SCREENING OF BIOACTIVE PROPERTIES IN *Padina tetrastromatica* HAUCK AND *Sargassum tenerrimum* J.AGARDH. FROM ANJUNA COAST, GOA

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COMPLETION CERTIFICATE

This is to certify that the dissertation report "**SCREENING OF BIOACTIVE PROPERTIES IN *Padina tetrastromatica* Hauck AND *Sargassum tenerrimum* J.Agardh FROM ANJUNA COAST, GOA**", is a bonafide work carried out by **Ms. Surbhi Suresh Karapurkar**, under my supervision/ mentorship in partial fulfilment of the requirement for the award of degree of M.Sc. in the Discipline Botany at the School of Biological Sciences and Biotechnology, Goa University.

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Ms. Surbhi Suresh Karapurkar

CONTENTS

ABBREVIATIONS

TPC- Total Phenolic Content

TFC- Total Flavonoid Content

GAE- Gallic Acid Equivalent

QE- Quercetin Equivalent

DPPH- 2,2-diphenyl-1-picrylhydrazyl

IC_{50} – Half- maximal inhibitory concentration

MHA-Mueller–Hinton agar

MEA- Malt-Extract Agar

TLC- Thin Layer Chromatography

R_f - Retention Factor

UV-VIS- Ultra Violet Visible Spectrophotometry

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ABSTRACT

The presence of bioactive compounds reflects multiple health-enhancing properties, which may lead to their exploitation in food, pharmacological, and cosmetic industries. However, the bioactivity of various seaweeds across Goa's coastlines is yet to be explored. The present study evaluated the impact of two different solvents on extraction yields of the total phenolic, flavonoid Content, antioxidant capacity, antimicrobial activity and pigment analysis of two brown macroalgae viz., *Padina tetrastromatica* Hauck and *Sargassum tenerrimum* J.Agardh. The results showed that solvents were essential in the extraction yield, the chemical component's content, and the tested biological activities. Methanol was identified as the most effective solvent for the extraction, with the highest flavonoid content in both algae. The highest content of phenols was observed in the methanolic extract of *S. tenerrimum* and the ethanolic extract of *P. tetrastromatica*, with no significant differences in the values. Likewise, the ethanolic extract of *P. tetrastromatica* exhibited high antioxidant capacity from the DPPH assay, while *S. tenerrimum* exhibited higher DPPH activity in the methanolic extract. However, the reducing power assay showed the maximum antioxidant activity in the methanolic extracts of *P. tetrastromatica and S. tenerrimum*. Additionally, both extracting solvents showed increasingly higher antibacterial activity of *S. tenerrimum* with *Escherichia coli* and *Bacillus cereus.* On the other hand, the methanolic extracts of *S. tenerrimum* inhibited fungal growth. Algal photosynthetic pigments, chlorophyll a, chlorophyll c, β-carotene and fucoxanthin were extracted and identified utilizing Thin Layer Chromatography and UV-VIS spectra.

Chapter 1

INTRODUCTION

1. INTRODUCTION

Oceans occupy about three fourth of the world and are home to several fantastic flora and fauna. They are also essential for the interplay of nature's basic elements that help in the survival of living beings. In addition to seafood, oceans harbour seaweeds, one of the world's ecologically and economically critical living resources of the world (Rajapakse and Kim, 2011). Seaweeds or marine macroalgae are potential renewable resources in the marine environment. About 6000 species of seaweeds have been identified and are grouped into different classes, which include green (Chlorophytes), brown (Phaeophytes), and red (Rhodophytes) algae (Abbott, 1995). They can biosynthesise secondary metabolites that can mediate a broad range of intra and inter-specific ecological interactions between marine organisms, including chemical defences against herbivores (Kavitha and Palani, 2016; Hay and Steinberg, 1992).

India, bordering the Indian Ocean, ranks first among all countries in the number of recorded specific and intra-specific seaweed taxa ahead of Australia and South Africa (Manilal *et al.*, 2009). Approximately 841 species of marine algae have been found in both the inter-tidal and deep-water regions of the Indian coast. It has been estimated that about 90% of marine plant species are algae, and about 50% of global photosynthesis is contributed by algae (Dhargalkar and Pereira, 2005). They grow in intertidal, shallow marine areas up to 180 m deep in estuaries and back-waters on solid substrates such as rocks, coral, pebbles, shells and other plant material.

Algae is a diverse group of photosynthetic eukaryotic organisms. They have thallus and lack tissue differentiation. Algal thallus ranges from unicellular to giant multicellular forms. Their habitat ranges from fresh waters to marine waters to extreme conditions. As they are diverse in structure and habitat, there is much diversity in their reproduction and perennation strategies.

Marine algae have attracted much attention in the last few years. Besides their crucial ecological role in the ocean, marine algae contain significant quantities of vitamins, minerals, dietary fibres, proteins, polysaccharides, and various functional polyphenols, while nutrient contents can vary with species and geographical location, season, and temperature. Algae contain many phycocolloids such as agar, alginate, and carrageenan, which have been utilised as gelling agents and emulsifiers in the food and pharmaceutical industries (Liu *et al.,* 2012; Andrade *et al.*, 2013).

Macroalgae are extraordinary sustainable resources in the marine ecosystem and possess a wide variety of natural compounds with interesting properties (Shahnaz and Shameel, 2009). More than 15,000 primary and secondary metabolites from different metabolic pathways have been reported from macroalgae, and different applications were assigned to them. Much of this is based on edible farming species or on producing agar, carrageenan and alginate. These wide varieties of seaweeds are found to possess beneficial untapped biochemical compounds, which might be a potential source of drug leads in the future (Huang et al., 2006). In recent years, there have been many reports of macroalgae-derived compounds with a broad range of biological activities, such as antibiotic, antiviral, antioxidant, antifouling, anti-inflammatory, cytotoxic and antimitotic activities (Ely, 2004).

Asian countries like China, Japan, the Philippines and other parts of Southeast Asia have a long history of seaweed utilisation, where it has been used as a traditional medicine for promoting good health by providing nutritional benefits for treating diseases such as iodine deficiency (goitre, base Dow's disease) and also as food ingredients and beverages (Folmer *et al.,* 2010). The ancient Romans used seaweeds to treat wounds, burns and rashes. Their use as an alternative medicine for skin-related diseases (eczema, scabies, psoriasis), gallstones, renal diseases, asthma, atherosclerosis, heart disease, ulcers and cancer has also been documented (Ali *et al.,* 2000; Li *et al.,* 2011; Wijesekara *et al.,* 2011). In addition, seaweeds have been

employed for treating intestinal disorders (vermifuges), as hypocholesterolemic and hypoglycemic agents and as ointments for gynaecology and cosmeceuticals (Kim *et al.,* 2008). Thus, being a rich source of natural compounds, the importance of marine algae to human beings is self-evident.

About 221 macroalgal species have been listed worldwide as commercially utilised (White and Ohno, 1999). In India, about 220 genera and 740 species of marine algae were recorded, of which 60 species are of economic value (Kolanjinathan *et al.,* 2014). Each year an increasing number of novel marine metabolites are being reported in the literature, indicating that the marine environment is likely to continue to be a prolific source of more natural products for many years to come.

1.1 BROWN ALGAE:

Brown algae are the second most abundant group of seaweeds in the ocean (Davis *et al.,* 2003; Mestechkina and Shcherbukhin, 2010; Reddy and Urban, 2009). The body of all brown algae is termed a thallus, indicating that it lacks the complex xylem and phloem of vascular plants; its cell walls are composed of alginic acid, which can be extracted as algin or alginate, used for various industrial purposes. Most of the members of this division are almost exclusively of marine occurrence. Most brown algae grow in the intertidal belt and the upper littoral region (Lewmanomont, 1978). The algal structure may range from simple, freely branched filaments to highly differentiated forms. The brown algae are rich in photosynthetic pigments (chlorophyll a & c, carotene, xanthophylls and fucoxanthin) and polysaccharides with many biological activities (Ruperez and Saura-Calixto, 2001; Siriwardhana *et al*., 2004). The brown algae are distinguished by their colour, which varies from olive green through light golden to a rather deep shade of brown. This is because of the presence of a golden brown xanthophylls

pigment fucoxanthin ($C_4H_5O_6$) in their chromatophores, which masks the remaining pigments. Species of brown seaweed are well known to contain large amounts of cell-wall polysaccharides, most of which are the sulfated polysaccharide fucoidan (Asker *et al*., 2007), which is not found in terrestrial plants.

Brown seaweeds are mainly used in conditions like hypothyroidism, fatigue, cellulite, cough, asthma, stomach ailments and headache. Brown seaweeds also promote weight loss and aid in skin care (Cumashi *et al*., 2007). The potential antioxidant compounds were identified as some pigments and polyphenols (Yoshie and Suzuki, 2000). Those compounds are widely distributed in plants or seaweeds and are known to exhibit antioxidative activities via reactive oxygen species scavenging activity and the inhibition of cell apoptosis (Yan *et al*., 1999; Heo *et al*., 2005; Sriwardhana *et al*., 2003).

1.2.1. PHYTOCHEMICAL STUDIES:

Phytochemicals are non-nutritive plant chemicals. The phytochemical screening of seaweeds is on the rise to search for valuable bioactive compounds with beneficial properties (Ali *et al.*, 1999; Rajauria *et al*., 2016). Plants synthesise phytochemicals for many reasons, including protecting themselves against insect attacks and plant diseases. Plant and plant-based products are natural sources of phytochemicals such as phenols, flavonoids, and alkaloids. Phenols and flavonoids are the most common phytoconstituents of different fruits, vegetables, aromatic plants, and seaweeds, responsible for antioxidant activities (Scalbert *et al*., 2005).

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate and phenylpropanoid pathways in plants. More than 8,000 polyphenolic compounds are identified in various plant species. Polyphenols are highly structured molecules consisting of multiple phenol groups. Polyphenols are classified based on the number of phenol

rings that contain the structural elements that bind these rings to one another. They are broadly divided into Phenolic acids, flavonoids, stilbenes and lignans.

Seaweeds are considered an excellent source of polyphenols in comparison to terrestrial plants because terrestrial plants contain polyphenols with a lower number of rings in the structure and are derived from gallic and ellagic acid. In contrast, seaweed polyphenols are multiple-ringed (up to eight) and derived from phloroglucinol units (1,3,5- trihydroxy benzene) (Burtin, 2003). Polyphenols containing more rings in their structure exhibit more significant antioxidant activity (Gupta and Ghannam, 2011). Polyphenols are predominantly found in brown seaweeds, and phlorotannin (multi-ringed phloroglucinol-based tannins), such as the well-studied eckol, are exclusively found in brown algae. Other polyphenols, such as bromophenol, are present in all classes of seaweeds. Phlorotannins can account for up to 40% of the dry weight of some brown seaweed (Arnold *et al*., 1995).

Mohammad et al. (2021) investigated the effect of solvents on bioactive compounds and antioxidant activity of *Padina tetrastromatica* and *Gracilaria tenuistipitata* seaweeds collected from Bangladesh during which the six crude extracts for two seaweed species were screened for the occurrence of six phytochemicals named saponin, terpenoid, cardiac glycoside, phlobatannin, phenolic, and flavonoid. They observed that every extract contained varying amounts of active secondary metabolites (phytochemicals) such as saponin, terpenoid, cardiac glycoside, phlobatannin, phenolic and flavonoid.

Sruthy and Chitra (2022) also reported the phytochemical, biochemical profile and antioxidant activity of *Sargassum tenerrimum* J.Agardh and showed abundant phytochemicals in solvents like distilled water, methanol and chloroform. It showed alkaloids and steroids in distilled water and chloroform, respectively and confirmed the presence of flavonoids, phenol, coumarin, terpenoids and saponins in all three solvent systems.

1.2.2. PHENOLIC ACIDS:

For many years, phenolic compounds from brown algae have been widely studied for their biological activities. Phenolic acids account for about a third of the polyphenolic compounds in our diet and are found in all plant materials but are particularly abundant in acidic fruits. Caffeic, gallic, and ferulic acids are common phenolic acids. The phenolic compounds are the most effective antioxidant present in brown algae (Nagai and Yukimoto, 2003). Phenolic compounds (Phlorotannins) are secondary defence metabolites synthesised during development as components of algal cell walls (Schoenwaelder and Clayton, 1999) or as a chemical defence in response to abiotic and biotic stress conditions, such as UV radiation, grazing, bacterial infection, and epiphytism, as well as in intra and interspecific communication (Burtin, 2003; Koivikko, 2008). Phlorotannin corresponds to the polymerisation of phloroglucinol monomer units containing phenyl and phenoxy (Li *et al*., 2011). Accumulation of these phloroglucinol-based phenolics contributes to the potent antioxidant activity against free radical-mediated oxidation damage (Kang *et al*., 2010; Fujii *et al*., 2013). They also show other potential bioactivities such as bactericidal, antiviral, anticancer activity, radioprotective and anti-allergic effects (Li *et al*., 2011; Eom *et al*., 2012; Lann *et al*., 2016). The phenolic content of brown algae varies from 20-30% dry weight (Ragan and Glombitza, 1986). Sarojini et al. (2013) analysed seasonal variations in phenol distribution and DPPH radical scavenging activity in *Sargassum vulgare* and *Padina tetrastromatica*

1.2.3. FLAVONOIDS:

Flavonoids are members of a class of natural compounds that have recently been the subject of considerable scientific and therapeutic interest. Flavonoids constitute the largest class of phenolic compounds with more than 3,000 structures. These consist of two aromatic rings bound together by three carbon atoms that form an oxygenated heterocycle (hydroxylated phenolic substances) (Tsao, 2012). These are known to be synthesised by plants in response to microbial infection, and they are antimicrobial substances against a wide array of microorganisms in vitro.

Flavonoids contain a broad spectrum of chemicals and biological activities, including antioxidant and free radical scavenging properties, antibacterial, antiviral, anticancer, anti-inflammatory, anti-allergic and potential therapeutic agents against a wide variety of diseases. Meenakshi et al. (2009) evaluated the total flavonoid and *in vitro* antioxidant activity of two seaweeds, *Ulva lactuca* and *Sargassum wightii*. Sarojini et al. (2012) reported the total flavonoids from fifteen seaweeds from the Visakhapatnam coast.

1.3. ANTIOXIDANT ACTIVITY:

Antioxidant compounds that restrain or delay the oxidation of molecules by reacting with free radicals are known as antioxidants. In the biological system, antioxidants are "substances that, when present in low concentration with an oxidisable substrate, would significantly tetrad or prevent oxidation of the substrate" (Halliwell *et al.,* 1995). The oxidisable substrate could be a molecule in food or biological materials, including carbohydrates, DNA, lipids and proteins (Shenoy and Shirwaikar, 2002).

Free radicals are responsible for ageing, and their presence in excess constitutes the cause of various human diseases. Different studies show that antioxidant substances that scavenge free radicals are essential in preventing free radical-induced diseases (Ismail and Hong, 2002).

Antioxidants in the human body help the body to neutralise radicals before they may lead to damage (Ames *et al*., 1993). Reactive oxygen species (ROS) such as hydroxyl, superoxide and peroxyl radicals are formed in human cells by endogenous factors and result in extensive oxidative damage, which can lead to age-related degenerative conditions, cancer and a wide range of other human diseases (Revan and Witzum, 1996; Aruoma, 1999). The antioxidant helps in providing prevention against cancer and many other health problems as a consequence of oxidation. Different antioxidants function in different ways; for example, they could operate by obstructing singlet oxygen, diminishing oxygen concentration, inhibiting the initiation of the first chain by quenching initial radicals such as hydroxyl, decomposing primary products to non-radical compounds, binding metal ion catalysts and chain breaking to retard continued hydrogen abstraction from the substrate (Benzie and Strain, 1999).

Over the years, the search for new antioxidant compounds from natural products has mounted due to health concerns regarding synthetic antioxidants' potentially toxic side effects and changes in consumer preference for natural products (Safher, 1999). Many commercialised synthetic antioxidants, such as Butylated Hydroxy Anisole (BHA) and Butylated Hydroxy Toluene (BHT), are used under strict regulation in certain countries because of their health hazards. It is commonly used in the food industry for preserving food and its quality, but those have been suspected of toxic and excreting carcinogenic effects (Gupta and Ghannam, 2011). Thus, the search for alternative antioxidants from natural products has increased, and among them, marine plants have gained focus.

The absence of oxidative damage in the structural components of macroalgae and their stability to oxidation during storage suggests that their cells have protective antioxidative defence systems (Matsukawa *et al*., 1997). Algae have protective enzymes (superoxide dismutase, peroxidase, glutathione reductase, catalase) and antioxidative molecules such as phlorotannins, ascorbic acid, tocopherols, carotenoids, phospholipids, chlorophyll related compounds, bromophenols, catechins, mycosporine-like amino acids, polysaccharides which are similar to those of vascular plants (Tutour *et al*., 1998; Rupérez *et al*., 2002; Yuan *et al*., 2005). Although different compounds may be responsible for the antioxidant properties of seaweeds, polyphenols seem to be of particular interest since they can display antioxidant properties at low concentrations by being employed in the food industry to protect foods from oxidative degradation (Tutour *et al*., 1998).

Marine macroalgae are rich sources of various natural antioxidants (Meyer *et al*., 1982). Therefore, the consumption of antioxidants and the addition of antioxidants in food materials protect the body and food against these events (Chandini *et al*., 2008). Chandini et al. (2008) investigated invitro antioxidant activity of selected Indian brown seaweeds viz., *Sargassum marginatum, Padina tetrastomatica and Turbinaria conoides* in five different fractions (petroleum ether (PE), ethyl acetate (EA), dichloromethane (DCM), butanol (BuOH) and aqueous).

