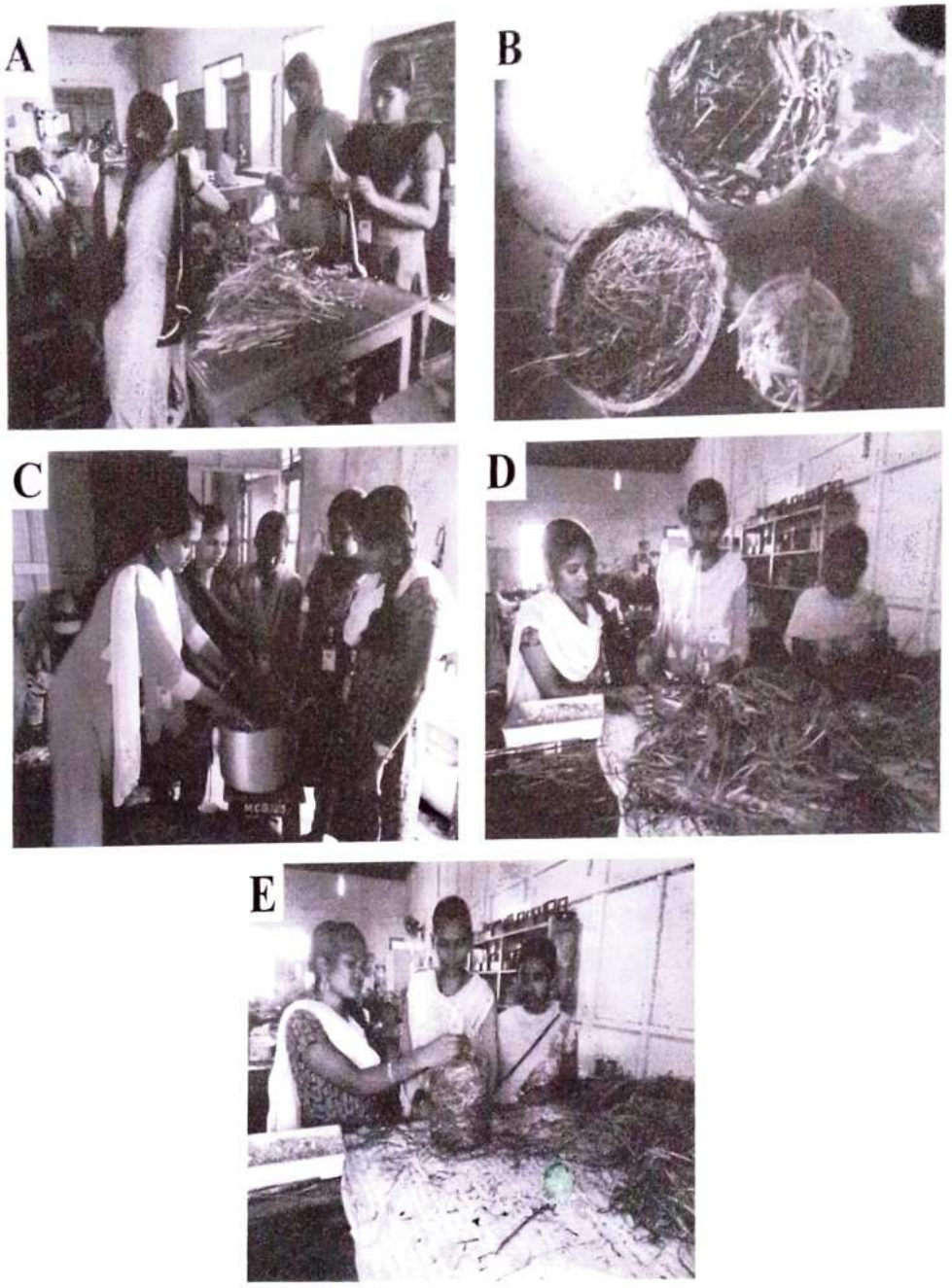
Soaked substrates were sterilized at 121°C for 20-30 minutes by using pressure cooker. After sterilization, the excess amount of water content was removed and cooled down by shade drying in the room temperature.

Bag Preparation:

Before starting the packing, hands were washed thoroughly with the help of antiseptic lotion. Polypropylene bags with the size of 60 x 30 cm and with a thickness of

Plate 1

BAG PREPARATION FOR MUSHROOM CULTIVATION



A –Substrate Preparation

B – Soaking

C –Sterilization

D & E- Bag Preparation

80 gauges were used for the cultivation. The bottom end of the bag was tied with the help of thread and turned toward the inside.

Layering of Substrate:

The sterilized substrate was filled in the bag to a height of 3 inches. Handful of grain-based spawn was sprinkled over the layer. Likewise, few layers were placed on the bag. Finally, the bag was pressed gently and tied with a thread. Few holes were made on the bags to facilitate ventilation and for the removal of excess water.

Spawn Running:

The spawned bags were kept in a dark room for 1 week to facilitate the spawn running and colonization. Then the bags were transferred to cropping room.

Temperature and Humidity:

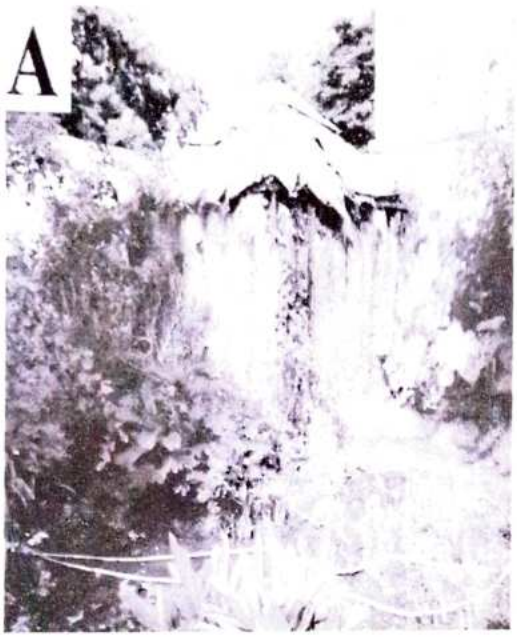
The optimum temperature (22°C - 25°C) and required humidity (85%) were maintained by spraying water on the walls of the mushroom unit four to five times in a day.

