PHYTOCHEMICAL SCREENING AND STUDIES ON THE ANTIBACTERIAL AND ANTIOXIDANT POTENTIAL OF GRACILARIA CORTICATA (J. Agardh) J. Agardh

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN BOTANY

By

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CERTIFICATE

This is to certify that the dissertation entitled "Phytochemical screening and studies on the antibacterial and antioxidant potential of *Gracilaria corticata* (J. Agardh) J. Agardh " is an authentic record of research work carried out by Miss NEETHU ROBERT (AM20BOT014) under the supervision and guidance of Smt. I. K. Nishitha Assistant Professor, Department of Botany and Centre for Research, St. Teresa's College (Autonomous), Ernakulam, in partial fulfilment of the requirements for the award of the degree of Bachelor of Science in Botany. I further certify that no part of this work embodied in this project has been submitted for the award of any degree or diploma.

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DECLARATION

I hereby declare that the project entitled "Phytochemical screening and studies on the antibacterial and antioxidant potential of Gracilaria corticata (J. Agardh) J. Agardh*"* submitted to Mahatma Gandhi University, Kottayam, in partial fulfilment of the requirement for the Degree of Master of Science in Botany is an original project done by under the supervision and guidance of Smt. I.K Nishitha Department of Botany and Centre for Research, St. Teresa's college (Autonomous), Ernakulam.

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CONTENT

INTRODUCTION

Algae are diverse group of relatively simple, chlorophyll containing, photo-autotrophic and oxygen evolving aquatic thalloid (without differentiation into True roots, stems, leaves or leaf like organs) organisms. The word “algae” has its origin from Latin, where ‘alga’ means seaweed. The term algae was first used by Carl Linnaeus in 1753.

Pond scums, seaweeds, freshwater and marine phytoplankton etc are different algal forms. They are principal photo synthesizers in the globe and control our atmosphere in several ways. Their role in environment is enormous (Barsanti & Gualtieri, 2006; Graham et al., 2009). Marine algae from Indian coasts amounting to 844 species are distributed among 217 genera. They grow in the intertidal, shallow and deep-sea areas up to 180meter depth and also in estuaries, backwaters and lagoons on solid substrates such as rocks, dead corals, pebbles, shells, mangroves and other plant materials (Anatharaman et al., 2007; Sakthivel, 2007). Although seaweeds have been utilized in traditional and folk medicine for a long time their use in modern medicine has been realized only after 1950 (Lincoln et al., 1991).

The term "seaweed" refers to a variety of marine plants and algae that can be found in the ocean, rivers, lakes, and other bodies of water. Some seaweeds, such as phytoplankton, are small and remain floating in the water column, providing the foundation for most aquatic food chains. Some are massive, such as the giant kelp that grow in dense forests. The large percentage are medium-sized, with red, green, brown, and black colours, and sometimes may wash up on beaches and shorelines. (Guiry, Michael D, 2014). Mostly seen seaweeds are macro algae. Seaweeds are classified as Green algae (Chlorophyta), Brown algae (Phaeophyta), Red algae (Rhodophyta) and some filamentous Blue-green algae (Cyanobacteria). Most of the seaweeds are red (6000 species) and the rest known are brown (2000 species) or green (1200 species) (Richard et al., 1998). They differ significantly in many ultrastructural and biochemical functions, including photosynthetic pigments, storage molecules, cell wall composition, presence/absence of flagella, mitosis ultrastructure, linkage between cells, and structure of the chloroplasts, in addition to pigmentation.

Algae are found in both marine and freshwater habitats. Algae are relatively undifferentiated organisms which, unlike plants, have no true roots, leaves, flowers or seeds. As these organisms have a short doubling time, they are considered among fastest growing creatures. They have different pathways to fix atmospheric carbon dioxide and to efficiently utilize the nutrients to convert it into biomass. In recent years, focus towards these organisms has increased due to

their food and fuel production capability. In fuel industry algae biofuels have emerged as a clean, nature friendly, cost-effective solution to other fuels. More recently algae have been identified and developed as renewable fuel sources, and the cultivation of algal biomass for various products is transitioning to commercial-scale systems. Large-scale cultivation of algae merges the fundamental aspects of traditional agricultural farming and aquaculture (Emily M Trentacoste et al., 2015). Algae fuels are categorized into bio-ethanol, biogas, bio-hydrogen, biodiesel and bio-oil. Algae can be used in the preparation of Biodiesel, Bioethanol, Biobutanol and Hydrogen gas (Raja et al., 2013).

Algae have been in use as human food for centuries in various parts of the world. Algae are taken in several ways according to the choice and taste of the people. Their nutritional value is quite high, as they contain a good amount of proteins, carbohydrates, fats and vitamins, especially A, B, C and E. Not only is algae considered worldwide to be a low cost source of protein, but it also contains a number of important minerals such as iron, potassium, magnesium, calcium, manganese, and zinc. Commonly used species are -Chlorophyta– Chlorella, Ulva lactuca (Sea lettuce). The large Brown and Red algae are used as organic fertilizers, especially on land close to the sea. Many forms of marine algae, Phaeophyta and Rhodophyta, are highly valuable for certain commercial products, chiefly agar-agar, algin or alginic acid and carrageenin. The important use of agar is in microbiology and tissue culture (in the preparation of culture media for growing algae, fungi and bacteria in the laboratories). Other uses are in the cosmetics, paper and silk industries, etc. Digenia simplex, a red alga, provides an antihelmnitic drug. Agar-agar, for its absorptive and lubricating action, is used medicinally in the prevention of constipation. The antibacterial product chlorellin, obtained from Chlorella is well known and is used against coliforms and other related intestinal bacteria. Algae are important sources of vitamins, minerals, proteins, fatty acids etc (Pulz & Gross, 2004).