Megha *et. al.,* (2014) studied the antioxidant activity of three species of *Sargassum viz. S. cinereum, S. ilicifolium and S. tenerrimum*, and were used for antioxidant assays such as DPPH radical scavenging Activity, Ferric Reducing Antioxidant Power assay (FRAP), Ferrous Ion Chelating ability (FIC), Reducing Power, Hydrogen Peroxide Scavenging Activity and Total Antioxidant Capacity (TAC).

1.4 ANTIMICROBIAL ACTIVITY:

Microorganisms are naturally available in the environment around us, and therefore they can easily reach food during harvesting, slaughtering, processing, and packaging (Hatab *et al*., 2018). Food spoilage is a complex ecological phenomenon caused mainly by the biochemical activity of specific groups of microorganisms. Bacteria, yeast, and molds are common microorganisms that grow by utilising nutrients and produce metabolites that cause food spoilage and are responsible for the spoilage of many food and food products (Lianou *et al.*, 2016; Parlapani *et al.*, 2017). Various adverse conditions used in food preservation, such as low temperature, vacuum packaging, and modified atmospheric packaging, allow these microorganisms to survive (Dimitrijević *et al.*, 2007; Provincial *et al.*, 2013; Saraiva *et al.*, 2016; Säde *et al.*, 2017).

The importance of microbes to food products of animal origin has been demonstrated by recent outbreaks of foodborne illness associated with consuming meat, milk and dairy products contaminated with pathogenic organisms or toxins. Undesirable microorganisms are the primary hazard to food products' safety, quality, and wholesomeness. Thus, there is considerable concern among both consumers and food industries regarding the risk of using synthetic additives, as there are an increasing number of cases related to food infections. Hence, an essential target for the food industry is to avoid food contamination and its related foodborne illnesses, which led to decreased use of these chemicals in food preservation (Gyawali and Ibrahim, 2014; Caleja *et al.*, 2016; Kalem *et al.*, 2017).

Therefore, new eco-friendly methodologies are required to reduce the growth of pathogenic bacteria and prolong the shelf-life of food products without using chemical preservatives. There is an urgent need to find new antibacterial agents due to the widespread drug-resistant bacteria and the lack of new antimicrobials to combat them. (Palaniappan and

Holley, 2010). Recently, many researchers investigated the possible utilisation of some plant extracts as effective natural preservatives (Fernández-López et al., 2005; Suppakul *et al*., 2016; Clarke *et al.,* 2017). Seaweeds have been proven to be a good source of antibacterial agents (Bansemir *et al*., 2006; Kuda *et al*., 2007).

The antibacterial agents found in the algae include amino acids, terpenoids, phlorotannins, acrylic acid, phenolic compounds, steroids, halogenated ketones, alkanes, cyclic polysulphides and fatty acids. Green, brown and red algae contain various inorganic and organic compounds that benefit human health (Kuda *et al*., 2002). Algae have proven to possess high nutritional value and various biological properties due to their content in active compounds. Among these capabilities, macroalgae are recognised for having antimicrobial properties and have been used in traditional medicine for many years against various diseases (tuberculosis, arthritis, colds and influenza) (Kandale *et al*., 2011; Malhotra, 2008).

Subba Rangaiah et al (2010) carried out the antimicrobial potentiality of the marine algae, two species of brown algae, namely *Sargassum ilicifolium, Padina tetrastromatica* and one red algae *Gracilaria corticata* collected from different coastal regions of Visakhapatnam.

Manivannan et al. (2011) studied the antimicrobial activity of *Turbinaria conoides, Padina gymnospora and Sargassum tenerrimum using* seven different solvents (methanol, acetone, petroleum ether, ethanol, ethyl acetate, chloroform, diethyl ether) against bacterial strains *Bacillus subtilus, Klebsiella pneumonia* and fungal strains *Cryptococcus neoformans* and *Aspergillus niger*.

There is currently very little information on various algal species' phytochemicals, antioxidants and secondary metabolites and pharmacological applications. Because of the need for new alternative bioactive compounds with different biochemical activities, algae could potentially be a great source. This study observed two brown algae, *Padina tetrastromatica*

and *Sargassum tenerrimum*, for their bioactivity, antibacterial, and antifungal activity using different extraction solvents.

OBJECTIVES

This study aims at understanding the effects of two extraction solvents on the antifungal and antibacterial activity of the two algae, *Padina tetrastromatica* and *Sargassum tenerrimum*. TLC and spectrophotometric methods studied the quantitative and qualitative analysis of phenolic compounds, flavonoids, antioxidants and pigments. The detailed objectives are as follows:

- Using two extraction solvents, extract the bioactive compounds like total phenolics and total flavonoids from *Padina tetrastromatica* and *Sargassum tenerrimum*.
- To quantitate the amount of Total Phenolic Content and Total Flavonoid Content in methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum*.
- To investigate the antioxidant activity of methanolic and ethanolic extracts *of Padina tetrastromatica and Sargassum tenerrimum* using DPPH radical scavenging assay and Reducing Power assay.
- To evaluate the antifungal and antibacterial activity of methanolic and ethanolic extracts of *Padina tetrastromatica and Sargassum tenerrimum* on fungal sp. (*Aspergillus* niger) and two bacterial strains (*Escherichia coli* and *Bacillus cereus*).
- To carry out TLC profiling and UV-VIS spectra for algal pigments in the algae *Padina tetrastromatica* and *Sargassum tenerrimum*.

Chapter 2

MATERIALS AND METHODS

2. MATERIALS AND METHODS

2.1 COLLECTION OF SAMPLES

Two species of brown marine algae, namely, *Padina tetrastromatica* and *Sargassum tenerrimum,* were collected from the coast of Anjuna, (15˚35'04.0" N 73˚44'13.6" E) Goa, India, from the rocky surfaces during low tide between November and December 2022. The Collected thallus was washed with clean seawater, and the impurities were removed with tap water and rinsed with sterile distilled water. The algae were then shade-dried and Oven-dried at 40˚C for 1 h.

2.2 IDENTIFICATION OF COLLECTED ALGAL SPECIMEN

The sample identification was accomplished by taking the help of Prof. Vijaya Kerker, Botany discipline, School of Biological Sciences and Biotechnology, Goa University.

2.3 PREPARATION OF ALGAL EXTRACTS

The dried seaweed sample was grounded to make a fine powder using liquid nitrogen. Fine seaweed powder (8g) was soaked in 200 mL of solvent (methanol and ethanol) to prepare an extract by solvent extraction. The sample was kept dark for 24 h with intermittent shaking for better extraction. After incubation, the solution was filtered with Whatman's filter paper No. 4 (20-25 μm), retaining hygienic conditions. After filtration, the remaining wet powder was extracted in their respective solvents for 12 h and then filtered to get the maximum out of the sample powder. The methanol and ethanol extracts were then concentrated using a rotary evaporator at 36˚C. The extract of each specimen was weighed after drying. The extractive values were calculated (Kokabi *et al*., 2013) on a dry weight basis using the following formulae:

$$Extractive\ value\ (yeild\ \%) = \frac{weight\ of\ dry\ extract}{weight\ taken\ for\ extraction} \times 100$$

Finally, the working solution was prepared as 1mg/mL and was further diluted into various concentrations for different experiments (Sobuj *et al*., 2021).

2.4 QUANTITATIVE ANALYSIS

2.4.1 Determination of Total Phenolic Content (TPC)

Total Phenolic content was estimated by Folin Ciocalteu's method. Standard Gallic acid solution was prepared by dissolving 10 mg in 10 mL of distilled water (1mg/ mL). The total phenolics were expressed as mg of Gallic acid equivalents (GAE)/ g of extract. Various concentrations of gallic acid solutions in distilled water (50, 100, 150, 200, and 250 µg/ mL) were prepared from the standard solution. Concentrations of 50, 100, 150, 200, and 250 µg/ mL of algal extracts were prepared in respective solvents (methanol and ethanol). 0.5 mL of each sample was introduced into test tubes. Then 5 mL of 10-fold dilute Folin Ciocalteu's reagent was mixed and shaken. After 5 minutes, 5 mL of 7.5% sodium carbonate was added. The test tubes were covered with aluminium foil and incubated for 1 h at room temperature. Take 2 mL from this mixture in another test tub and make the final volume 5 mL with distilled water. Take absorbance at 760 nm spectrophotometrically. All determinations were performed in triplicate. Folin Ciocalteu's reagent, sensitive to reducing compounds including polyphenols, produced a blue colour upon reaction measured spectrophotometrically. The calibration curve was plotted using standard gallic acid (Savitree *et al*., 2004). The concentration of total phenolics was calculated as mg of Gallic acid equivalent per gram.

2.4.2 Determination of Total Flavonoid Content (TFC)

Total Flavonoid Content was estimated by Aluminium chloride colourimetric assay. Standard Quercetin solution was prepared by dissolving 10mg in 10 mL, 80% methanol (1mg/mL). The

total flavonoids were calculated as mg of quercetin equivalent per gram. Various concentrations of quercetin solutions in 80% methanol (50,100, 150, 200 and 250 µg/ mL) were prepared from the standard solution. A concentration of 1mg/mL of algal extracts was prepared in respective solvents (methanol and ethanol) and (50, 100, 150, 200 and 250 µg/ mL) from the stock solution using a respective solvent. 500 µL of the sample was added to a test tube with 1.5mL 95% ethanol (v/v). To the test tube, 0.1mL 10% aluminium chloride was added and after 5 min, 0.1 mL of 1M potassium acetate was added, and the total volume was made up to 5 mL with distilled water. The solution was mixed well and incubated at room temperature for 30 min. The absorbance of the reaction was measured at 420 nm spectrophotometrically. All determinations were performed in triplicate. The calibration curve was plotted using standard quercetin (Woisky *et al*., 1998). The concentration of total flavonoids was calculated as mg of quercetin equivalent per gram.

2.5. ANTIOXIDANT ACTIVITY

2.5.1. DPPH radical scavenging assay

Free radical scavenging activity of methanolic and ethanolic extracts of algae *Padina sp.* and *Sargassum sp.* was determined according to the DPPH methods (Ahmed *et al*., 2013; Brand-Williams *et al*., 1995). The hydrogen atom donating ability of the algal extracts was determined by the colouration of ethanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH produces a violet colour in ethanol solution and fades to shades of yellow in the presence of antioxidants. The stock solution of DPPH radical (24 mg /100 mL in ethanol) was prepared and stored at -20˚C until further use. A working solution was prepared by diluting the stock solution of DPPH with ethanol (10 mL/45 mL ethanol). Stock solutions of algal extracts were prepared in methanol and ethanol with a 3mg/ mL concentration. Different dilutions from 2-10 µL/ mL were made from this. The test mixture contained 3mL of DPPH working solution and

1mL of a sample. The mixture was incubated at room temperature for 30 min in the dark. The absorbance of each sample was recorded at 517nm. For a negative control, 1 mL of methanol and ethanol was added in place of the algal extract, respectively. Ascorbic acid, 10 mg/ 10mL distilled water was prepared and diluted to different concentrations for positive control. Each extract was analyzed in triplicate. % Inhibition curves were made, and IC_{50} values were calculated for all samples. The percentage of inhibition was calculated by using the formula:

$$\% \text{ Inhibition} = \{(A \text{ blank} - A \text{ sample})/ A \text{ blank}\} \text{ X } 100$$

Where, *A* blank is the absorbance of the negative control, and *A* sample is the absorbance of the sample/ standard (Ahmed *et al*., 2013; Brand-Williams *et al*., 1995).

2.5.2. Reducing power assay

The reducing power assay method is based on the principle that substances with reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric–a ferrous complex that has an absorption maximum at 700 nm. The diluted sample of 1 mL was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1 % potassium ferricyanide. The mixtures were incubated at 50 ˚C for 20 min in the water bath, followed by adding 2.5 mL of 10 % trichloroacetic acid. An aliquot of 1 mL of the reaction mixture was added with 1 ml of distilled water and 200 µL of 0.1 % $FeCl_3$ and incubated at room temperature for 10 minutes. The absorbance of the resulting solution was measured at 700 nm. Increased absorbance of the reaction mixture indicates the increased, reducing power. Here ascorbic acid was used as the positive control.(Rafiquzzaman *et al*., 2013).

2.6 ANTIMICROBIAL ACTIVITY

2.6.1 Fungal and bacterial species

The fungal strain *Aspergillus niger* was obtained from the Goa University fungal culture collection lab (GUFCC), Department of Botany, Goa University and was maintained in malt-extract agar (MAE) slant at RT.

The bacterial strains *Escherichia coli* and *Bacillus cereus* were obtained from PES's College of Arts and Sciences, Farmagudi and were maintained in nutrient agar slants at 4˚C for experimental studies.

2.6.2 Preparation of sterile disc

Whatman's No. 1 filter paper was punched into 6mm disc form and then sterilized by autoclaving; each sterile disc was individually soaked in different concentrations (50µg/mL, 200 µg/mL and 500 µg/mL) extracts for 5 minutes and then was allowed to air dry.

2.6.3 Assay of antimicrobial Assay using disc diffusion method

Malt extract Agar media and Mueller Hinton Agar (MHA) media were poured into sterile Petri plates. After solidification, 100 µL of fresh fungal *Aspergillus niger* and bacterial inoculum *Escherichia coli* and *Bacillus cereus* was spread plate onto their respective plates. For the antibacterial assay, the dried discs, soaked in extracts of 50µg/mL, 200 µg/mL and 500 µg/mL, were placed in the centre of each marked region (according to respective concentration) on the plates using sterile forceps.

The well-diffusion method was used for the antifungal assay, and extracts of different concentrations, 50µg/mL, 200 µg/mL and 500 µg/mL, were filled into the wells with the help of a micropipette and sterile tips. The plates were incubated for 48 hours at room temperature.

After incubation, the diameter of the inhibitory zones around the disk and wells was recorded (Mahalngam *et al.,* 2011).

2.6.4 Antimicrobial activity of commercially available antibiotics (Positive and negative control)

The antimicrobial activity of the algal extracts on fungal strain *(Aspergillus niger)* and bacterial (*Escherichia coli* and *Bacillus cereus*) were compared with the commercially available antibiotics. Ketoconazole fungal positive control and Ampicillin bacterial positive control (10mg/mL) were used. Malt extract Agar media and Mueller Hinton Agar (MHA) plates were prepared, and the test organisms were spread plate onto their respective agar plates. The antibiotic disc, Ampicillin, was placed in the centre of the plates using sterile forceps, and Ketoconazole was filled in the wells. The plates were incubated for 48 hours at room temperature, and after incubation, the diameter of the inhibition zones was recorded.

100% methanol and ethanol were used as negative control (Daniyan and Mahammad, 2008).

2.7 Thin layer Chromatography (TLC) and UV-VIS spectra

2.7.1 TLC of Algal pigments

Pre-coated TLC Silica Aluminium Plates (Merck) measuring 20 x 20 cm were used. The solvent system – Petroleum ether: Acetone: Chloroform (3:1:1) was used for this experiment. The methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum* were loaded as concentrated bands, 1.5- 2 cm from the edge of their respective TLC plates and allowed to dry. The plates, with dried samples, were gently lowered into the chromatography chamber, closed and left to develop. The plates were removed from the chromatography chamber when the solvent front had travelled $3/4^{th}$ of the plate's length. The position of the

solvent front was immediately marked with a soft pencil. The different bands' retention factor (Rf) values were then calculated using the equation (Wagner and Bladt, 1996).

2.7.2 UV-VIS spectra

The methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum* were examined under UV and visible light for proximate analysis using UV-VIS Spectrophotometer. The extracts were diluted to 1:9 with the respective solvents and scanned in the wavelength ranging from 190-700 nm. The characteristic peaks were detected.

Chapter 3

RESULTS

3.1. Collection of algal specimens

Seaweed, the natural resource obtained from the coastal areas of Goa, holds the potential to be exploited and utilized by the human population. Algal samples were collected from the coast of Anjuna Goa, India (15˚35’04.0” N 73˚44’13.6” E)from the rocky surfaces during low tide between November and December 2022.). **Fig.1** shows the collection site.

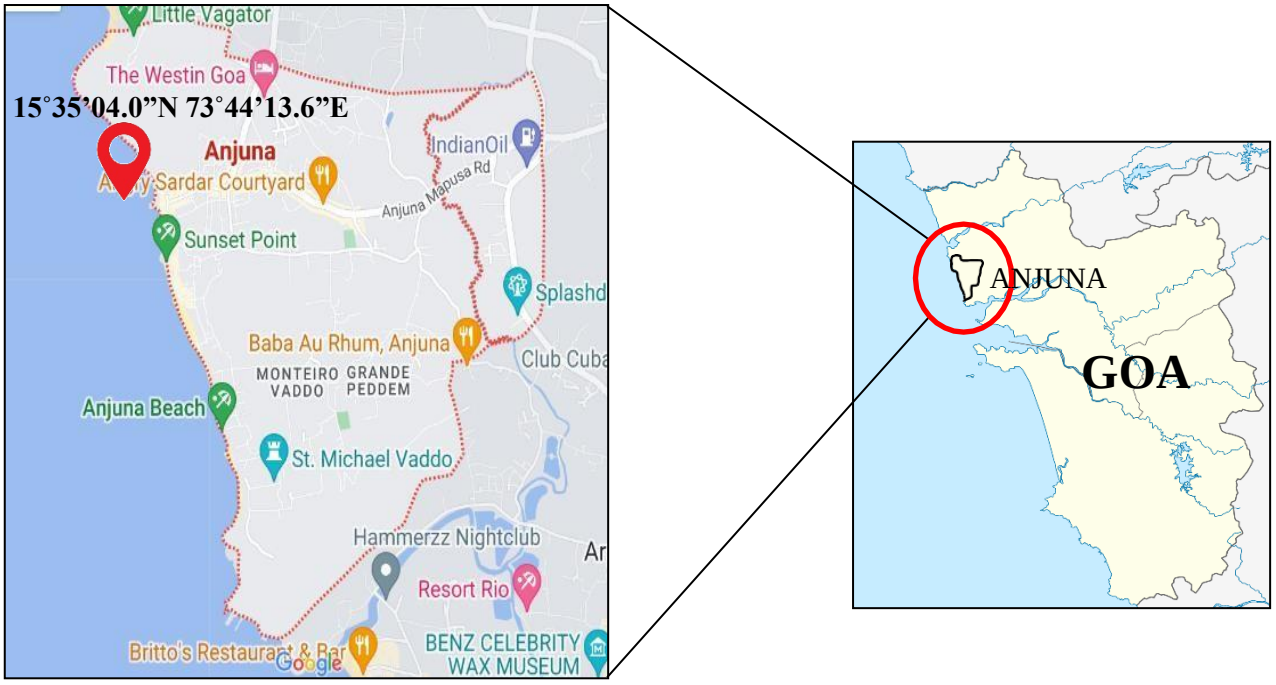


Fig.1. Location map of the sample collection area.