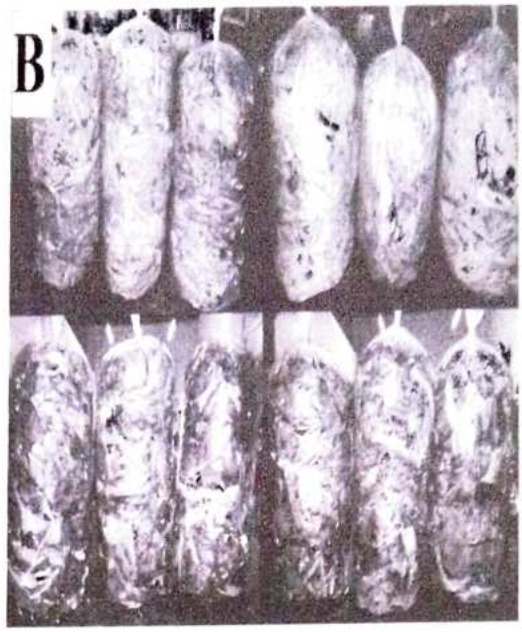
Harvesting:

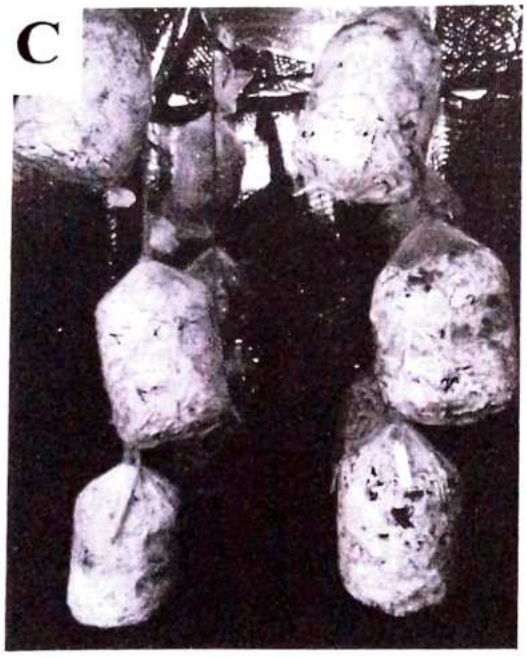
After colonization, the mycelium starts to produce its reproductive structure called fruiting bodies. Initially, it looks like a pin head and it was transformed to a full matured fruiting body within two days. After maturity, edges of the pileus start to shrink towards inside. At this stage the fruiting bodies were collected manually and used for further experiments.

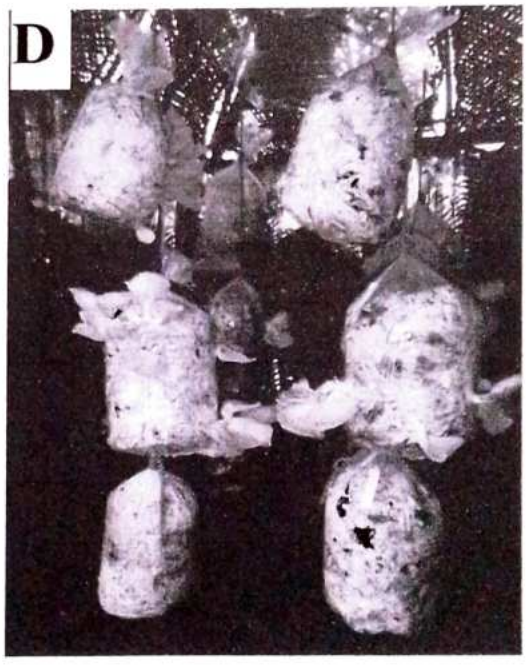
Plate 2

CULTIVATION OF PLEUROTUS OSTREATUS









A – Mushroom Cultivation Unit

B – Spawn Inoculated Bags

C – Colonized bags

D –Development of Fruiting bodies

Data Collection:

The bags were observed carefully from the preparation of bags till the last day of the yield. Number of days required for colonization, pin head formation, fruiting body maturation and yield were recorded.

MORPHOLOGICAL PARAMETERS:

Length and width of stipe and pileus were measured immediately after harvesting with the help of thread and measuring scale.

Biological Efficiency:

Biological efficiency was calculated with the help of the following formula

$$\text{Biological efficiency} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of the substrate}} \times 100$$

Moisture Content:

Known amount of sample was dried in shade for 12 hours and then moisture content was calculated by using the following formula

$$\text{Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

SAMPLE PREPARATION FOR BIOCHEMICAL ANALYSIS:

The fruiting bodies were shade dried and powdered with the help of mixer grinder. The powdered sample was sieved to get uniform size particle and stored in an airtight container. The extract was prepared with the help of different solvents such as hexane, chloroform, ethyl acetate, methanol, ethanol and water in 1:10 ratio (1gram sample in 10 ml solvent).

QUALITATIVE ANALYSIS

Phytochemical constituents were analyzed using different extracts of *Pleurotus ostreatus*. Standard procedures were followed for the same **(Horbone, 1984, Harborne, 1998, Kokate *et al.*, 1995)**

Test for Alkaloid (Wagner's test)**:**

About 1 ml of extract was taken and few drops of Wagner's reagent was added and the formation of a reddish-brown precipitate indicates the presence of alkaloids.

Test for Flavonoid (Shinoda Test):

A pinch of magnesium turnings was added to 1 ml of extract and then 1-2 drops of concentrated hydrochloric acid was added. Formation of pink color indicates the presence of flavonoids.

Test for Phenol (Lead acetate test):

1 ml of extract was taken and 0.5 ml of 1% lead acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolics compounds.

Test for Tannin (Ferric chloride test):

1 ml of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish black color indicates the presence of tannins.

Test for Steroid and Phyto Steroid:

1 ml of extract was mixed in 2 ml of chloroform and then equal volume of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring indicates the presence of steroids. Appearance of blush brown colour indicates the presence of phytosteroid.

Test for Carbohydrate (Benedict's test):

1 ml of Benedict's solution was added to 0.5 mg of extract and boiled in water bath. The appearance of brick red indicates the presence of reducing sugars.

Test for Saponin (Foam test):

0.5 ml of extract was diluted with 20 ml of distilled water and shaken well in a graduated cylinder for15 min. The formation of foam to a length of 1cm indicated the presence of saponin.

Test for Glycoside:

0.5 ml of extract was added in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

Test for Protein & Amino Acid (Ninhydrin test):

About 0.5 ml of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. The appearance of purple colour indicates the presence of proteins, peptides or amino acids.

Test for Terpenoid:

1 ml of the extract was mixed with 2 ml of chloroform and then concentrated sulphuric acid was added to form a layer. A reddish-brown coloration of the interface showed the presence of terpenoids.

Test for Phlobatannin:

Sample was boiled with 1% aqueous hydrochloric acid to produce red precipitate indicating the presence of phlobatannins.

Coumarin:

About 1 ml of 10% NaOH was added to 2 ml of extract. The formation of a yellow colour was an indication for the presence of coumarins.

Cardiac Glycosides (Keller-Killani Test):

2 ml of extract was treated with 2 ml of glacial acetic acid containing a drop of $FeCl_3$. A brown coloured ring or brown-violet under a brown greenish layer indicated the presence of cardiac glycosides.

QUANTITATIVE ESTIMATION OF NUTRIENT CONTENT

TOTAL SOLUBLE PROTEIN (Lowry *et.al.*, 1951)

Reagents:

Alkaline Copper Reagent

Solution A: 20% Sodium Carbonate in 0.1 N Sodium Hydroxide

Solution B: 1% Sodium Potassium Tartarate

Solution C: 0.5% Copper Sulphate

To prepare 100 ml alkaline copper reagent, 98 ml of solution A, 1ml of solution B and 1 ml of solution C were mixed together freshly.

Folin-Ciacalteau Reagent (commercial reagent was diluted in the ratio of 1:1 with distilled water at the time of use).

Procedure:

100 mg of sample was homogenized in 10 ml of distilled water and filtered through a muslin cloth and centrifuged at 3000 rpm for 10 minutes. To the supernatant, 10%trichloro acetic acid (TCA) was added in 1:1 ratio (equal volume of supernatant and trichloro acetic acid) and left in an ice bath for 30 minutes to precipitate protein. Then centrifuged at 3000 rpm for 5 minutes and discarded the supernatant. The precipitate was dissolved in 0.1 N sodium hydroxide and diluted to a known volume.

5 ml of alkaline copper reagent was added to 0.5 ml of protein extract. After thorough mixing, 0.5 ml of folin ciocalteau reagent was added and allowed to stand for 30 minutes; the blue colour appeared and absorbance was measured at 650 nm using UV visible spectrophotometer (Model No: UV 2371). The analysis was performed in triplicates and the amount of protein was calculated and expressed as mg/g DW. Bovine serum albumin (BSA) was used as standard.

ESTIMATION OF CARBOHYDRATE (Dubois *et al.*, 1956)

Reagents:

5% Phenol (5 ml phenol + 95 ml distilled water)

96% Sulphuric acid (96 ml sulphuric acid + 4 ml distilled water)

Procedure:

100 mg of sample was grounded with 10 ml distilled water. It was then filtered and centrifuged. The filtrate was collected. To 0.1 ml of the filtrate, 0.9 ml of distilled water, 1 ml of 5% phenol and 5 ml of 96% H_2SO_4 were added. After 30 minutes,

absorbance was measured at 490 nm using UV visible spectrophotometer (Model No: UV 2371).The analysis was performed in triplicates and the results were expressed as mg/g DW. Glucose was used as standard.

ESTIMATION OF FREE AMINO ACID (Moore and Stein, 1948)

Total free amino acids (Ninhydrin method) were determined according to the procedure given by Moore and Stein.

Procedure

1 ml of the sample was mixed with 1 ml of Ninhydrin reagent in a test tube. Tubes were kept in water bath for 20 minutes and then added 5 ml of diluents (equal volume of water and n-propanol) incubated at room temperature for 15 minutes and absorbance was read at 570 nm against a blank. The analysis was performed in triplicates and the results were expressed as mg/g.

DETERMINATION OF TOTAL LIPID (Folch *et al.*, 1957)

500 mg of dried sample was taken in a screw capped test tube, 10 ml of 2:1solvent mixture (chloroform: methanol) was added. The tube was loosely capped and heated in a water bath at 60°C for 30 minutes. After cooling the solution, the volume was made up to 10ml with the solvent mixture. 0.4 ml of the extract was pipetted in a separate test tube, allowed to dry completely and digested with 0.4 ml of concentrated H_2SO_4 by boiling in a water bath for 10 minutes. After cooling the tube, 5 ml of phosphovanillin reagent was added and allowed to stand for 30 minutes for colour development. The absorbance was then measured at 520 nm against a blank using spectrophotometer (Model No: UV 2371). Cholesterol was used as standard.

RESULT AND DISCUSSION

Four different types of substrates were compared with respect to the production of oyster mushroom. The various substrates used in this study showed variations in spawn run, duration of first fruiting, days to harvest, length of stipe, diameter of pileus, total yield and weight of final substrate.

DAYS FOR THE COMPLETION OF SPAWN RUNNING

Time required for the completion of spawn running varied on different substrates ranged from 22 to 37 days **(Figure 1a).** As per the findings of this study, the growth of *P. ostreatus* mycelia was relatively faster on paddy straw (22 days). Our findings in the present experiment are almost similar to the findings of **Lalithadevi and Many (2014)** who reported that spawn running day was between 16 – 25 days on paddy straw. It was followed by banana leaves (29 days) and mix of all the three substrates (29 days) used for the study. The longest spawn running was observed in the case of sugarcane bagasse (37 days). The findings of the spawn run on sugarcane bagasse did not agree with the report of **Hossain (2017)** who stated that *P. ostreatus* completed the spawn run in 17 days on sugarcane bagasse. Increase in number of days for spawn running on lignocellulosic waste materials might be due to slow hyphal growth of mushroom on substrates **(Mandeel *et al.*, 2005).**

The difference in days for full mycelia running on different substrates might be due to variation in their chemical composition and C:N ratio as reported by **Bhatti *et al.* (1987). Tan (1981)** reported that the spawn running took 16 – 25 days after

inoculation. The variation in the number of days taken for a spawn to complete colonization of a given substrate depends on the function of the fungal strain, growth condition and substrate type.

NUMBER OF PINHEADS

P. ostreatus produced different number of pinheads on different substrates as shown in **Figure 1b.** Maximum numbers of pinheads (51) were recorded on paddy straw followed by banana leaves (33) and the mix of paddy straw + banana waste + sugarcane baggase (29). Our findings are further supported by **Hague (2004)** and **Al Amin (2004)** who reported that highest number of pinheads of Oyster mushroom was found on paddy straw. Minimum numbers of pinheads were observed on sugarcane baggase (12). Almost similar results are reported by **Hasan *et al.* (2015)** who observed minimum number of pinheads of Oyster mushroom on sugarcane bagasse.