3.2. Identification of collected algal specimens

The Collected samples were identified as *Padina tetrastromatica* Hauck and *Sargassum tenerrimum* J.Agardh. The thallus was washed with clean seawater, and the impurities were removed with tap water, rinsed with sterile distilled water, and photographed (**Fig. 2a and 2b**).

Table1. Taxonomical classification of *Padina tetrastromatica* (Hauck, 1887) and *Sargassum tenerrimum* (J.Agardh, 1894)

Domain	Eukaryota	Eukaryota
Kingdom	Chromista	Chromista
Division	Heterokontophyta	
Class	Phaeophyceae	Phaeophyceae
Order	Dictyotales	Fucales
Family	Dictyotaceae	Sargassaceae
Genus	*Padina*	*Sargassum*
Species	*Terastromatica* **(Hauck, 1887)**	*Tenerrimum* **(J.Agardh, 1848)**

3.2.1. *Padina tetrastromatica* Hauck

Padina tetrastromatica Hauck is a striped, yellowish-brown fan-shaped alga that turns olive to dark green on drying (Mica, 1966). The thalli are irregularly cleft into narrow lobes, having an involute apical margin, and it is attached with the help of a small, compact,holdfast, 0.5 – 1.5 cm broad and 0.7 – 2.0 cm long. It grows in shallow and sand-covered rocky coastal surfaces. It proliferates in tropical waters (**Fig.2a**). These algae are mainly used as seasoning in dried flakes and table salt replacementsfor high blood pressure patients (Novaczek and Athy, 2001). These studies showed that it contains alginic acid, a

major polysaccharide that shows high anticoagulant (Prasada Rao, Sastrey, and Venkata Rao, 1984) and antiviral properties (Chatterji *et al*., 2004).

Fig.2a: Photograph showing the freshly collected ***Padina tetrastromatica,*** **Hauck** specimen.

3.2.2. *Sargassum tenerrimum* J.Agardh

It has soft, slender light brown to greenish brown thallus and is bushy, 30-50 cm high. The centralaxis is very short (5 mm), terete (1 mm broad), bearing above irregularly alternately arrangedprimary cylindrical branches with similar secondary branches and branchlets. The lower phylloid of primary branches are oblong-lanceolate with an elongate base and short stipe, tapering to round apices, 6-7 cm long, 12-15 mm broad. The midrib is delicate, vanishing below the apex, and the margins are irregular. The vesicles are stalked, spherical,

ovate and often pointed. Receptacles 4-6 mm long, 0.5-1.5 mm broad, fusiform or slightly compressed, two- or three-edged, toothed at margins, forked. The holdfast is discoid (**Fig.2b**). Growing on subtidal rocks. This seaweed extract can be used as a gel enhancer (Shitole *et al.*, 2014).

Fig.2b: Photograph showing the freshly collected ***Sargassum tenerrimum,*** **J.Agardh** specimen.

3.3. Preparation of algal extracts

The collected algal specimens of *Padina tetrastromatica* and *Sargassum tenerrimum* were shade-dried and Oven-dried at 40˚C for 1 hour (**Fig.3A and 3B**). Then the dried algae were powdered (**Fig.3C and 3D**) and extracted using two different solvents: ethanol and Methanol (**Fig.3E and 3F**).

3.4. Extractive value

The weight of the extracts was determined using an electronic balance. The extractive value and colour of Ethanolic and Methanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum* were investigated and represented in **Table 2** and **Fig.4**.

The present study found that the extractive value of Methanolic extracts of *Sargassum tenerrimum* was a maximum of 14.53% g/Dw compared to the ethanolic extract, which was very low, viz 3.18% g/Dw. *Padina tetrastomatica* showed a higher yield in the methanolicextract by 7.53% g/Dw compared to the ethanolic extract, which was slightly lower viz. 4.36% g/Dw.

The colour of *Sargassum tenerrimum* extracts observed was Dark green in methanol and ethanol extracts and Dark brown in ethanol and methanol extracts of *Padina tetrastromatica*.

2.7.2.1 B.

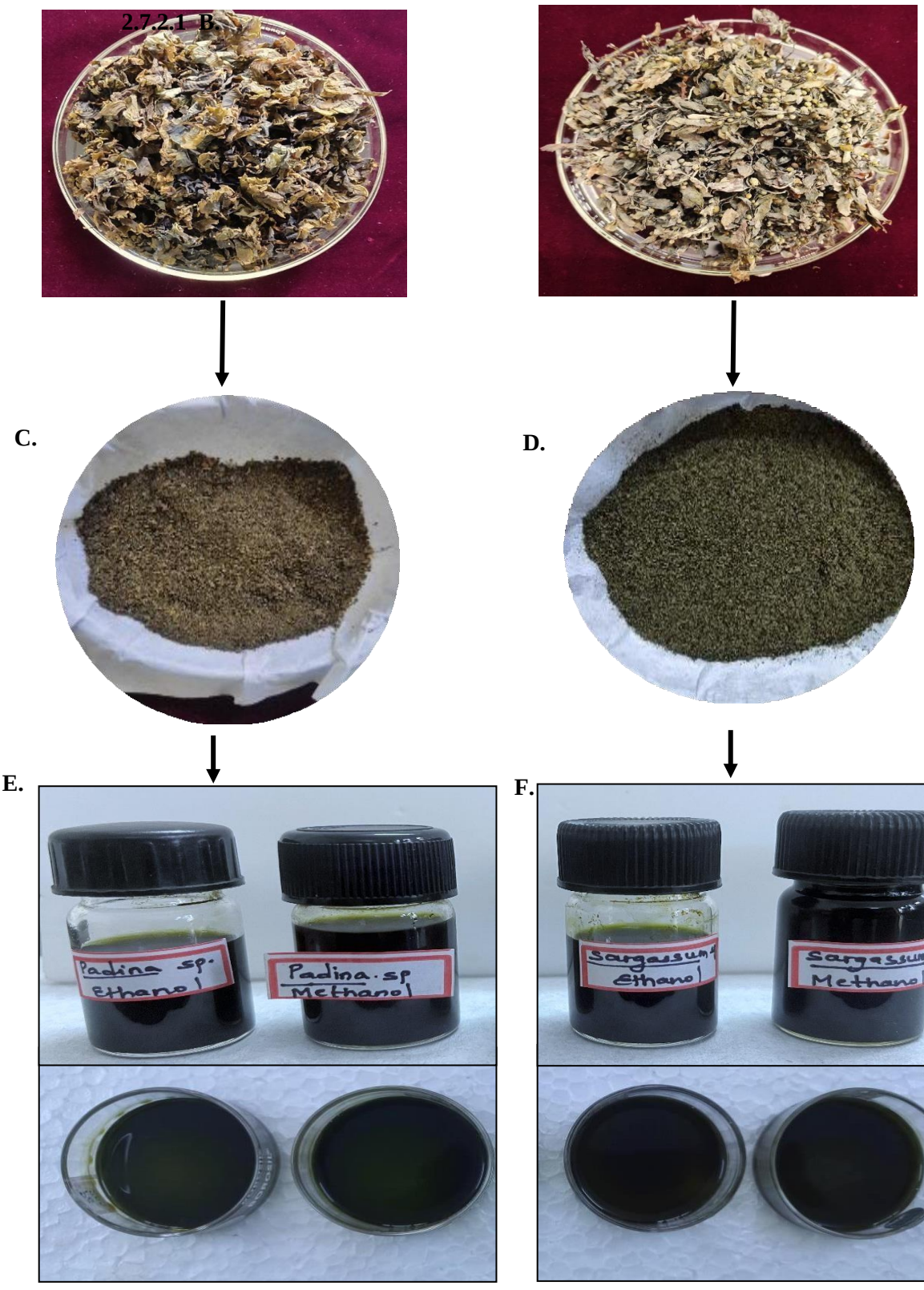


Fig.3: Preparation of algal extract**: 3A-** Dried *Padina tetrastromatica* **3B-** dried *Sargassum terrenimum* **C-** Powdered *Padina tetrastromatica* **D-** powdered *Sargassum terrenimum* **E-** ethanolic and methanolic extracts of *Padina tetrastromatica* **F-** ethanolic and methanolic extracts of *Sargassum terrenimum.*

Table2: Extractive value (yield %) of ethanolic and methanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum.* Data represent mean values ± Standard Deviation (n=3).

Algal sample	Weight of powdered algae (g)	Solvents	Colour of extracts	Extractive value % (g/Dw)
Padina tetrastromatica	8	Ethanol	Dark brown	4.36±0.38
	8	Methanol	Dark brown	7.53±0.17
Sargassum tenerrimum	8	Ethanol	Dark green	3.18±0.27
	8	Methanol	Dark green	14.53±0.68

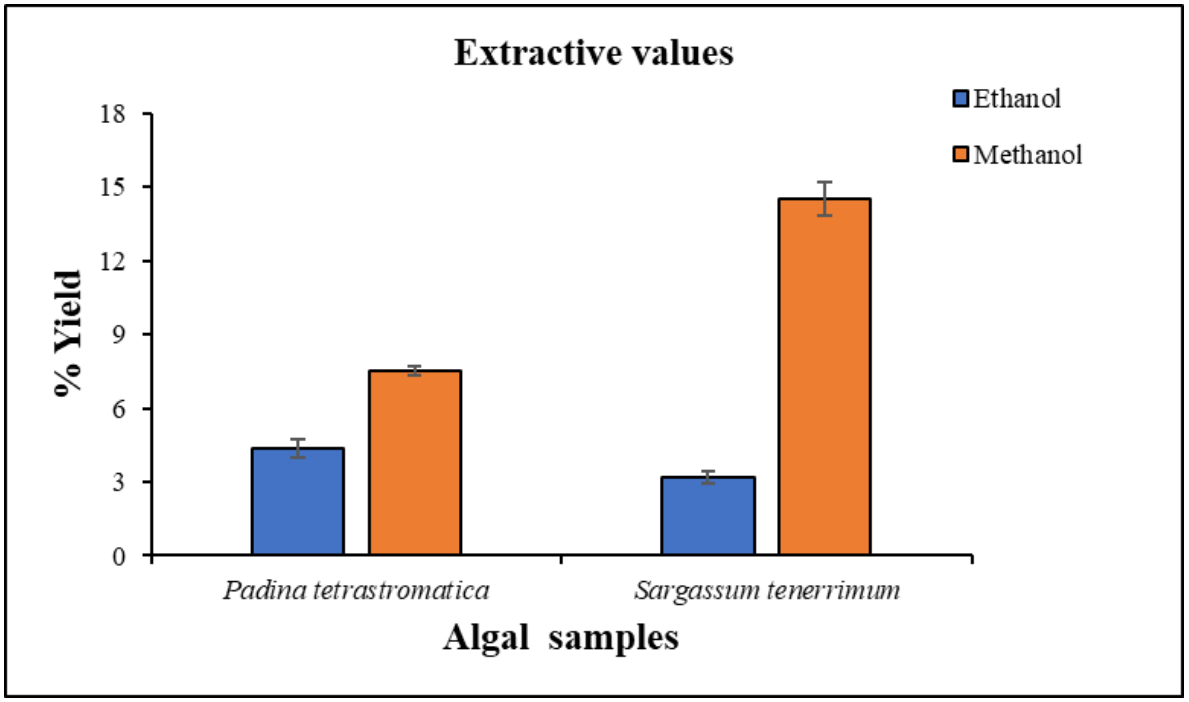


Fig. 4: Effect of solvents ethanol and methanol on the extractive values of *Padina tetrastromatica* and *Sargassum tenerrimum.*.

3.5. Quantitative estimation of Total Phenolic Content (TPC)

Total phenolic content in different concentrations of methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum* were measured using Folin–Ciocalteu (F-C) reagent and external calibration with Gallic acid as standard are represented in **Table 3, Fig.5** and **Fig.6.** At higher concentration of methanolic extracts of 250 µg/mL of *Padina tetrastromatica* showed higher total phenolic content of 36.94% compared to the methanolic extract of *Sargassum tenerrimum of* 24.79% at the same concentration. Similarly, the same trend was observed at a lower 50 µg/mL concentration. *Padina tetrastromatica* showed a higher TPC of 26.56% compared to the *Sargassum tenerrimum* showing a TPC of 16.11%.

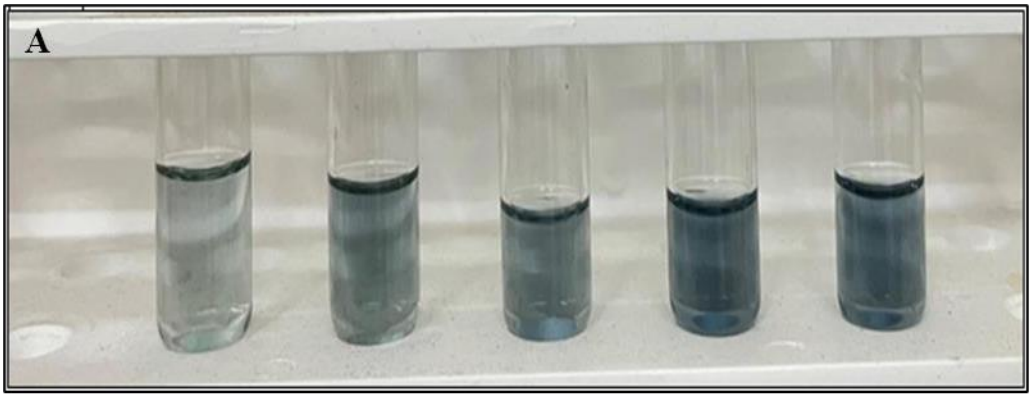
On the other hand, the ethanolic extracts at a higher concentration of 250 µg/mL of *Padina tetrastromatica*also showed a TPC of 37.75% compared to *Sargassum tenerrimum* showing 21.45% of TPC at the same concentration. However, at a lowerconcentration of 50 µg/mL, Padina tetrastromatica showed a lower TPC of 8.78%as compared to the Sargassum tenerrimum, which showed a TPC of 14.33%.

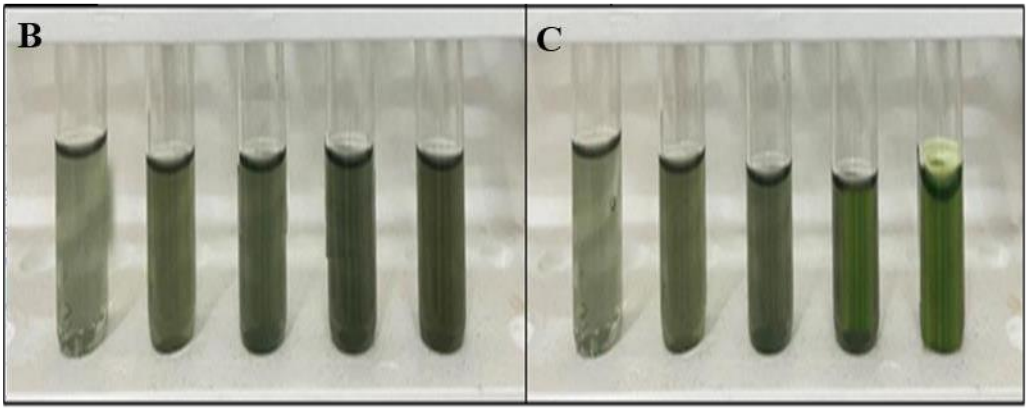
Overall, *Padina tetrastromatica* showed a higher total phenolic content (TPC) than *Sargassum tenerrimum.*

Table3: The amount of Total Phenolic Content (TPC) in methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum.*

Solvents	Concentration (µg/mL)	Total Phenolic Content (mg of GAE/g of extract)	
		Padina tetrastromatica	*Sargassum tenerrimum*
Methanol	50	26.56±0.90	16.11±1.30
	100	31.06±0.90	21.05±0.45
	150	32.56±0.76	21.19±0.17
	200	34.14±0.22	23.3±0.81
	250	36.94±0.36	24.79±0.68
Ethanol	50	8.78±0.9	14.33±0.90
	100	14.95±0.45	18.27±0.55
	150	23.79±0.3	18.35±0.17
	200	37.47±0.22	19.13±0.22
	250	37.75±0.10	21.45±0.27

GAE: Gallic Acid Equivalent. Data represent mean values ± Standard Deviation (n=3).