PERCENTAGE OF FRUIT BODIES DEVELOPED FROM PIN HEADS

The fruit body is the edible part of mushroom. Data presented in **Figure 1c** and **Plate 3 & 4** showed that there a difference between the substrates for the percentage of fruit bodies developed from pinheads. Highest percentage of fruit bodies (84%) were produced by banana waste followed by paddy straw + banana waste + sugarcane baggase mix (75 %), paddy straw (66 %) and sugarcane baggase (64 %). It was observed that the number of pinheads were maximum on paddy straw compared with other substrates whereas fruit bodies developed from pinheads were minimum on paddy straw compared with other substrates. Highest percentage of fruit bodies from banana waste may be due to the high-water holding capacity.

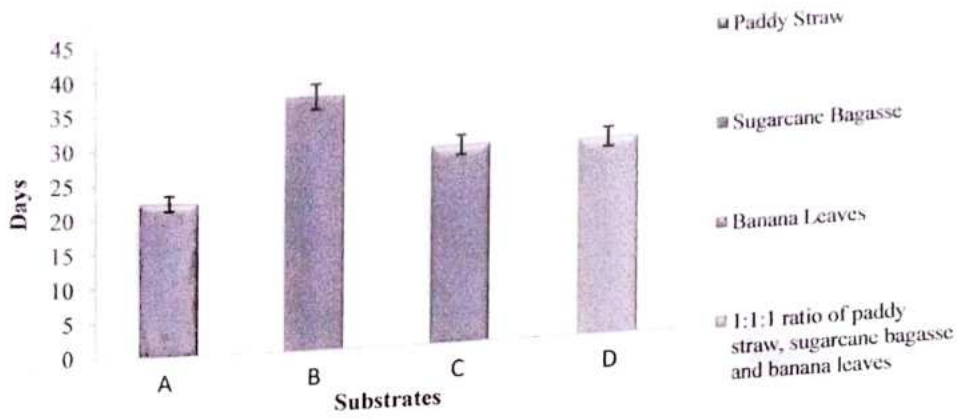


Figure 1a: Effect of Different Substrates on Spawn Running Days of *Pleurotus ostreatus*

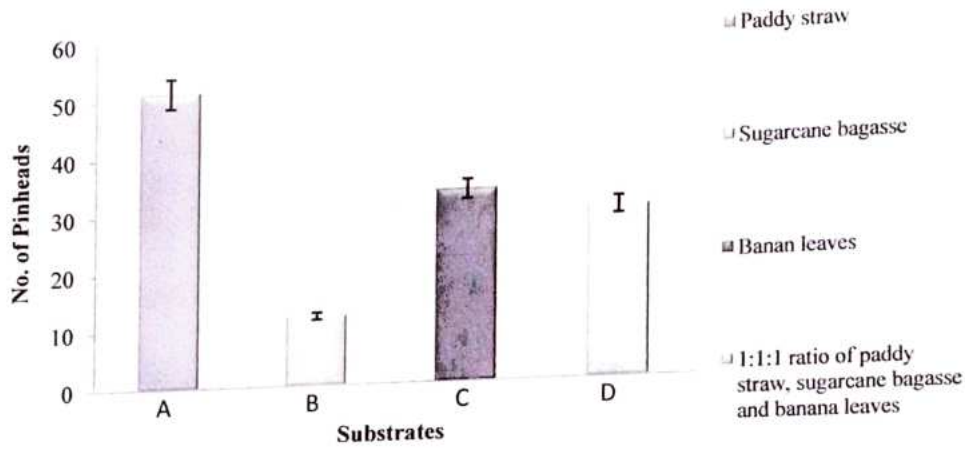


Figure 1b: Effect of Different Substrates on Number of Pinheads of *Pleurotus ostreatus*

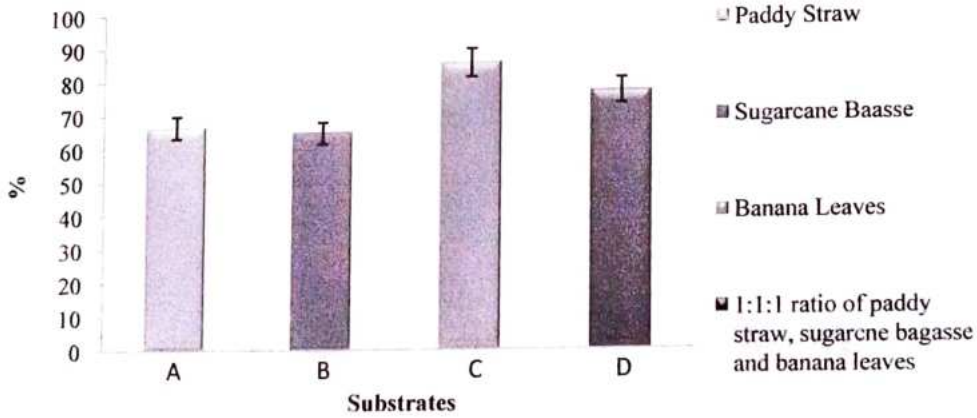
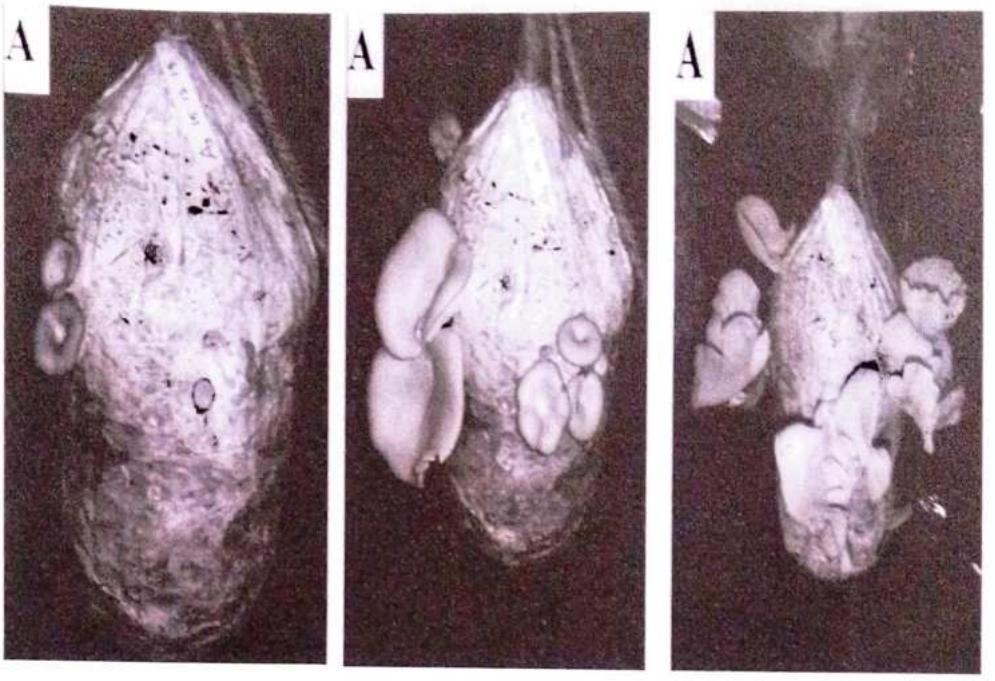


Figure 1c: Effect of Different Substrates onFruiting Body Development from Pinheads

Plate 3

DEVELOPMENT OF FRUTING BODIES ON PADDY STRAW



DEVELOPMENT OF FRUTING BODIES ON SUGARCANE BAGASSE

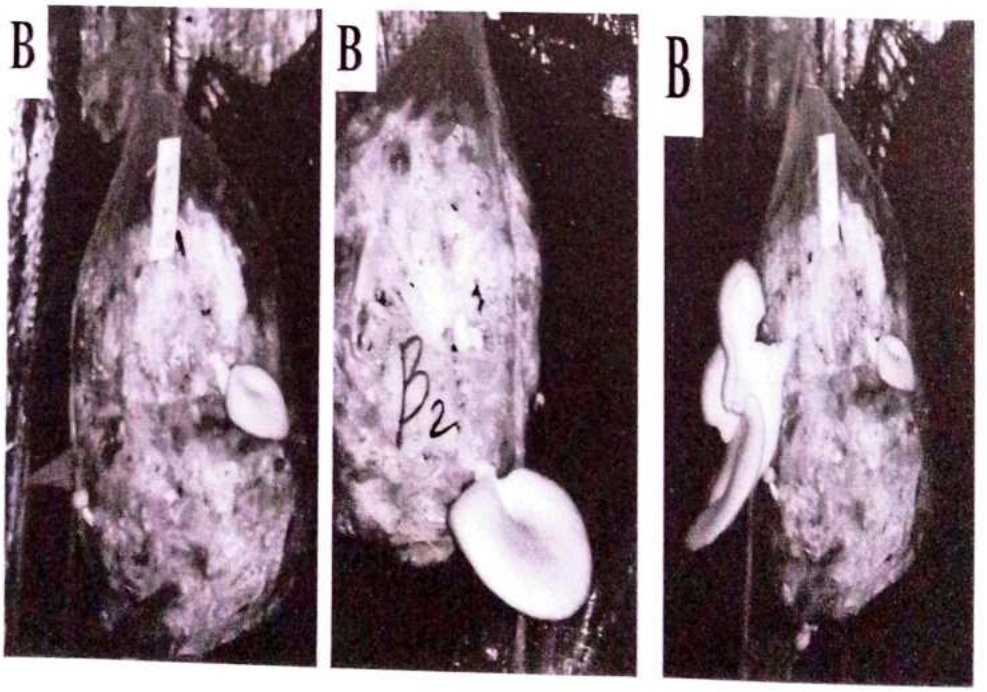
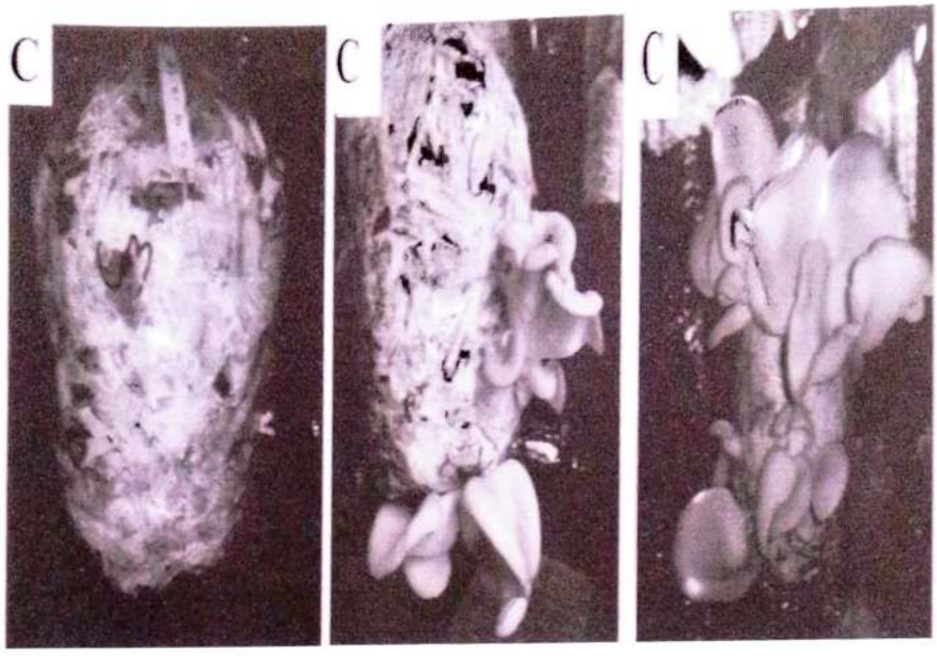
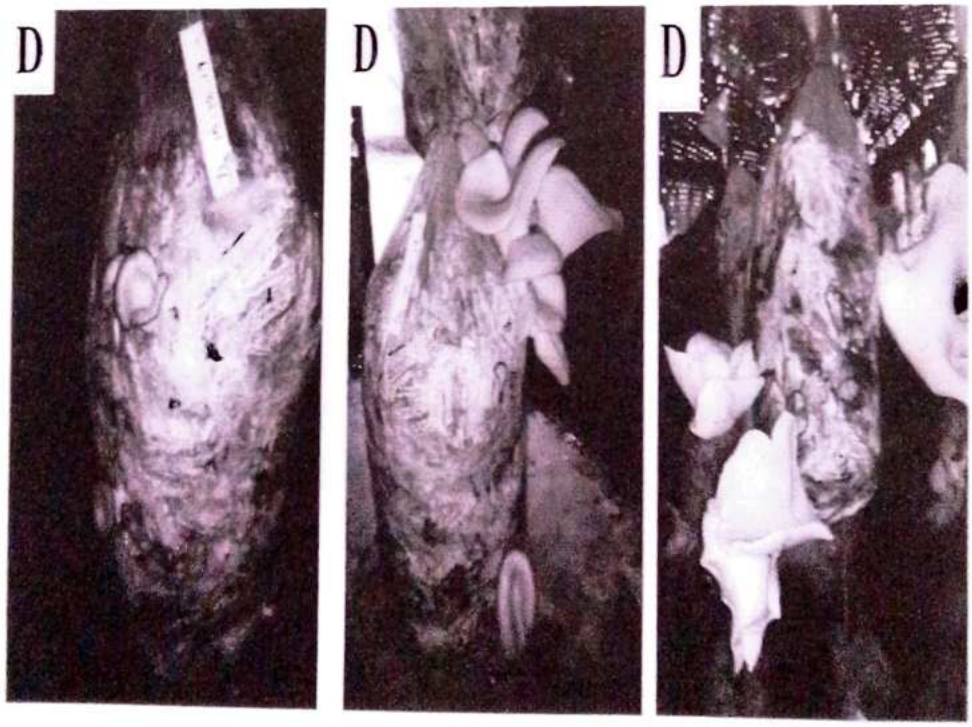