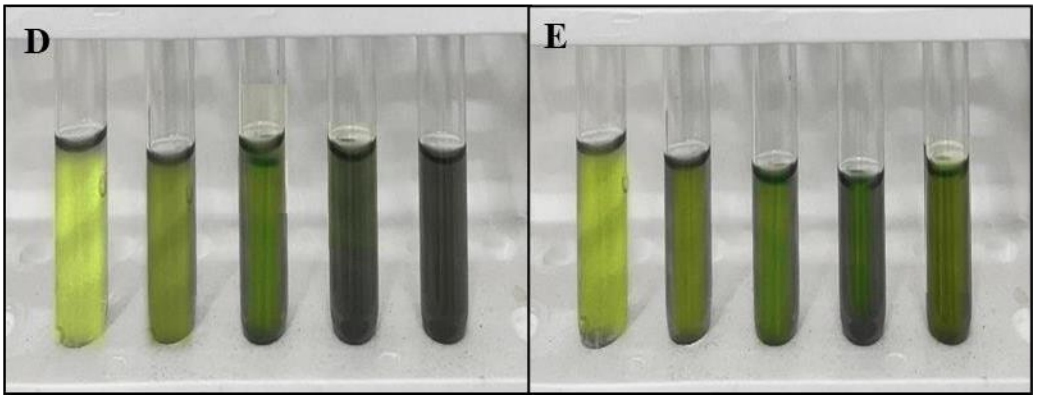


Fig.5: Determination of Total Phenolic Content (TPC) of : **A**- Gallic acid standard; **B**- Methanolic extracts of *Padina tetrastromatica;* **C**- Ethanolic extracts of *Padina tetrastromatica;* **D**- Methanolic extracts of *Sargassum tenerrimum*; **E**- Ethanolic extracts of *Sargassum tenerrimum*

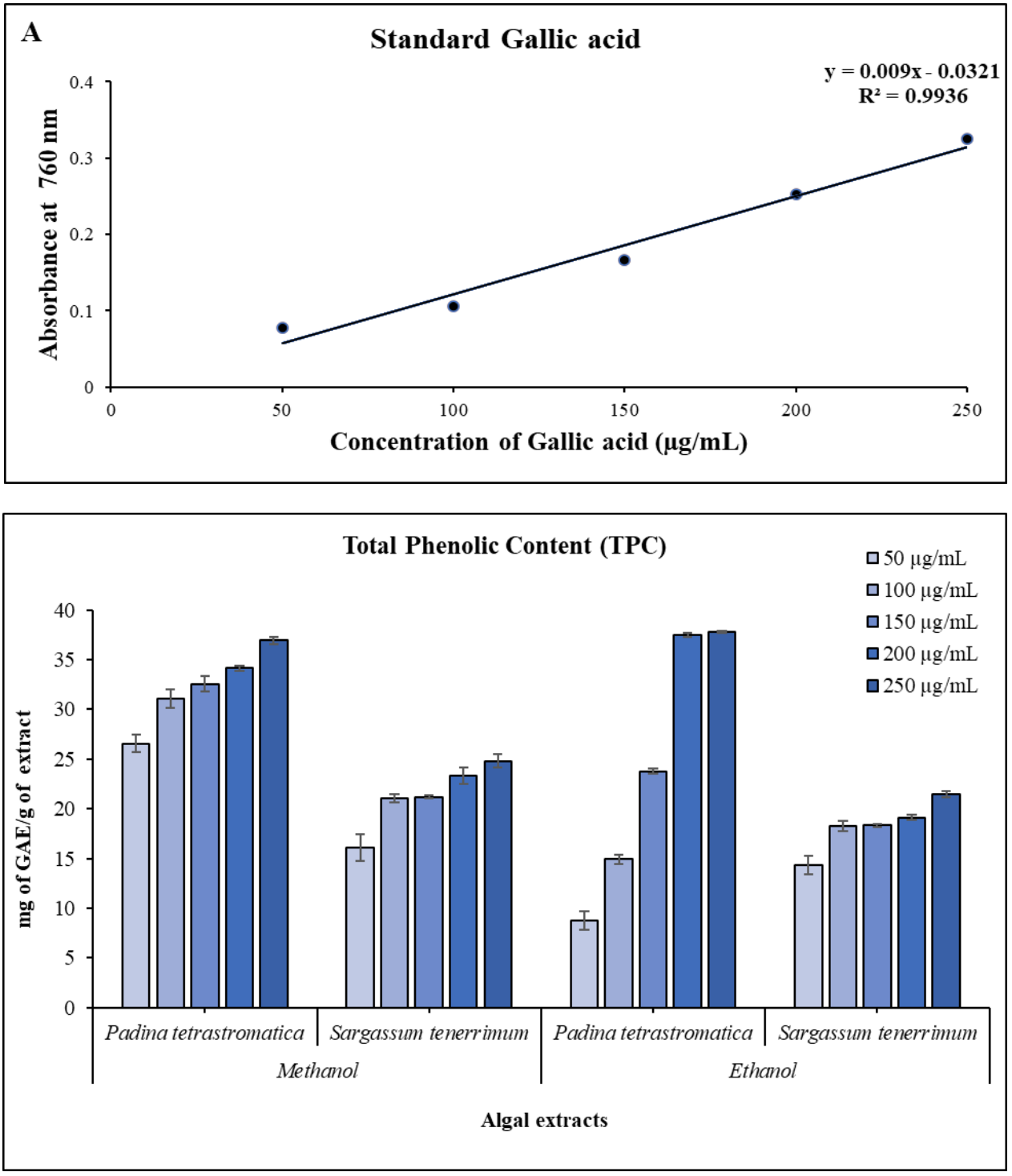


Fig.6: Determination of Total Phenolic Content (TPC) of *Padina tetrastromatica and Sargassum tenerrimum.* **A-** Calibration Curve for Gallic acid; **B-** Total Phenolic Content (mg of Gallic acid equivalent (GAE)/g of extract) at varying concentrations. Bars represent mean values ± SD (n=3).

3.6. Quantitative determination of Total Flavanoid Content (TFC)

Total Flavonoid Content (TFC) in different concentrations of methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum* were measured using the aluminium chloride colourimetric method and external calibration with quercetin as standard are represented in **Table 4, Fig.7** and **Fig. 8**.

The higher concentration of methanolic extracts at 250 µg/mL of *Padina tetrastromatica* showed a higher TFC of 12.43% compared to *Sargassum tenerrimum* of 9.29% at the same concentration. Similarly, at a lower concentration of 50 µg/mL, it was observed that *Padina tetrastromatica* showed a higher TFC of 9.33% as compared to the *Sargassum tenerrimum* showing 3.14% of TFC.

On the other hand, the higher concentration of 250 µg/mL of ethanolic extracts of *Padina tetrastromatica* also showed a higher TFC of 11.2% compared to *Sargassum tenerrimum* showing 7.58 % of TFC at 250 µg/mL. Similarly, at lowerconcentrations of 50 µg/mL, *Padina tetrastromatica* showed a much higher TFC of 6.47 % compared to the *Sargassum tenerrimum* 0.76%.

Overall, *Padina tetrastromatica* showed a higher TFC as comparedto *Sargassum tenerrimum.*

Table4: The amount of Total Flavonoid Content (TFC) in methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum.*

Solvents	Concentration (µg/mL)	Total Flavonoid Content (mg of QE/g of extract)	
		Padina tetrastromatica	*Sargassum tenerrimum*
Methanol	50	9.33±0.67	3.14±1.16
	100	9.42±0.58	6.57±0.58
	150	10.09±0.38	6.76±0.77
	200	11.02±0.33	8.52±0.60
	250	12.43±0.71	9.29±0.58
Ethanol	50	6.47±0.67	0.76±0.67
	100	7.28±1.16	4.19±0.33
	150	8.34±0.89	5.33±0.38
	200	9.23±.0.73	6.5±0.58
	250	11.2±0.84	7.58±0.71

QE: Quercetin equivalent. Data represent mean values ± Standard Deviation (n=3).

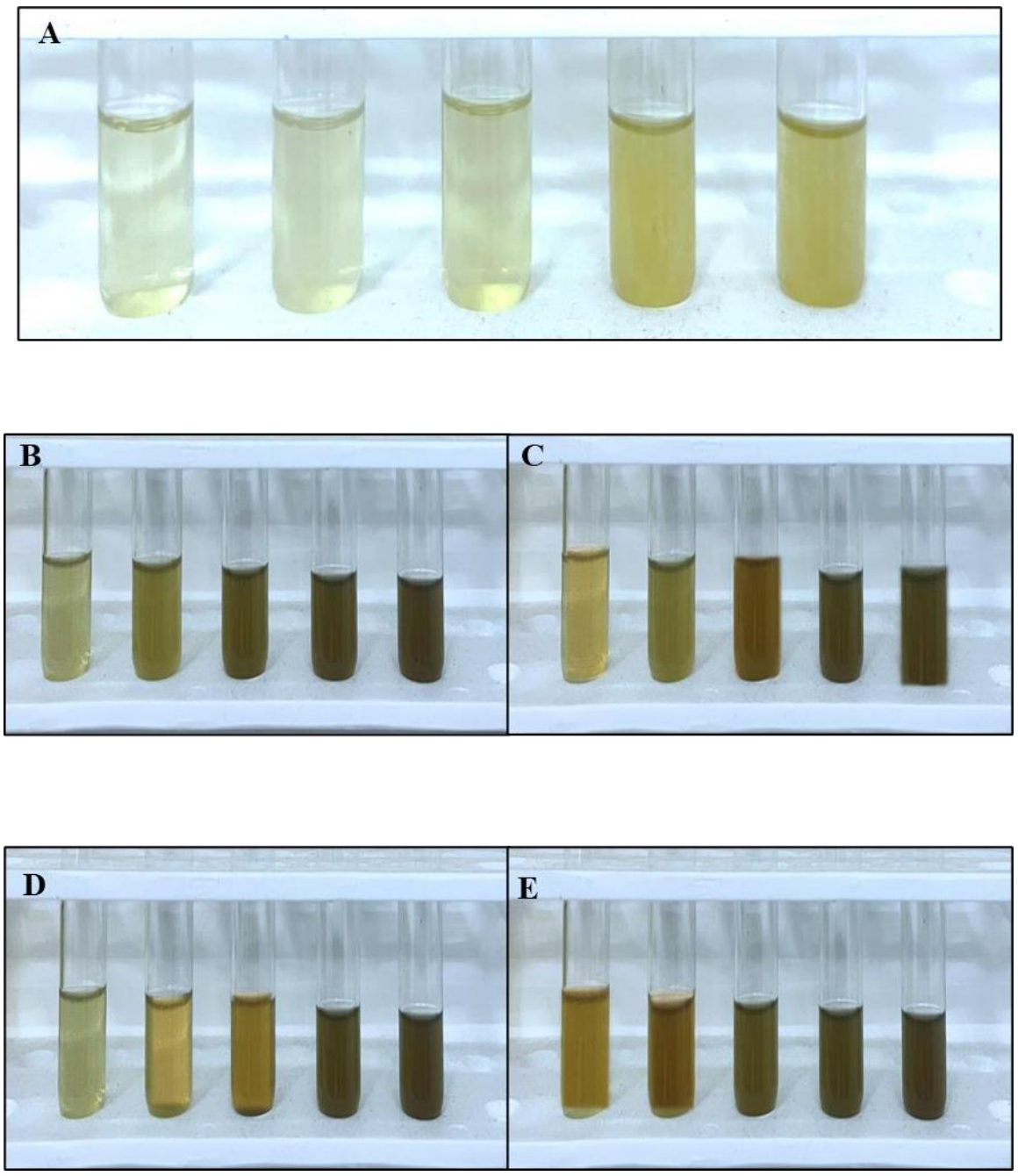


Fig.7: Determination of Total Flavanoid Content (TFC) of: **A**- Quercetin standard; **B**-Methanolic extracts of *Padina tetrastromatica;* **C**- Ethanolic extracts of *Padina tetrastromatica;* **D**-Methanolic extracts of *Sargassum tenerrimum*; **E**- Ethanolic extracts of *Sargassum tenerrimum.*

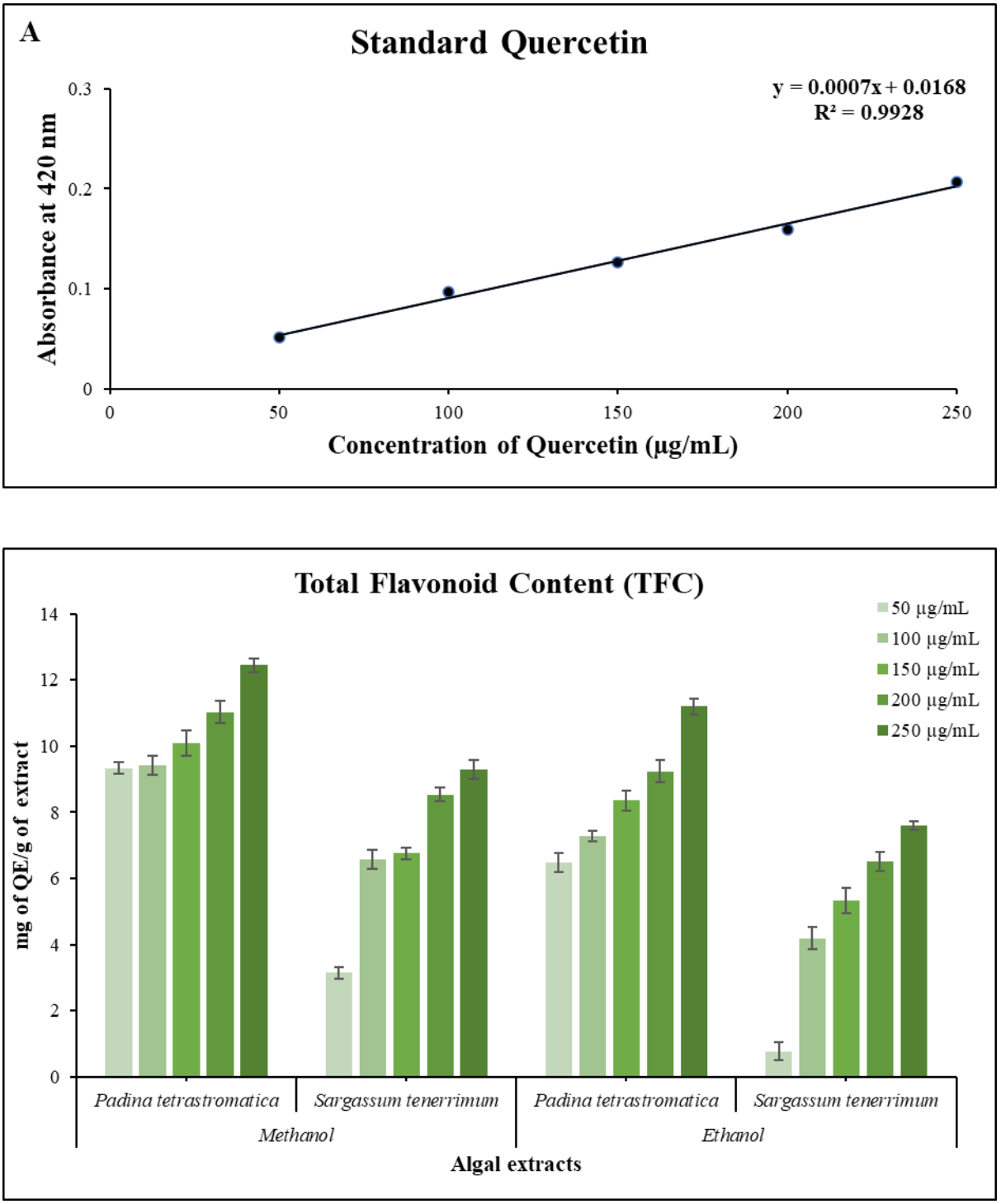


Fig.8: Determination of Total Flavanoid Content (TFC) of *Padina tetrastromatica and Sargassum tenerrimum.* **A**- Calibration Curve for Quercetin; **B**- Total Flavonoid Content (mg of Quercetin equivalent (QE)/g of extract) at varying concentrations. Bars represent mean values ± SD (n=3).

3.7. Antioxidant activity

In the present study, methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum* were tested for two assays; DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity and reducing power assay were carried out in varying concentrations viz. 2 μg/mL, 4μg/mL, 6 μg/mL, 8 μg/mL and 10 μg/mL.

3.7.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

In this process, nitrogen-free radical in the DPPH is readily scavenged by the antioxidant compounds like phenolic acids, polyphenols and flavonoids, thus inhibiting the oxidative mechanisms. The antioxidants clear the purple colour of the DPPH solution. DPPH scavenging activity of methanolic and ethanolic extract of *Padina tetrastromatica* and *Sargassum tenerrimum* are shown in **Tables 5, 6 and Fig.9, 10.**

The per cent (%) scavenging activity of methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum* are compared with the standard L-Ascorbic acid. The findings show that the % scavenging activity of altered crude seaweed extracts increases as the concentration of extracts increases.

Table 5 and **Fig. 9** show a high per cent (%) scavenging activity in the methanolic extracts of *Padina tetrastromatica* compared to the methanolic extracts of *Sargassum tenerrimum.* Similarly, the ethanolic extracts of *Padina tetrastromatica* showed higher % scavenging activity than the ethanolic extracts of *Sargassum tenerrimum.* (**Table 6 and Fig.10**). Methanolic extracts of both *Padina tetrastromatica* and *Sargassum tenerrimum* showed higher per cent (%) scavenging activity than their ethanolic extracts.