Plate 4

DEVELOPMENT OF FRUTING BODIES ON BANANA LEAVES



DEVELOPMENT OF FRUTING BODIES ON THE MIXER OF PADDY SRAW, SUGARCANE BAGASSE AND BANANA LEAVES



YIELD AND BIOLOGICAL EFFICIENCY

Total yield and biological efficiency of *P. ostreatus* cultivated on various substrates are presented in **Table 1**. Among all the four substrates, paddy straw showed highest percentage of biological efficiency and yield (90% and 588 g) followed by banana leaves (76% and 571 g) respectively. The lowest biological efficiency and yield (14% and 101 g) of *P. ostreatus* were obtained on sugarcane baggase. Our results agree with the result of **Sardar *et al.* (2016)** who reported that lowest biological efficiency was obtained on sugarcane baggase. Higher the biological efficiency of different substrates represents its higher suitability for the cultivation of mushroom. The increase in the yield of *P. ostreatus* on paddy straw is due to easier way of getting sugars from cellulosic substances **(Ponmurugan *et al.*, 2007)**. Superiority of paddy straw over other substrates in cultivation of *P. ostreatus* with respect to yield and biological efficiency has been reported earlier by **Pala *et al.* (2012)**. Our results also agree with the result of **Ragunathan *et al.* (1996)** who reported that maximum yield was obtained by cultivation *P. sajor – caju* on paddy straw.

LENGTH AND WIDTH OF PILEUS

Among mushroom quality characteristics, pileus diameter, stipe length, stipe diameter are very important attributes **(Mondal *et al.*, 2010)**. The data regarding the length and width of pileus of *P. ostreatus* is shown in **Table 1**. In the present study, maximum length and width of pileus was obtained (11.3 ± 2.3 cm and 21.5 ± 6.7 cm) on banana leaves followed by (11.3 ± 3.4 cm and 20.3 ± 6.1 cm) the mix of paddy straw +

TABLE 1: EFFECT OF DIFFERENT SUBSTRATES ON THE PERFORMANCE OF *PLEUROTUS OSTREATUS*

S.No	Substrate	Pileus		Stipe		Yield (g)	Biological Efficiency (%)	Organic Mass Loss (%)
		Length (cm)	Width (cm)	Length (cm)	Width (cm)			
1.	A	9.8 ± 2.2	18.5 ± 7.8	2.3 ± 1.5	2.6 ± 1.1	587.7	78.4	26.3
2.	B	5.8 ± 0.8	8.2 ± 2.9	2.6 ± 1	1.2± 0.3	101.7	13.6	12.5
3.	C	11.3 ± 2.3	21.5 ±6.7	1.3 ± 0.3	2.0± 0.4	571	76.1	24.1
4.	D	11.3 ± 3.4	20.3± 6.1	3.1 ± 1.5	2.3 ± 0.7	525.7	70.1	21.6

sugarcane baggase + banana leaves respectively. The minimum length and width of pileus was noted (5.8 ± 0.8 cm and 8.2 ± 2.9 cm) on sugarcane baggase. Our results are in consistence with the findings of **Sardar *et al.* (2016)** who observed minimum diameter of pileus (4.10 ±0.07 cm) on sugar cane baggase.

LENGTH AND WIDTH OF STIPE

Stipe length and width of *P. ostreatus*was observed on different substrates in the present study and found significant difference on different substrate is shown in **Table 1**. Maximum length of stipe (3.1 ± 1.5 cm) was obtained on the mix of paddy straw + sugarcane baggase + banana leaves and paddy straw alone (3.0± 1.5 cm). Similarly, Maximum width of stipe (2.6 ± 1.1 cm) was obtained paddy straw alone and on the mix of paddy straw + sugarcane baggase + banana leaves (2.3±0.7 cm). Minimum length of stipe (1.3 ± 0.3 cm) was observed on banana leaves while the minimum width was noticed (1.2 ± 0.3 cm) on sugarcane baggase. Oyster mushroom quality depends on the length of stipe. **Mondal *et al.* (2010)** found that the higher the stipe length, the poorer the quality of the mushroom. Hence growers should use substrates that do not promote excessive growth of stipe length at the expense of marketable yield.

PERCENTAGE OF BIOMASS LOSS

The mushroom has the ability to degrade lignocellulosic materials during the idiophase stage following severe nitrogen and carbon depletion **(Manson *et al.*, 1989).** Paddy straw biomass loss was 26.3% which shows that degradation and solubilization was more intensive in the paddy straw **(Table 1).**

QUALITATIVE ANALYSIS

The present study was carried out in preliminary phytochemical analysis of *P. ostreatus*. The phytochemical characteristics of P. ostreatus of various extracts investigated were summarized in **Table 2**. The various extracts of *P. ostreatus* revealed the presence of medicinally important bioactive ingredients. The alcoholic extracts of *P. ostreatus* showed the presence of alkaloid, protein, carbohydrate, phenol, tannin, flavonoid and saponin whereas the aqueous extract was found to contain protein, carbohydrate, tannin and coumarin. These phytochemicals have also been observed in mushrooms by others workers. Some phytochemicals such as steroid, glycoside, quinone and phytosterol was found of absence in all the extracts investigated in the present study.