Lower the IC_{50} value, higher the antioxidant activity. It was observed that the IC_{50} value of standard ascorbic acid was 5.77 μg/mL, and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum* were 6.99 and 11.96 μg/mL, respectively. In contrast, the IC_{50} value

of methanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum* were 7.36 and 7.54 µg/mL, respectively. Overall, the ethanolic extracts of *Padina tetrastromatica* show the highest antioxidant activity compared to its methanolic extracts, compared both the extracts of *Sargassum tenerrimum* but slightly lower than standardL-Ascorbic acid (**Table 7**).

Table5: Antioxidant activity: DPPH free radical scavenging by ascorbic acid and methanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum.* Data represent mean values ± Standard Deviation (n=3).

Sr. No.	Concentration (µg/mL)	L-Ascorbic acid	Methanolic extracts	
			Padina tetrastromatica	*Sargassum tenerrimum*
1	2	27.97±0.15	15.98±0.48	11.22±1.66
2	4	36.34± 0.11	20.06±0.96	20.40±0.83
3	6	45.37± 0.18	30.61±0.83	30.27±0.96
4	8	60.07± 0.07	47.27±0.48	43.19±1.27
5	10	88.61± 0.07	80.61±0.83	81.63±1.44

Table6: Antioxidant activity: DPPH free radical scavenging by ascorbic acid and methanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum.* Data represent mean values ± Standard Deviation (n=3).

Sr. No.	Concentration (µg/mL)	L-Ascorbic acid	Ethanolic extracts	
			Padina tetrastromatica	*Sargassum tenerrimum*
1	2	27.97±0.15	12.43±0.70	5.97±1.21
2	4	36.34± 0.11	24.37±0.70	18.40±0.70
3	6	45.37± 0.18	41.79±1.21	33.33±0.70
4	8	60.07± 0.07	58.70±0.70	43.78±1.40
5	10	88.61± 0.07	73.63±1.40	60.19±0.70

Table7: Antioxidant activity: IC50 values of methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum* with L-Ascorbic acid as standard

Sr. No.	Samples/Standard	IC50 Values (µg/mL)	
		Ethanolic extract	Methanolic extract
1	*Padina tetrastromatica*	6.99	7.36
2	*Sargassum tenerrimum*	11.96	7.54
3	L-Ascorbic acid (Standard)	5.77	

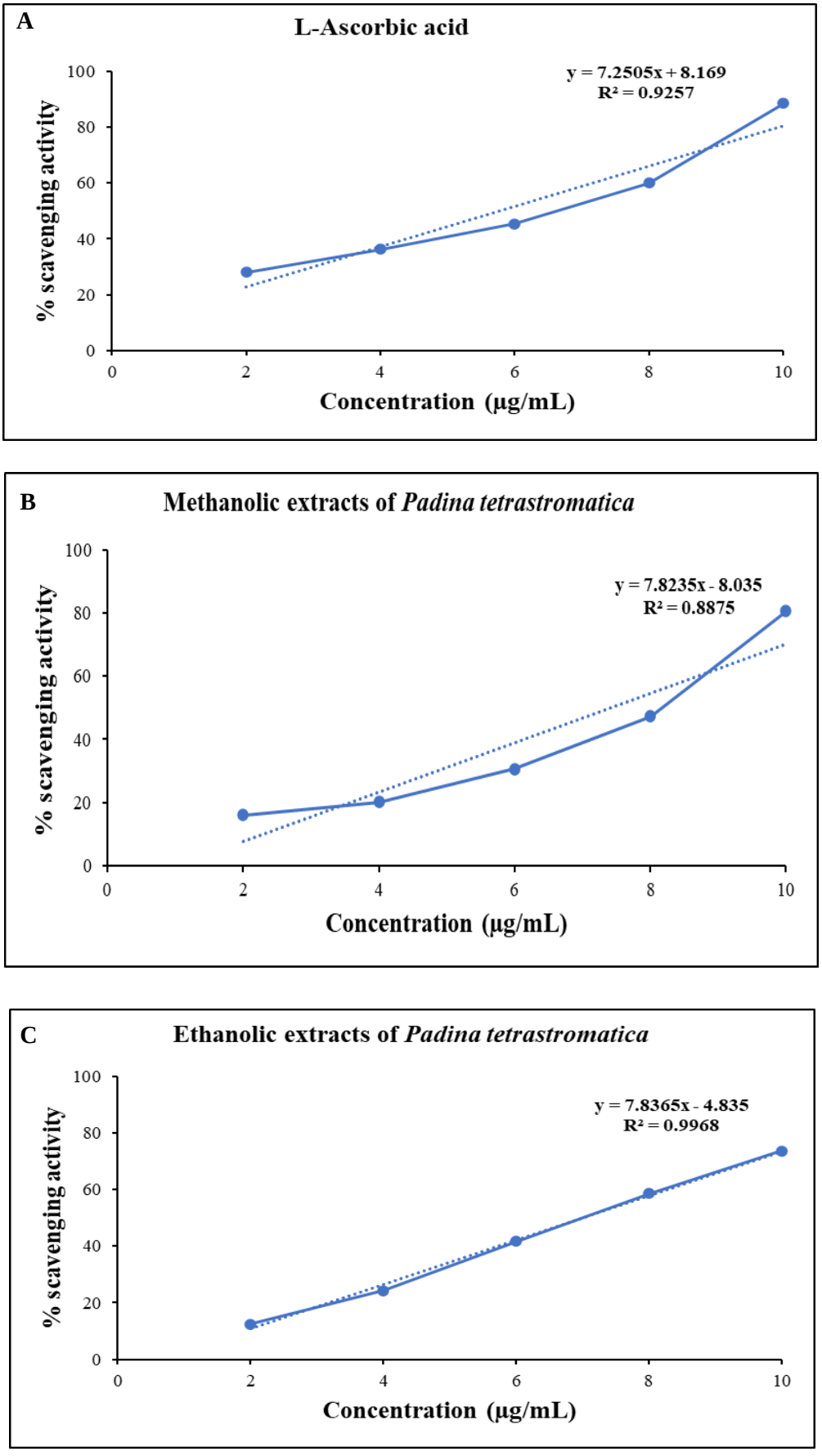
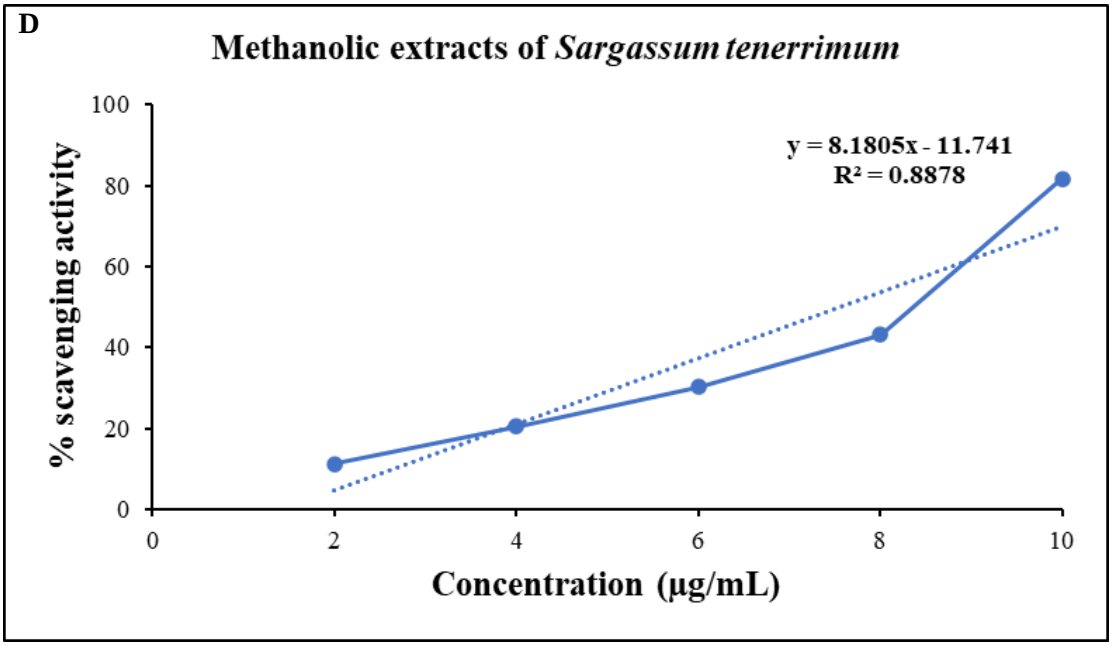


Fig.9: Antioxidant activity- Linear regression graphs of A- L-Ascorbic acid; *Padina tetrastromatica*: B- Methanolic Extract; C- Ethanolic extracts.



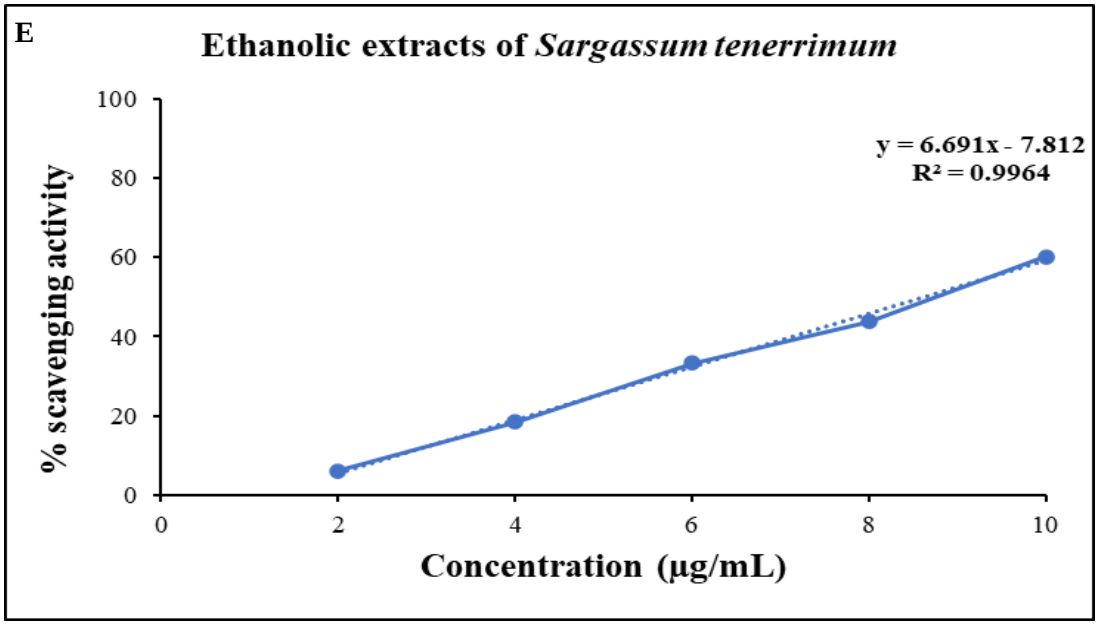
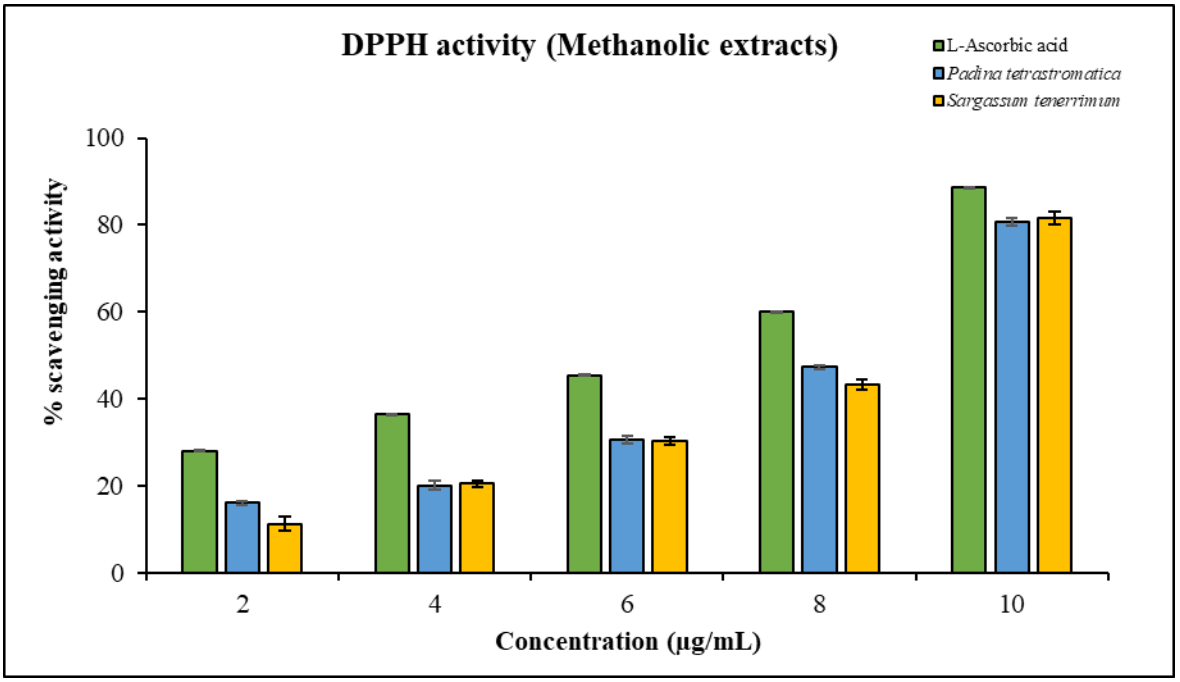


Fig.9: Antioxidant activity- Linear regression graphs of *Sargassum tenerrimum* : D- Methanolic Extract ; E- Ethanolic extracts



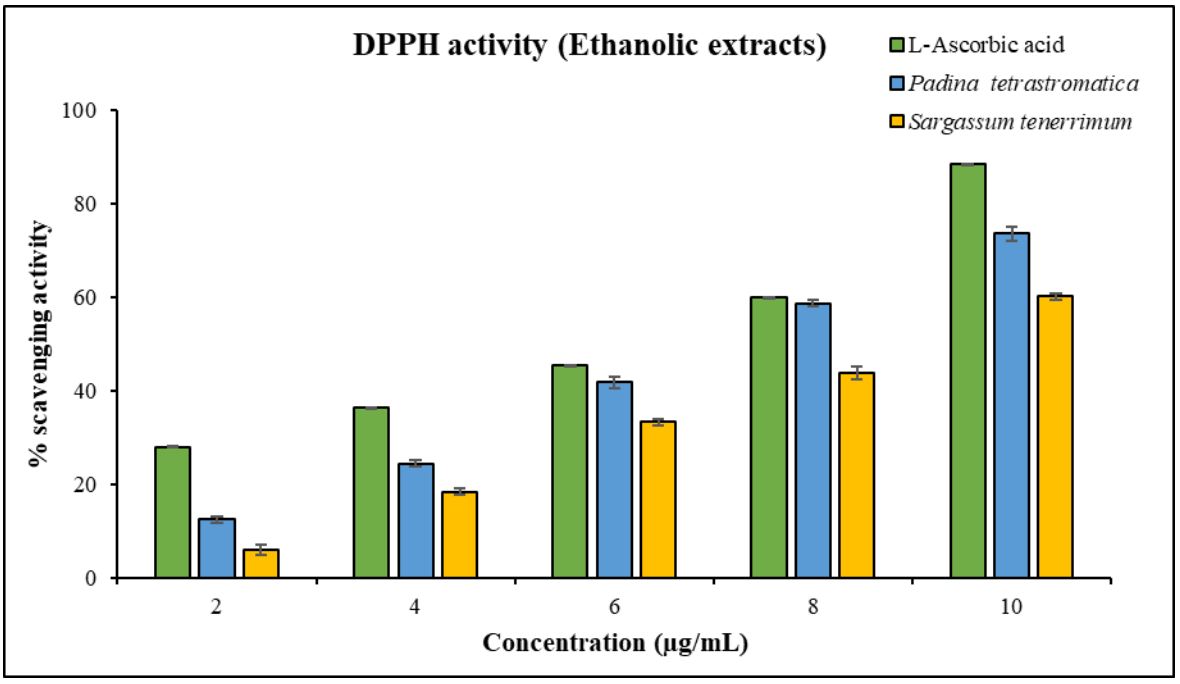


Fig.9: Antioxidant activity- DPPH free radical scavenging activity of *Padina tetrastromatica and Sargassum tenerrimum.* A- Methanolic Extracts; B- Ethanolic extracts. Bars represent mean values ± SD (n=3).

3.7.2. Reducing power assay

The reducing power assay evaluated the antioxidant activity of altered crude extracts of *Padina tetrastromatica* and *Sargassum tenerrimum*. This assay depends on the hydrogen ion in antioxidants reducing ferric (Fe^{3+}) to ferrous (Fe^{2+}) products, changing the substance's colour to shades of green to blue depending on the antioxidant function. (**Fig.11**).

Here methanolic extracts of *Padina tetrastromatica* showed significantly higher absorbance (A_{700nm} 0.360-0.496) than its ethanolic extracts (A_{700nm} 0.30-0.405). Similarly, the absorbance of methanolic extracts of *Sargassum tenerrimum* (A_{700nm} 0.252- 0.356) is comparatively higher than its ethanolic extracts A_{700nm} 0.213-0.306).

Overall, the methanolic extracts of *Padina tetrastromatica* showed the highest absorbance compared to its ethanolic extracts and methanolic and ethanolic extracts of *Sargassum tenerrimum.*

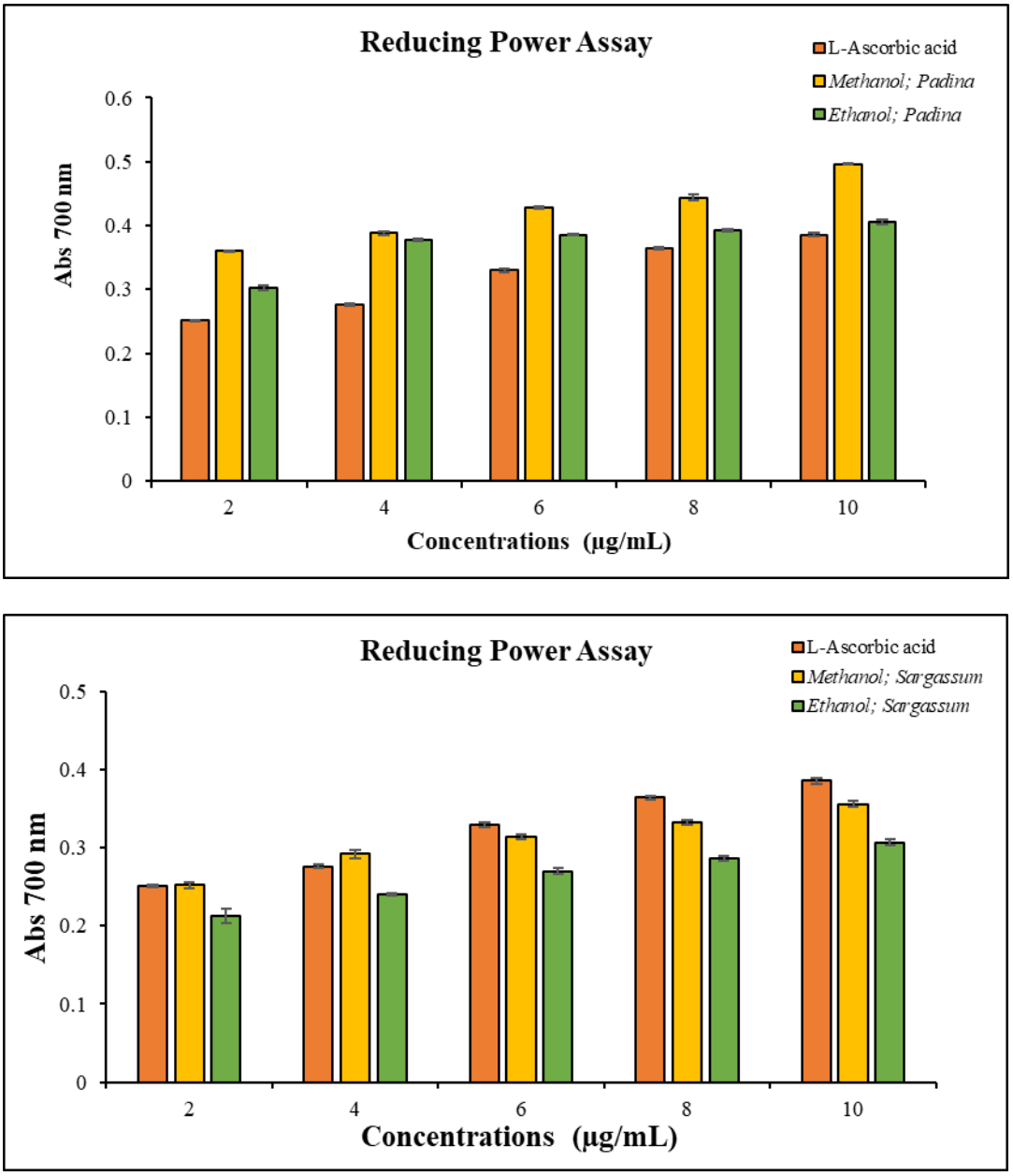


Fig. 10: Antioxidant activity- Reducing power assay. **A-** Methanolic and Ethanolic Extract of *Padina tetrastromatica;* **B-** Methanolic and Ethanolic Extract of *Sargassum tenerrimum.*; Bars represent mean values ± SD (n=3).

3.8. Antimicrobial activity

3.8.1. Antibacterial assay

The antibacterial activity of methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum* on the gram-negative bacteria *Escherichia coli* and gram-positive bacteria *Bacillus cereus* are shown in **Table 8** and **Fig.11**.

The Methanolic and ethanolic extracts of *Padina tetrastromatica* showed better results in the gram-positive bacteria *Bacillus cereus* viz. 6.33-11.33 mm and 6-10.82mm, than in the gram-negative bacteria *Escherichia coli* showing 6.67-10.33 mm and 7-9mm respectively.

The methanolic and ethanolic extracts of *Sargassum tenerrimum* showed more inhibitionof growth on *Escherichia coli* viz. 9.67-13.67 mm and 8-12.33 mm, respectively, than on the bacteria *Bacillus cereus* showing 6-8.33 mm and 7-11.33 mm, respectively.

Overall, methanolic and ethanolic extracts of *Sargassum tenerrimum* showed more inhibition of growth of the bacteria on both *Escherichia coli* and *Bacillus cereus* than the methanolic and ethanolic extracts of *Padina tetrastromatica.*

Table8: Antibacterial assay- Inhibition zone of *Escherichia coli* and *Bacillus cereus* in the presence of different concentrations of methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum.* Data represent mean values ± Standard Deviation (n=3).

Algal Sample	Extraction Solvents	Zone of inhibition for Bacterial strains					
		Escherichia coli			*Bacillus cereus*		
		50 µg/mL	200 µg/mL	500 µg/mL	50 µg/mL	200 µg/mL	500 µg/mL
Padina tetrastromatica	Methanol	6.67±0.47	8.33±0.94	10.33±0.47	6.33±0.47	7.67±0.94	11.33±0.47
	Ethanol	7±0.00	7.67±0.47	9±0.00	6±0.00	8±0.82	10±0.82
Sargassum tenerrimum	Methanol	9.67±0.47	11.33±0.47	13.67±0.94	6±0.00	6.67±0.47	8.33±0.47
	Ethanol	8±0.00	10.33±0.47	12.33±0.47	7±0.00	8.33±0.47	11.33±0.47
Positive control (Ampicillin)		17±0.81			17.67±0.94		
Negative control (Ethanol)		No activity			No activity		
Negative control (Methanol)							

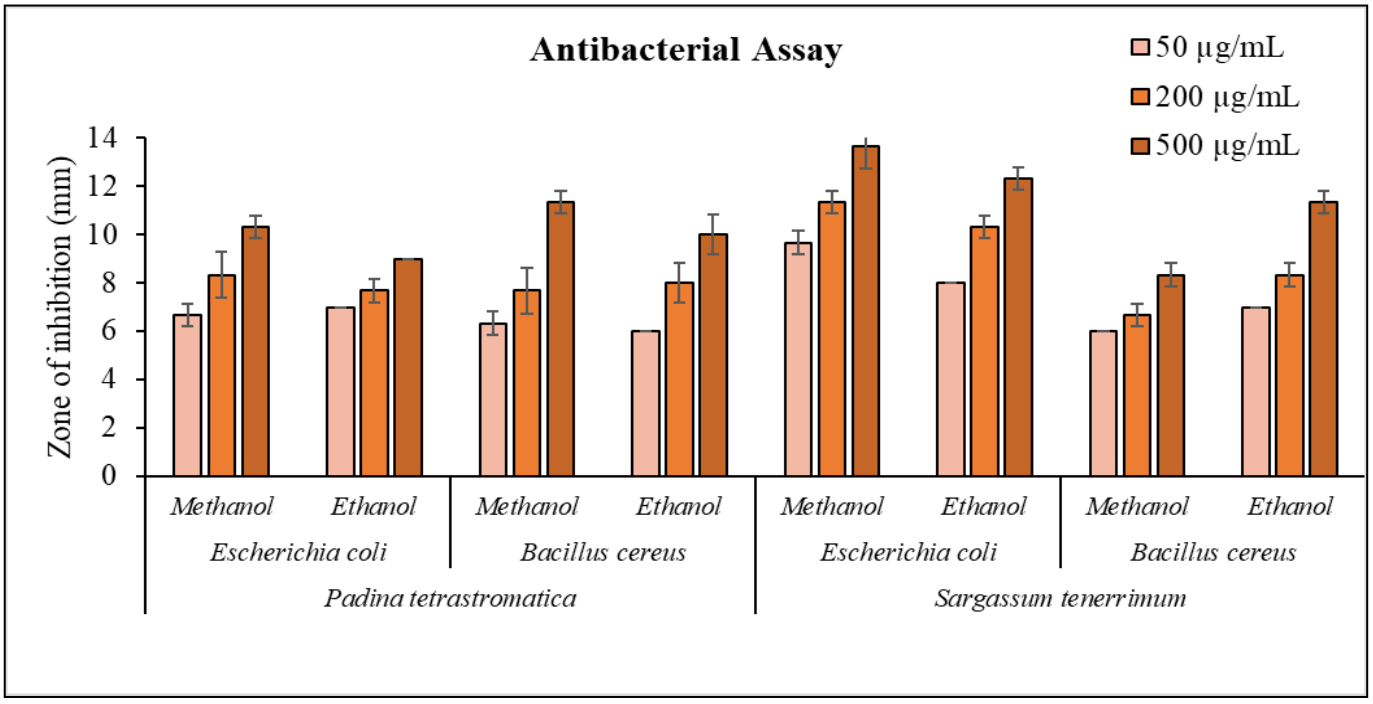


Fig.11: Antibacterial assay- Inhibition zone of *Escherichia coli* and *Bacillus cereus* in the presence of different concentrations of methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum.* Bars represent mean values ± SD (n=3).

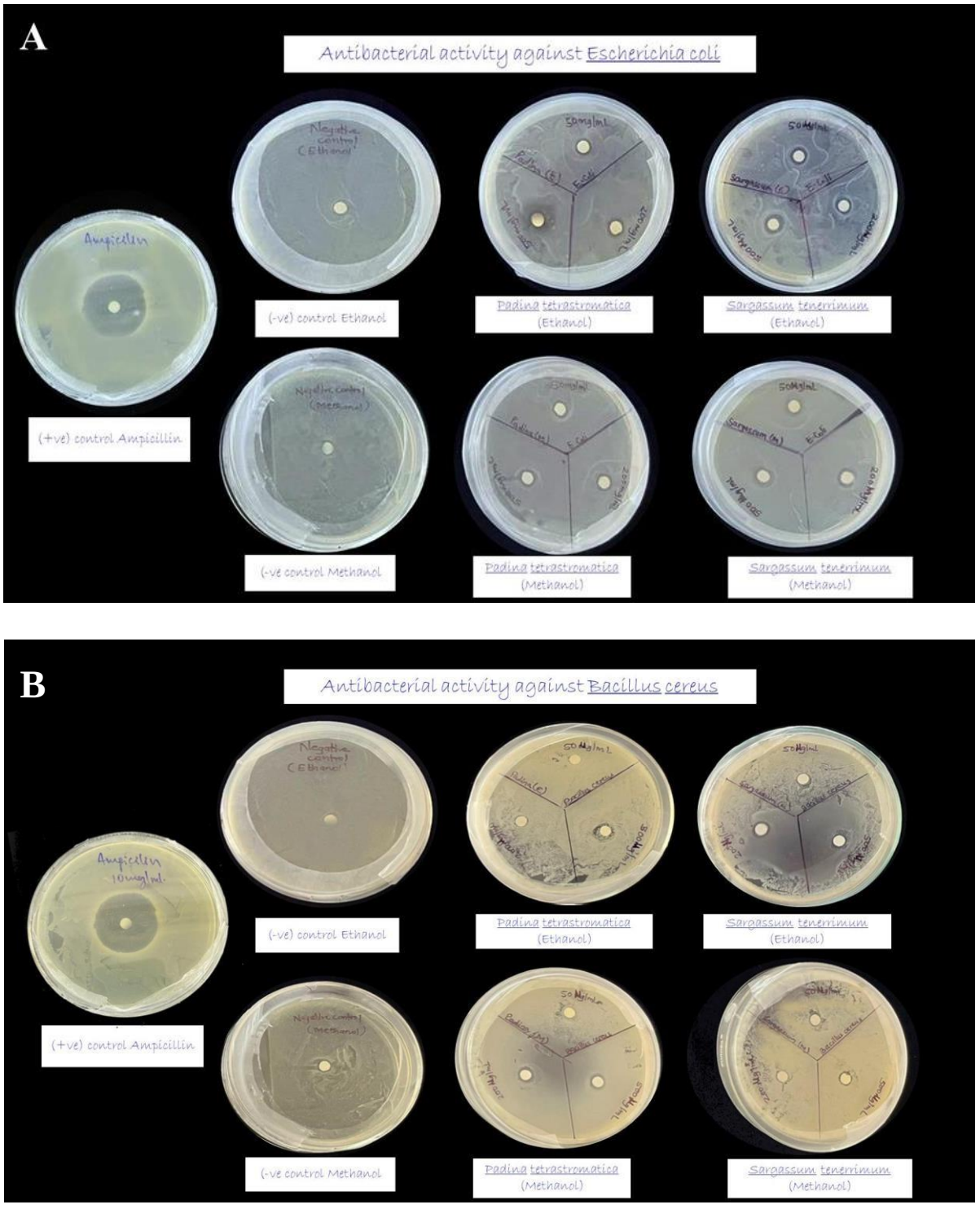


Fig.11: Antibacterial activity of methanolic and ethanolic extracts of *Padina tetrastromatica*, *Sargassum tenerrimum* against bacterial- A- *Escherichia coli*; B-. *Bacillus cereus*

3.8.2. Antifungal assay

The antifungal activity of methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum* on the fungal strain *Aspergillus niger* are shown in **Table 9** and **Fig.12**.

The methanolic extracts of *Padina tetrastromatica* showed a more significant inhibition zone of 10.67- 17.3mm than its ethanolic extracts of 6.33-12.33 mm. The ethanolic extracts of *Sargassum tenerrimum* showed less inhibition, 8.33-16mm, than its methanolic extracts, 10.33-22.33mm.

Overall, the methanolic extracts of *Sargassum tenerrimum* showed the highest result compared to its ethanolic extract and ethanolic and methanolic extracts of *Padina tetrastromatica.*

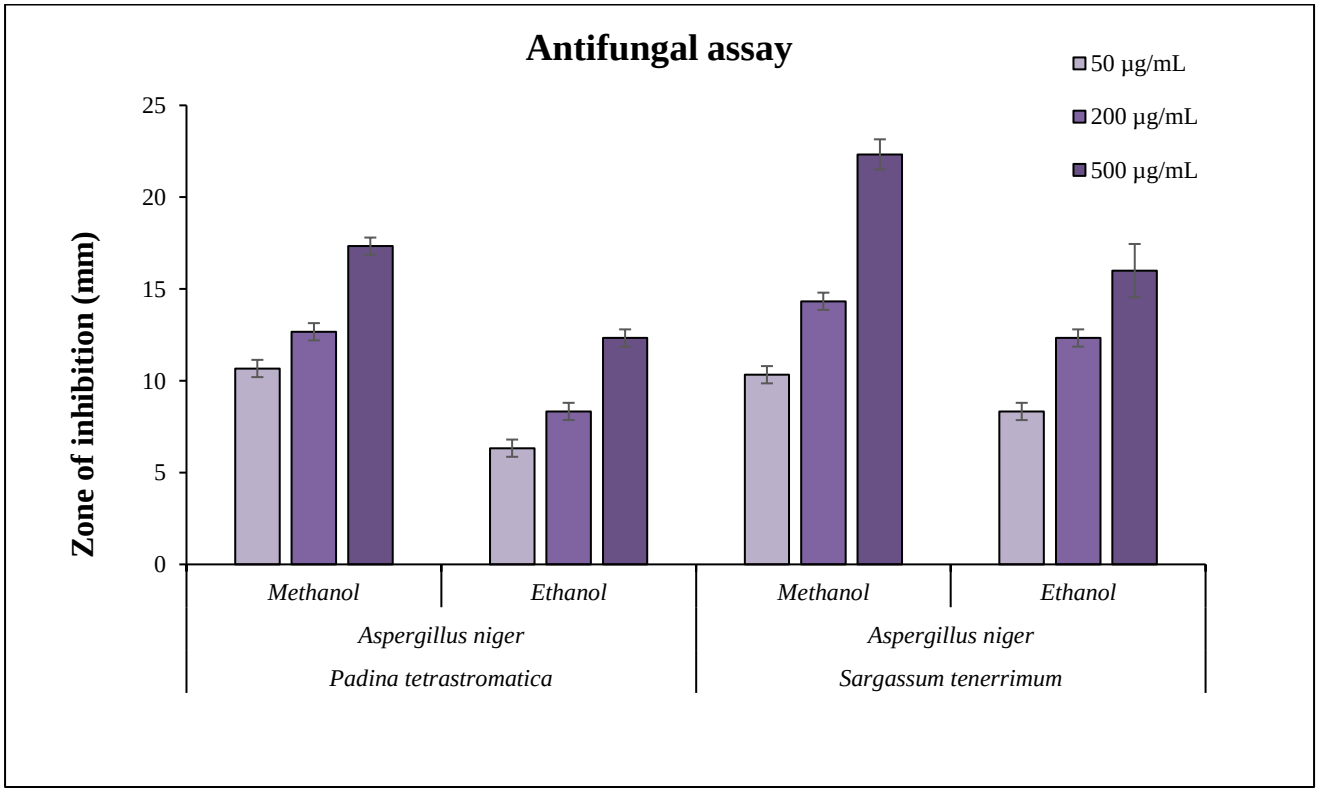


Fig.12: Antifungal assay- Inhibition zone of *Aspergillus niger* in the presence of different concentrations of methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum*. Bars represent mean values ± SD (n=3).

Table9 : Antifungal assay- Inhibition zone of *Aspergillus niger* in the presence of different concentrations of methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum.* Data represent mean values ± Standard Deviation (n=3).