NUTRITIONAL PARAMETERS

The results of moisture, protein, carbohydrate, lipid and amino acid contents are depicted in **Table 3**. The freshly cultivated *P. ostreatus* contains high moisture content. Moisture percentage in mushroom depends on the maturity of fruiting bodies, species and storage conditions and during packaging or processing. The present study revealed that the highest moisture content was observed in sugarcane baggase (91%) followed by banana leaves (90.3%), mix of paddy straw + sugarcane baggase + banana leaves (90.1%) and paddy straw (89.8%).

The carbohydrate content of mushrooms represents the bulk of fruiting bodies ranged from 5.6 to 11.6 mg/g on dry weight basis in ethanolic extract. Carbohydrate content was found to be maximum in dry thallus cultivated on sugarcane baggase

TABLE 2: QUALITATIVE ANALYSIS OF PLEUROUS OSTREATUS

Test	Water				Hexane				Chloroform				Ethyl acetate				Ethanol				Methanol			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Alkaloid	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+
Protein	+	+	+	+	-	-	-	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+
Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+
Glycoside	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saponin	-	-	-	-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	+	+	+	+
Phenol	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+
Tannin	+	+	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+
Flavonoid	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+	+
Steroid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phytosterol	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Quinone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terpenoid	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
Cardioglycoside	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+	-	+	+	+
Coumarin	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-
Phlobatannin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

'+' Indicates Presence '–' Indicates Absence

TABLE 3: EFFECT OF DIFFERENT SUBSTRATES ON THE NUTRIENT CONTENT OF *PLEUROTUS OSTREATUS*

Substrate	Moisture content (%)	Carbohydrate (mg/g DW)		Protein (mg/g DW)		Aminoacid (mg/g DW)		Lipid (mg/g DW)	
		Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
A	89.8	3.02	5.6	80.5	134.2	136.6	206.5	1.8	5.3
B	91	3.7	11.6	144.1	194.1	155.9	220.3	4.7	8.6
C	90.3	3.6	8.6	91.4	169.3	147.4	229.7	1.7	5.7
D	90.1	3.3	6.8	99.1	184.2	144.1	243.8	3.5	7.8

(11.6 mg/g) followed by fruit bodies developed on banana leaves (8.6 mg/g). However, the fruitbodies developed on paddy straw had the lowest carbohydrate content (5.6 mg/g) (**Figure 2a**).

Protein is an important constituent of dry matter of mushrooms. Protein content of mushrooms depends on the composition of substratum, size of pileus, harvest time and species of mushrooms (**Bano and Rajarathnam, 1982**). Protein content *of P. ostreatus* was found maximum in sugarcane baggase treated (194 mg/g) followed by the mix of paddy straw + sugarcane baggase + banana leaves (184 mg/g) while the fruit bodies developed from paddy straw had the lowest protein content (134 mg /g). Protein content of the mushroom mycelium can be controlled by the amount of nitrogen supplied in the growth media. The Carbon: Nitrogen influences the protein and the fat content in the mushroom mycelium (**Shah *et al.*, 2004**). They differ according to the species but this difference depends on the substratum, atmospheric conditions, age and part of the fruitification (**Figure 2b**).

The mushroom protein is known to contain almost all the essential amino acids. Apart from essential amino acids, considerable amount of alanine, arginine, glycine, histidine, glutamic acid, aspartic acid, proline and serine can be found in mushroom. The free amino acid content of *P. ostreatus* ranged from 137 mg /g to 156 mg / g in alcoholic extract of dry thallus and 207 mg/g to 244 mg/g in alcoholic extract of dry thallus (**Figure 2c**). The highest amount of amino acids was recorded in ethanolic extract of dry thallus cultivated on sugarcane baggase while the lowest amount of amino acids was registered in ethanolic extract of dry thallus cultivated on paddy straw. The quantitative

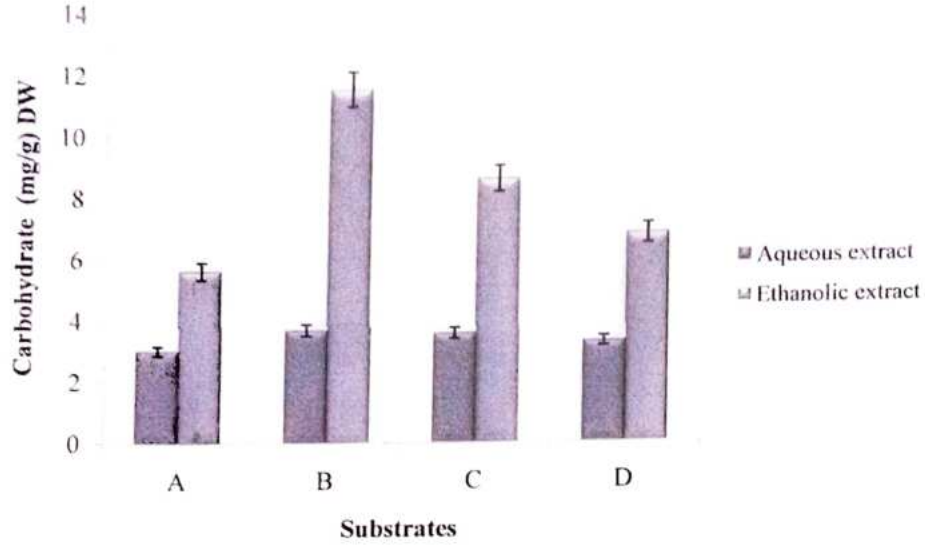


Figure 2a: Effect of Different Substrates on Carbohydrate Content of *Pleurotus ostreatus*