Algal Sample	Extraction Solvents	Zone of inhibition		
		Aspergillus niger		
		50 µg/mL	200 µg/mL	500 µg/mL
Padina tetrastromatica	Ethanol	6.33±0.47	8.33±0.47	12.33±0.47
	Methanol	10.67±0.47	12.67±0.47	17.33±0.47
Sargassum tenerrimum	Ethanol	8.33±0.47	12.33±0.47	16±0.82
	Methanol	10.33±0.47	14.33±0.47	22.33±1.45
Positive control (Ampicillin)		27.67±0.94		
Negative control (Ethanol)		No activity		
Negative control (Methanol)		No activity		

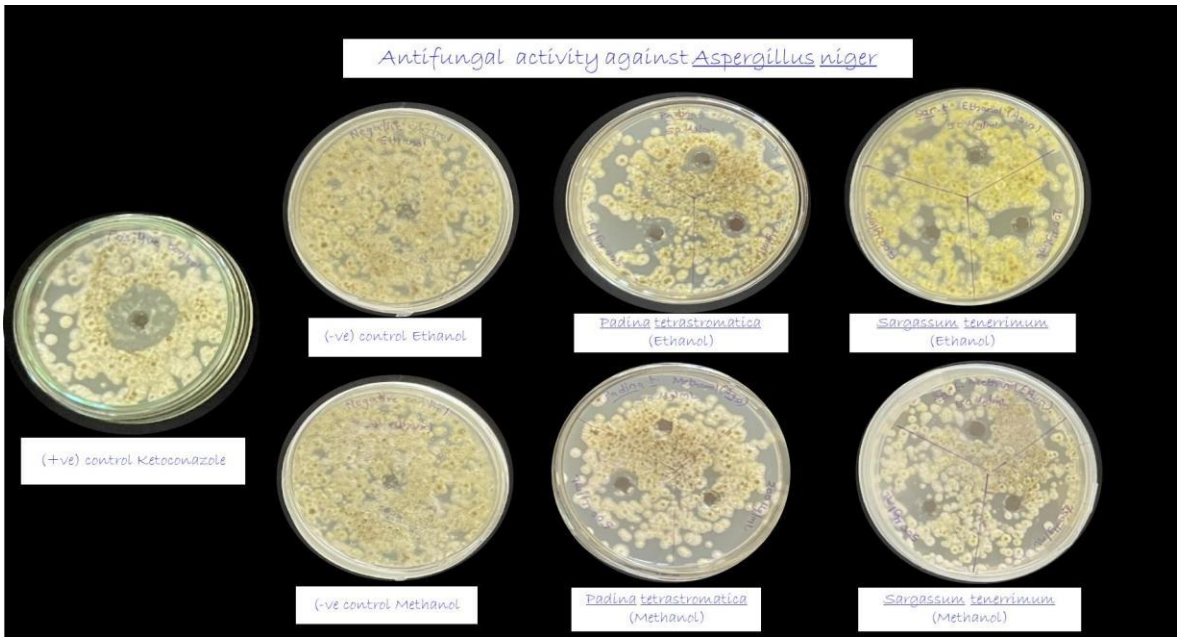


Fig.12: Antifungal activity of methanolic and ethanolic extracts of *Padina tetrastromatica, Sargassum tenerrimum* against *Aspergillus niger*

3.7. Thin layer Chromatography (TLC) and UV-VIS spectra

Thin layer chromatography (TLC) and UV-VIS spectra was carried out for separation of algal pigments in methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum* **.** Retention values (Rf) were calculated for the bands obtained and the pigments identified were corelated with absorption peaks obtained by UV-VIS spectroscopy.

3.7.1. Separation of photosynthetic pigments

The photosynthetic pigments were identified using thin layer chromatography (TLC). Photosynthetic pigments such as chlorophyll a, chlorophyll c, β-carotene and Fucoxanthin were observed in *Padina tetrastromatica* and *Sargassum tenerrimum* (**Table.10, 11,12 and 13 and Fig.13,14**).

In ethanolic extract of *Padina tetrastromatica,* four photosynthetic pigments tentatively identified are chlorophyll a with Retention factor (Rf) 0.65, chlorophyll c with Rf-0.06, β-carotene with Rf-0.95 and fucoxanthin with Rf- 0.54, showing colour blue-green, yellow, light orange and orange brown respectively (**Table.10 and Fig.13A**).

In methanolic extract of *Padina tetrastromatica,* four photosynthetic pigments tentatively identified are chlorophyll a with Rf- 0.67, chlorophyll c with Rf-0.03, β-carotene with Rf-0.93 and fucoxanthin with Rf- 0.52, showing colour blue-green, yellow, light orange and orange brown respectively. (**Table.11 and Fig.13B**).

Similarly, in ethanolic extracts of *Sargassum tenerrimum,* four photosynthetic pigments tentatively identified are chlorophyll a with Rf- 0.65, chlorophyll c with Rf-0.04, β-carotene with Rf-0.90 and fucoxanthin with Rf- 0.51, showing colour blue-green, yellow, light orange and orange brown respectively. (**Table.12 and Fig.14A**).

And in methanolic extracts of *Sargassum tenerrimum,* four photosynthetic pigments tentatively identified are chlorophyll a with Rf- 0.67, chlorophyll c with Rf-0.03, β-carotene

with Rf-0.93 and fucoxanthin with Rf- 0.52, showing colour blue-green, yellow, light orange and orange brown respectively. (**Table.13 and Fig.14B**).

Table10 : Retention factor (Rf) values of Thin Layer Chromatography (TLC) separation and spectral analysis of photosynthetic pigments showing respective colouration in ethanolic extract of *Padina tetrastromatica*.

Sr. No	Rf values	Theoretical Rf values	Colour of spots	Absorbance maxima (λmax) nm	Absorbance maxima (Reference) nm	Algal pigments
1	0.65	0.68	Blue-green	667	430, 662 (Jeffrey et al., 1997)	Chlorophyll a
2.	0.95	0.94	Light orange	412, 500	400-500 (Riswi Haryat Frehni et.al., 2015)	β -Carotene
3.	0.54	0.51	Orange brown	532	450-540 (Shade 'A Ahmed et al., 2022)	Fucoxanthin
4	0.06	0.03	Yellow	610	449, 610, 625 (Rabinowitch and Govindjee 1969)	Chlorophyll c

Table11 : Retention factor (Rf) values of Thin Layer Chromatography (TLC) separation and spectral analysis of photosynthetic pigments showing respective colouration in methanolic extract of *Padina tetrastromatica*.

Sr. No	Rf values	Theoretical Rf values	Colour of spots	Absorbance maxima (λmax) nm	Absorbance maxima (Reference) nm	Algal pigments
1	0.67	0.68	Blue-green	660	430, 662 (Jeffrey et al., 1997)	Chlorophyll a
2.	0.93	0.94	Light orange	413	400-500 (Riswi Haryat Frehni et.al., 2015)	β Carotene
3.	0.52	0.51	Orange brown	-	450-540 (Shade 'A Ahmed et al., 2022)	Fucoxanthin
4	0.03	0.03	Yellow	440	449, 610, 625 (Rabinowitch and Govindjee 1969)	Chlorophyll c

Table12 : Retention factor (Rf) values of Thin Layer Chromatography (TLC) separation and spectral analysis of photosynthetic pigments showing respective colouration in ethanolic extract of *Sargassum tenerimum*.

Sr. No	Rf values	Theoretical Rf values	Colour of spots	Absorbance maxima (λmax) nm	Absorbance maxima (Reference) nm	Algal pigments
1	0.65	0.68	Blue-green	664	430, 662 (Jeffrey et al., 1997)	Chlorophyll a
2.	0.90	0.94	Light orange	409	400-500 (Riswi Haryat Frehni et.al., 2015)	β Carotene
3.	0.51	0.51	Orange brown	541	450-540 (Shade 'A Ahmed et al., 2022)	Fucoxanthin
4	0.04	0.03	Yellow	611	449, 610, 625 (Rabinowitch and Govindjee 1969)	Chlorophyll c

Table13 : Retention factor (Rf) values of Thin Layer Chromatography (TLC) separation and spectral analysis of photosynthetic pigments showing respective colouration in ethanolic extract of *Sargassum tenerimum*.

Sr. No	Rf values	Theoretical Rf values	Colour of spots	Absorbance maxima (λmax) nm	Absorbance maxima (Reference) nm	Algal pigments
1	0.67	0.68	Blue-green	660	430, 662 (Jeffrey et al., 1997)	Chlorophyll a
2.	0.93	0.94	Light orange	413	400-500 (Riswi Haryat Frehni et.al., 2015)	β Carotene
3.	0.52	0.51	Orange brown	-	450-540 (Shade 'A Ahmed et al., 2022)	Fucoxanthin
4	0.03	0.03	Yellow	440	449, 610, 625 (Rabinowitch and Govindjee 1969)	Chlorophyll c

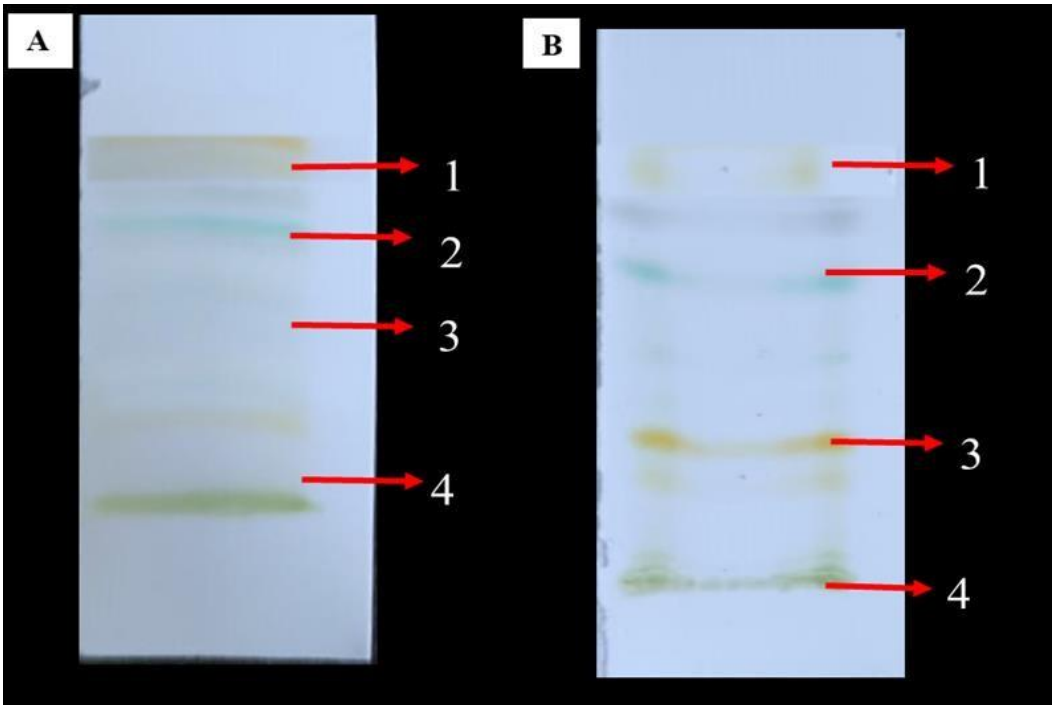


Fig. 13: The chromatograms of TLC profile of resolubilized crude pigments from brown algae *Padina tetrastromatica* **A**-ethanolic extract and **B**- methanolic extract. Obtained separated bands: **1**-β-carotene **2**-Chlorophyll a, **3**-Fucoxanthin and **4**- Chlorophyll c.

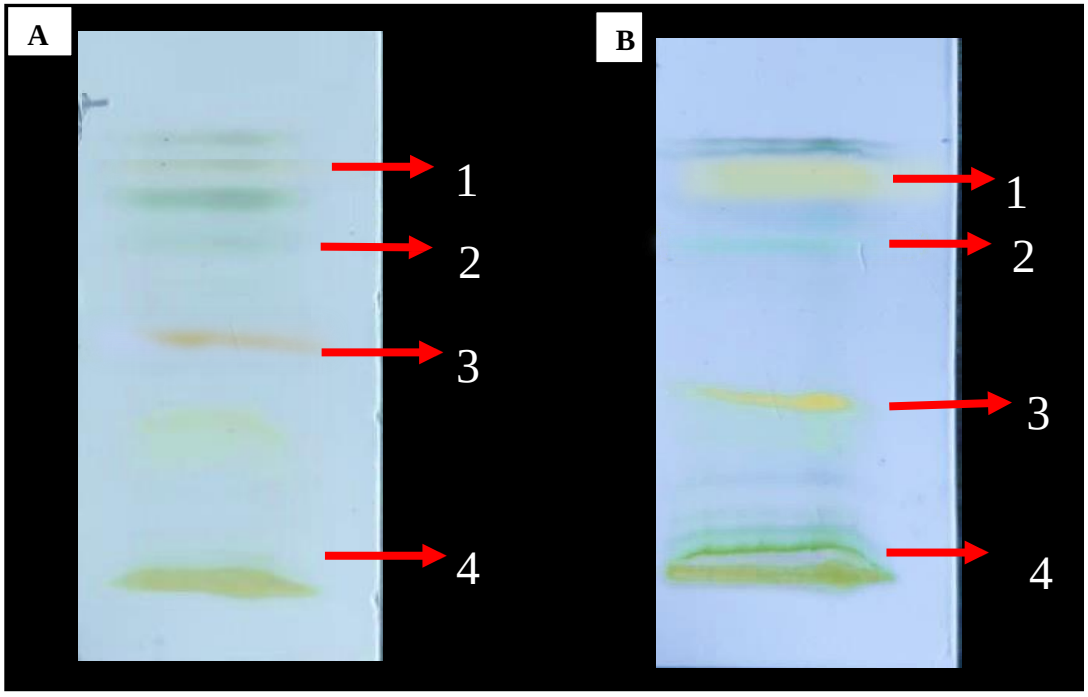


Fig. 14: The chromatograms of TLC profile of resolubilized crude pigments from brown algae *Sargassum tenerrimum* **A**- ethanolic extract and **B**- methanolic extract. Obtained separated bands: **1**-β-carotene **2**-Chlorophyll a, **3**-Fucoxanthin and **4**- Chlorophyll c.

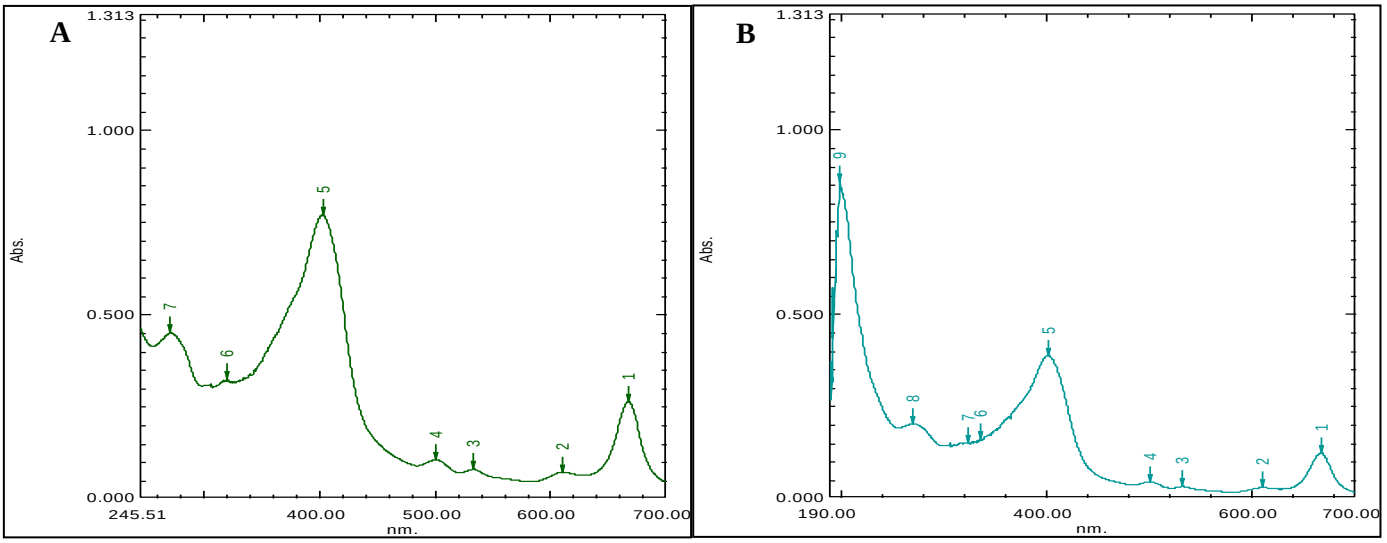


Fig.15 : UV-VIS spectra (190-700 nm) of A- ethanolic B- methanolic extracts of *Padina tetrastromatica*.

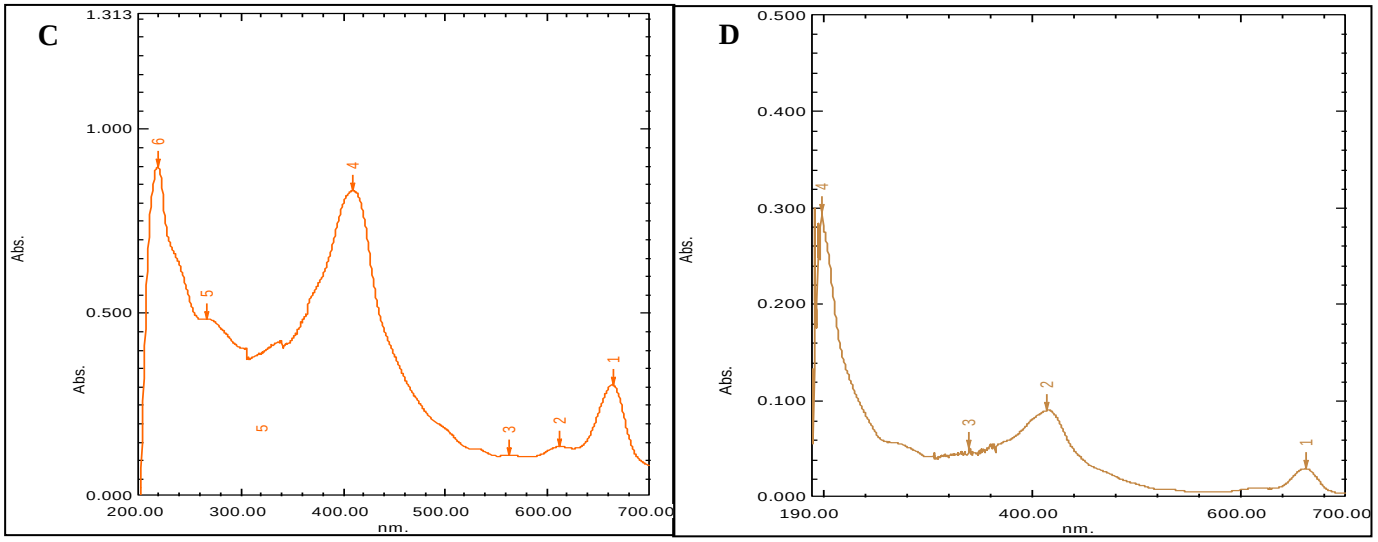


Fig.7: UV-VIS spectra (190-700 nm) of A- ethanolic B –methanolic extracts of *Padina tetrastromatica*; C- ethanolic D- methanolic extracts of *Sargassum tenerrimum*.

Chapter 4

DISCUSSION

DISCUSSION

Using bioactive compounds from natural sources as functional foods to promote human health and treat various diseases has increasingly attracted considerable attention. In the marine ecosystem, macroalgae are ecologically and biologically essential and provide nutrition for other living organisms (Wahbeh, 1997; Fleurence, 1999; Mcclanahan *et al.,* 2002; Wilson, 2002). Since ancient times, seaweeds have been used as food, fodder, fertilizer, and a source of medicine. The crude extracts of seaweed are amongst the foremost excessive fountainheads of unique, exceptional, and identified bioactive compounds (Peng *et al.,* 2015). Moreover, only a few studies have been conducted on Goa's seaweed assets emphasizing its bioactivity or secondary metabolites existence. Hence, it becomes time demanding to become familiar with unexplored seaweed assets as the abundance and accessibility of bioactive compounds of seaweeds are, to a significant extent, changes concurring to geographic area, natural condition, season, development and fair as the profundity of inundation (Graham *et al.,*1991). In the present study, two marine macroalgae viz., *Padina tetrastromatica* Hauck and *Sargassum tenerrimum* J.Agardh were used as a natural source of bioactive compounds such as phenolics, flavonoids, and photosynthetic pigments.

Several steps to attain bioactive compounds from the plant include grinding, milling, homogenization, and extraction (Do *et al.,* 2014). Amongst these steps, extraction is the primary process by which bioactive compounds may be isolated from biomass materials. The efficiency of the extraction is strongly affected by the extraction method, temperature, extraction period, the composition of phytochemicals, and the solvent used. The extraction process aims to maximize the amount of target compounds and obtain the maximum biological activity of these extracts (Chang *et al*., 2002). The present study used two organic solvents, methanol and ethanol, to extract bioactive compounds from the selected algal species. This

analysis showed that the methanol and ethanol resulted in distinctive extraction yields due to the differences in the polarity of the extraction solvents that could lead to a wide variation in the level of bioactive compounds in the extract. A higher extraction yield was observed in methanolic extracts than in ethanolic extracts (**Table 2** and **Fig.6**), indicating that the extraction efficiency favours the highly polar solvents. These results are consistent with the extraction yield of *P. pavonica* (Kokabi *et al.,* 2013) and other macroalgae such as *P. tetrastromatica and G. tenuistipitata* (Sobuj *et al*., 2021), which shows that methanolic extraction was higher than ethanolic and water extraction.The variation in the extraction efficacy of various solvents could be because the algal material contains high levels of polar compounds soluble in solvents with high polarity.

Phenolic compounds are commonly found in seaweeds and have been reported to have a wide range of biological activities, which includes antioxidant properties (Duan *et al*., 2006). Also, the polarity of any solvent plays a significant role in extracting phenolic compounds from some plants or fruit (Naczk and Shahidi, 2006). Previous reports have revealed that the phenolic components are one of the most effective antioxidants found in brown algae (Nagai and Yukimoto, 2003; Manach *et al.,* 2004). Our present study attained that ethanolic extract contained a significant amount of phenolics where *P. tetrastromatica* exhibited 37.75 mg of GAE/g and 21.45 mg of GAE/g in *S. tenerrimum*, with an insignificant difference in methanol extract (**Table 3**). The preceding reports by Sobuj et al. (2021) and Chia et al. (2015) underpin TPC's current observation, which also reported that methanolic and ethanolic extracts showed similar results compared to ethanolic and water extracts. Chia et al. (2015) and Vinayak et al. (2011) reported that the methanolic extracts of *P. tetrastromatica* showed 69.5 and 25.29 mg GAE/g, respectively, a more petite figure than the one obtained in our analysis, viz.,36.94

29 mg GAE/g. The comprehensive variety of results may be attributed to environmental conditions, the origin of the seaweed, or the varietal extraction method.

Flavonoids are the largest group of polyphenolic compounds known to contain a broad spectrum of biofunctional properties due to their unique structural characteristics, such as free radical scavenging and antioxidant properties (Velioglu *et al.,* 1998). Flavonoids are nature's tender drugs with numerous biological and pharmacological activities (Kahkonen *et al.,* 1999). In the present study, the flavonoid content of methanolic extracts of *Padina tetrastromatica* (12.43 mg QE/g) and *Sargassum tenerrimum* (9.29 mg QE/g) was higher than their ethanolic extracts (11.2 and 7.58 mg QE/g) depicted in **Table 4**. Sarojini et al. (2012) obtained results similar to our case, who also found that methanolic extracts of *S. asperum* were 11.34 mg/g, but higher than the brown alga *Padina tetrastromatica.* Furthermore, a study reported that the maximum quantity of TFC was found in methanolic extracts compared to ethyl acetate, butanol, or chloroform used as a solvent (Rafiquzzaman *et al*., 2016 and 2015).

Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent producing free radicals. In turn, in a cell, these radicals can start chain reactions causing damage or death to the cell. An **antioxidant** is a molecule that inhibits the oxidation of other molecules. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibiting other oxidation reactions helping the organism cope with minimal oxidative damage.

Quantifying the antioxidant efficacy of any natural extracts by performing a single assay is often difficult. In our study, *in vitro* antioxidant assays, DPPH (2,2-diphenyl-1-picrylhydrazyl) and reducing power assay were performed to evaluate the antioxidative

properties of the crude extracts of the selected seaweeds. In both antioxidative assays, the efficacy of altered crude extracts increased with increasing concentration, showing that these properties are dose-dependent. The influence of the amount of bioactive chemicals might be responsible for higher antioxidant activity with increased concentration. The potential antioxidant compounds were identified as a few algal pigments; fucoxanthin, astaxanthin, carotenoid and polyphenols viz. phenolic acid, flavonoid and tannins which are widely distributed in seaweeds (Yan *et al.,* 1999; Athukorala *et al.,* 2003a and 2003b). Similar antioxidant compounds were identified in the present study, including fucoxanthin pigment, phenols and flavonoids.

Hydrogen donation scavenges the DPPH radical by polyphenols, xanthophylls, and anthocyanins, forming the reduced DPPH-H. Therefore, the scavenging activity of DPPH radical is extensively used for screening antioxidants, such as polyphenols, xanthophylls, and anthocyanins from marine algae (Duan *et al.,* 2006; Wang *et al.,* 2009; Chandini *et al.*, 2008). The present investigation has shown that the methanolic extract residue of the brown seaweeds *P. tetrastromatica and S.tenerrimum* exhibited significant DPPH scavenging activity (**Table.5** and **6**). Compounds with reductones indicate their remarkable reducing ability by breaking the free radical chain by donating a hydrogen atom and can act as primary and secondary antioxidants by decreasing the reactive intermediates of lipid peroxidation (Jayanthi *et al*., 2011; Sikder *et al*., 2010). Likewise, the results obtained from this study show that the reducing ability was found to be highest in the methanolic extracts of *P. tetrastromatica and S.tenerrimum* (**Fig.10**)

These results of antioxidant assays are similar to the earlier findings (Do *et al.,* 2014; Rafiquzzaman *et al*., 2016; Arulkumar *et al*., 2018 and Chang *et al.,* 2012), which suggests that

it is because methanol extracts can have an H-donating property, allowing them to stop oxidation by transforming free radicals into stable compounds. However, according to Khaled et al. (2012), the highest effect was observed for ethyl acetate fraction in *P. pavonica*, whereas ethyl acetate and petroleum ether fraction in the case of *G. verrucosa (*Sreenivasan *et al*., 2007) and aqueous extract in *P. boergesenii (*Kumar et *al*., 2012) which are contradictory to the present findings. These differences might be due to the variation in the solvent used for analysis, differences in the analytical method and the presence of different bioactive compounds that potentially contribute to the antioxidant activity.

In recent years, due to the increase in microbial resistance against commercial antibiotics, there has been a growing need for new **active antimicrobial compounds** against pathogenic microbes. Recently, numerous reports of macro algae-derived compounds have a broad range of biological activities such as antibacterial, antifungal, antiviral, antineoplastic, antifouling, anti-inflammatory, antitumor, cytotoxic and antimitotic activities (Caccamesse *et al.*, 1980; Naqvi *et al.*, 1980). Most of the compounds of marine algae were found to possess beneficial untapped biochemical compounds, which might be a potential source of drug leads in the future. Several marine macroalgae produce bioactive metabolites in response to ecological pressures such as competition for space, maintenance of unfouled surfaces, deterrence of predation and the ability to reproduce successfully (Yamamoto *et al.*, 1984).

The present study evaluated the antibacterial activity of methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum* against gram-negative *Escherichia coli* and gram-positive bacteria *Bacillus cereus.* Moreover, the results revealed that the solvent extracts of *P. tetrastromatica* showed amelioration of the gram +ve bacteria *Bacillus cereus* than the gram -ve bacteria *Escherichia coli.* In comparison, the solvent extracts of *S.*

tenerrimum showed more growth inhibition on *Escherichia coli* than on *Bacillus cereus*. In our line of work, the algal extract exhibited a broad spectrum of antibacterial activity and inhibited both gram +ve and gram -ve strains. Methanol resulted in the effective extraction of the antimicrobial compounds. Antibacterial activity depends on both the species and the efficiency of the extraction method. Organic solvents always provide a higher efficiency in extracting compounds for antimicrobial activities than water-based methods (Ortega and Gonzales, 1990; Siddhanta *et al*., 1997). Kandhasamy and Arunachalam (2008) reported that the crude methanolic extract of *Ulva lactuca* showed the highest inhibiting activity against *Staphylococcus aureus*. Chiheb et al. (2009) and Subba Rangaiah et al. (2010) also reported that the methanolic extract of *Ulva lactuca* has a larger inhibition diameter against gram -ve bacteria *Escherichia coli* and gram +ve *Staphylococcus aureus* and *Enterococcus faecalis*. Kolanjinathan et al. (2011) stated that the methanolic extracts showed a maximum zone of inhibition against gram +ve *Bacillus cereus* and gram -ve *Enterobacter aerogenes*, whereas no zone of inhibition was recorded against gram +ve *Saccharomyces cerevisiae*.

It is impressive that the antibacterial activity towards Gram +ve species is mostly lower than that detected against Gram -ve bacteria compared with ampicillin as standard. (Rao and Parekh, 1981; Pesando and Caram, 1984; Reichelt, and Borowitzka, 1984). The outer membrane of Gram-negative bacteria is the main reason for resistance to a wide range of antibiotics (Pucci *et al*., 2017). These results hold good with the present study outcomes. Reports of Kandhasamy and Arunachalam (2008) were on par with observations of this study which suggests that the methanol extracts of tested seaweed showed a broad spectrum of antibacterial activity against human pathogenic bacteria. Furthermore, the antibacterial activities of seaweeds also varied with species division. Pesando and Caram (1984) found that the species of Phaeophyta exhibited the highest antibacterial activity. The complexity of

antimicrobial properties in seaweeds is due to their multiple inhibitory properties, possibly due to rapid and extensive defence activation induced by environmental conditions.

In the current investigation, the **antifungal** activity of the bioactive compounds in methanolic and ethanolic extracts of *Padina tetrastromatica* and *Sargassum tenerrimum* was evaluated against the fungal strain *Aspergillus niger*. Primary or secondary metabolites produced by these algae may be potential bioactive compounds of interest in the pharmaceutical industry (Findlay and Patil, 1984). The present investigation concluded that methanol extracts of the algal species showed maximum antifungal activity against the selected fungal strain. In a similar study by Elsie et al. (2011), the ethanolic extract of red algae *Gelidium acerosa* showed more significant inhibitory activity against *Candida albicans, Candida tropicals* and *Aspergillus niger.* Moreover, Tuney et al. (2006) reported that the ethanolic extract of *Padina pavonica* was active against *A.niger;* however, methanolic and acetone extracts of the same algae were inactive against *A.niger*.

In our study, the brown algae's methanolic extract residue exhibited maximum growth inhibition of *A.niger*. These data seemed to indicate that the efficiency of algal extracts against microorganisms is influenced by factors such as location and seasonality (Febles *et al*., 1995; Karthikaidevi *et al*., 2009; Kausalya *et al*., 2015).

Algae have been identified as a natural producer of bioactive commercial **pigments**. In particular, brown seaweeds exhibit a rich composition of pigments of different biosynthetic origins, providing their characteristic pigmentation: (i) chlorophylls a and c; (ii) carotenes, mainly represented by α-carotene and β-carotene; and (iii) xanthophylls, considered as the most prevalent family of pigments in these species, including neoxanthin's A and B, fucoxanthin and

violaxanthin as the major compounds (Aryee *et al*., 2018; Yuan *et al*., 2018). Notably, fucoxanthin and chlorophyll c are predominantly reported in the Phaeophyceae family, where brown algae belong (Kumar *et al.*, 2020). These pigments find applications as antioxidant, anti-inflammatory, immunoprophylactic, and antitumor activities (Jaime *et al*., 2005). The double bonds in their structure impart broad health applications while protecting other molecules from oxidation using several mechanisms induced by active radicals. Carotenoids are synthesized by certain species as major products; however, they also present as by-products in several species based on the pathway and genetic capability.

Fucoxanthin, a natural pigment of the carotenoid family, is one of the most prevalent xanthophyll variants in brown algae used as an additive stain in the chloroplasts, imparting them with an olive green or brown colour. Fucoxanthin has been found to have antioxidant, anticancer, antiobesity, antidiabetic, and antiphotoaging activity (Quirós *et al.,* 2006). *H. elongata* seaweed also showed antibiotic activity due to fucoxanthin (Rajauria *et.al.*, 2013). In this study, the preliminary identification of pigments was carried out through TLC and spectral analysis. The outcomes of TLC and UV-VIS spectroscopy showed the presence of chlorophyll a, chlorophyll c, β – carotene and fucoxanthin pigments. Previously, the fucoxanthin compound was identified in the crude extract of *Hymanthalia elongata* seaweed using UV-visible spectroscopy (Plaza *et al*., 2010). The presence of these pigments confirms their contribution to the results of antioxidants and antimicrobial properties of *P. tetrastromatica and S. tenerrimum* obtained through this study.

Chapter 5

CONCLUSION

CONCLUSION

Marine organisms constitute nearly half of the worldwide biodiversity; thus, they act as a vast resource for novel substances. Among these organisms, seaweeds or marine macroalgae produce a broad spectrum of bioactive compounds and hence is nature's best resource for such compounds. The current study explores the bioactive potential of the solvent extracts of marine brown algae, *Padina tetrastromatica,* Hauck *and Sargassum tenerrimum,* J.Agardh., collected from the intertidal regions of the Anjuna coast of Goa, India. Ethanolic and methanolic seaweed extracts were examined for various parameters. Results of extractive value reveal that methanolic extraction was higher in algae than ethanolic extraction. The amount of Total Phenolic Content (TPC) in ethanolic extracts of both algae was insignificantly higher than their methanolic extracts.

In comparison, the methanolic extracts of the two algae showed higher results in Total Flavonoid Content (TFC). The results of antioxidant activity (DPPH assay) show that the methanolic extract residue of the brown seaweeds *P. tetrastromatica* and *S. tenerrimum* exhibited significant DPPH scavenging activity; likewise, the methanolic extracts of both the algae showed higher reducing power. The antibacterial activity of methanolic extracts of *P. tetrastromatica* was higher against both gram +ve *Bacillus cereus* and the gram -ve *Escherichia coli,* and methanolic extract of *S. tenerrimum* showed higher results against the gram -ve *Escherichia coli*. In contrast, its ethanolic extract showed results against gram +ve *Bacillus cereus.* On the other hand, the methanol extracts of the algal species showed maximum antifungal activity against the fungal strain *Aspergillus niger*.

Furthermore, the photosynthetic pigments tentatively identified by Thin Layer Chromatography and UV-VIS Spectroscopy are chlorophyll a and chlorophyll c, β-carotene and fucoxanthin. Overall, the study indicates that methanol as a solvent resulted in considerable extraction of bioactive compounds in the case of the selected algal species.

Chapter 6

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