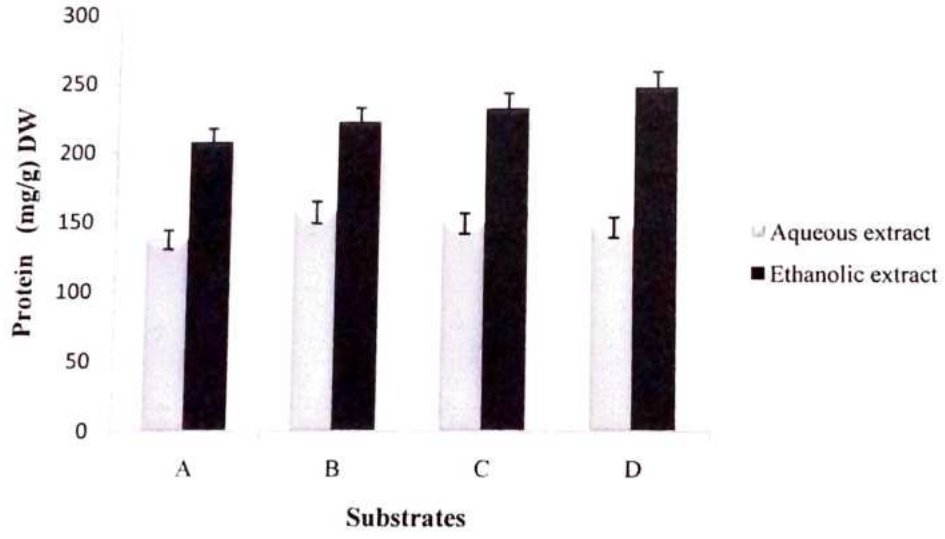


Figure 2b: Effect of Different Substrates on Protein Content of *Pleurotus ostreatus*

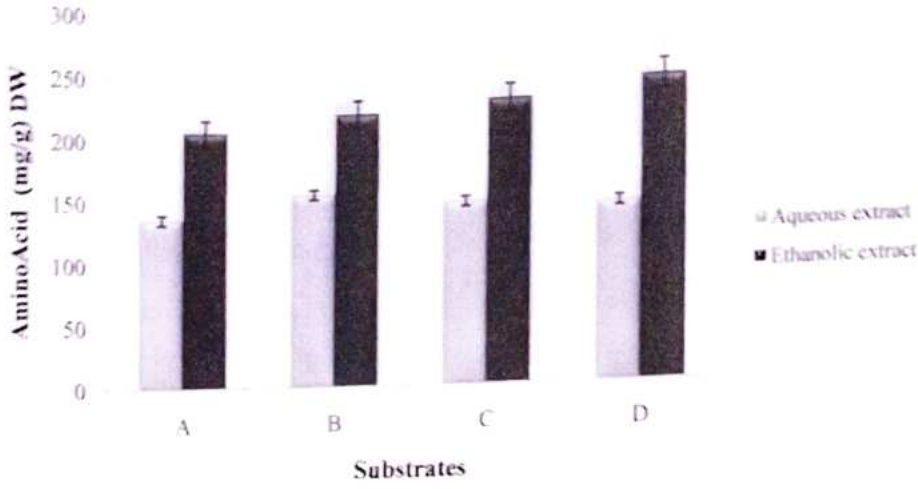


Figure 2c: Effect of Different Substrates on AminoAcid Content of

Pleurotus ostreatus

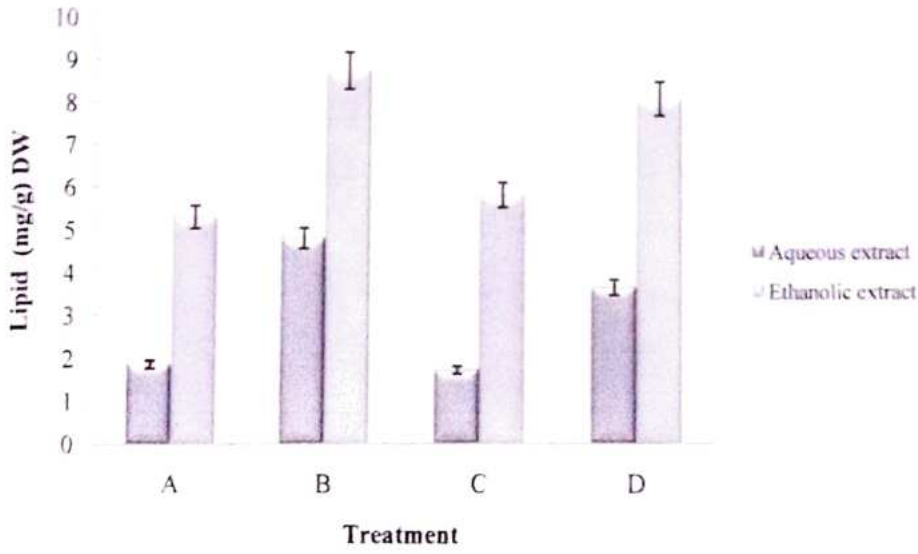


Figure 2d: Effect of Different Substrates on Lipid Content of

Pleurotus ostreatus

spectrum of essential amino acids has served as the basis to calculate biological value, nutritional value and protein score **(Haque, 1989).**

Lipid content of all the four investigated substrates showed variation. In mushrooms, the fat content is very low as compared to carbohydrates and proteins. The highest lipid content was found in fruit bodies developed on sugarcane baggase (8.6 mg/g) followed by the mix of paddy straw + sugarcane baggase + banana leaves (7.8 mg/g) while the fruit bodies developed from paddy straw had the lowest lipid content (5.3 mg/g) in ethanolic extract **(Figure 2d). Yilmaz *et al.* (2006)** and **Pedneault *et al.* (2006)** reported that fat fraction in mushrooms is mainly composed of unsaturated fatty acids.

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Edible mushrooms have been eaten and appreciated for their flavor, economical and ecological values and medicinal properties. They are able to grow under climatic conditions on cheap, readily available waste materials. These mushrooms are a clear example of how low value waste which is produced primarily through the activities of the agricultural, forest and food processing industries, can be converted to higher value material useful to mankind. For many reasons, the fungus *Pleurotus* genus had been intensely studied and cultivated in many different parts of the world. This mushroom demands environmental controls for cultivation and its fruiting bodies are not often attacked by diseases and pest and it can be cultivated in a simple and cheap way. Another advantage of growing oyster mushroom is that a high percentage of the substrate is converted to fruiting bodies, increasing profitability as compared to other mushrooms making *P. ostreatus* an excellent choice for mushroom cultivation.

1. The results of the present study support the efficient production of mushroom on substrates.
2. Utilizing these waste products as substrates for the production of mushrooms would reduce the adverse environmental effects of theses waste products.
3. An economical strategy for converting waste products into nutritious food source is represented in this study

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