Rhodophyta is a phylum of macroalgae that includes the classes Phaeophyceae and Chlorophyta, which are brown and green seaweeds, respectively. The Red algae are one of the oldest groups of eukaryotic algae, and also one of the largest, with about 5,000–6,000 species of mostly multicellular, marine algae, including many notable seaweeds.

Within Archaeplastida, Rhodophyta, or red algae, is a monophyletic lineage that contains glaucophyte algae, green algae, and terrestrial plants. Bangia-like species have been found in 1.2billion-year-old strata, indicating that Rhodophyta has a lengthy fossil history. The

morphology of red algae ranges from unicellular filamentous to multicellular thalloid forms, with certain species producing economically important products like agar and carrageenan. These species can be found in a variety of marine settings, ranging from the intertidal zone to deep oceans. There are also freshwater (e.g., Batrachospermum) and terrestrial lineages. A triphasic life cycle with one haploid and two diploid phases, with the carposporophyte borne on female gametophytes, is one of the Rhodophyta's significant advances.

Freshwater Rhodophyta has 66 species and 27 genera in North America, although these numbers will change as molecular investigations uncover more diversity. Freshwater red algae have a limited size range than marine species, with the majority (80%) of them measuring 1-10 cm in length. Gelatinous filaments, free filaments, and pseudoparenchymatous forms are the most prevalent types (Yoon, Hwan Su, et al., 2017).

In terms of the number of species, the genus *Gracilaria* is one of the largest genera of red algae. It's also a wide spread genus, with species found in all oceans except the Arctic. Nearly 28 species of *Gracilaria* have been reported from the Indian coast (Sahoo *et al*., 2001). Because of its size and extensive range, it's suitable for biogeographic investigation. The greatest number of *Gracilaria* species can be found in tropical waters. Large beds of *Gracilaria* usually grow in the eulittoral zone, or just below it in the beginning of the sublittoral, on sandy or muddy sediments that are protected from waves. Sometimes it can be found free-floating in tidal lakes of salt or brackish water. It can adapt to large variations in growing conditions such as freshwater dilution, increase in fertilizer concentration from runoff, and raised temperatures. Large biomasses can grow when there is little competition from other species, and vegetative propagation may be a normal method of reproduction (McLachlan, J., et al., 1984). In tropical and subtropical oceans, these are frequently red, green, or greenish brown.

Gracilaria are found as branched thalli, terete to flattened, branching sub-dichotomous to irregular. It has holdfast a disc or crust giving rise to one to many erect axes. The thalli are red, olive, green to purple, Spermatangia are seen in pits or shallow depressions. Sporophytes with tetrasporangia are scattered in the outer cortex, cruciately divide (R Iyer, et al., 2004)

Because they have phycocolloids, the major source of agar- (1, 4)-3, 6-anhydro-l-galactose and -(1,3)-d-galactose with low esterification in the cell wall, *Gracilaria* species are essential for industrial and biotechnological uses. Agar and other polysaccharides are found in *G. confervoides, G. dura, G. chilensi, and G. secundata* among the carbohydrates.

Infectious diseases are one of the main causes of high morbidity and mortality in human beings around the world, especially in developing countries (Waldvogel, 2004). Antibiotic resistance in bacteria and fungi is one of the major emerging health care related problems in the world, it became a greater problem of giving treatment against resistant pathogenic bacteria (Sieradzki et al., 1999). Decreased efficiency and resistance of pathogen to antibiotics has necessitated the development of new alternatives (Smith et al., 1994; Ireland, 1988). Aquatic organisms are a rich source of structurally novel and biologically active compounds (Ely et al., 2004). Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry (Febles et al., 1995). Algae have wide span of ecosystems contributes to the innumerable chemical compounds that they are able to synthesize. A number of antimicrobial compounds have been identified in microalgae as well as macroalgae (De Marsac and Houmard, 1993). A large number of microalgal extracts and or extracellular products have been proven antibacterial, antifungal, antiprotozoal and antiplasmodial activity (Mayer and Hamann, 2005; Cardozo et al., 2007). Harder (1917) was the first to note seaweed's antimicrobial properties. Bactericidal compounds have been discovered in a variety of algal organisms (Glombitza, 1979; Banerjee et al., 2009).

Marine algae were reported to produce a wide variety of bioactive secondary metabolites as antimicrobial, antifeedant, antihelmintic and cytotoxic agents and the bioactive substances included alkaloids, polyketides, cyclic peptide, polysaccharide, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols (Cabrita et al., 2010) and marine macro-algae are considered as the actual producers of some bioactive compounds with high activity (Shimizu, 1996). Seaweeds are a powerful group of secondary metabolites with a wide range of structures. These bioactive compounds provide resistance to herbivores, fouling species, and pathogens as well as reproduction, UV defence and allopathic agent resistance (Hay, 1996). The marine habitat of India has diverse seaweeds, spread through inter-tidal and deep-water regions of the Indian coast. Marine algae (Seaweeds) are a group of marine multicellular algae, plentiful in minerals, vitamins, and polysaccharides. They are considered as a potential source of bioactive substances such as proteins, lipids, and polyphenols possessing potent antibacterial, anticancer, antioxidant, antifungal, and antiviral properties (Sundaramurthy A et al., 2016).

Decreasing efficiency and resistant of pathogens to antimicrobial drugs made the search of a new antimicrobial agent an important strategy for the establishment of alternative therapies in difficult handling infections, eg: Escherichia coli, Staphylococcus aureus and Pseudomonas

aeruginosa that causes diseases like diarrhea, mastitis and upper respiratory complications (Levine, 1987; Jawetz et al., 1995). In recent years, multiple resistances in human pathogenic microorganism have developed due to the indiscriminate use of antibiotic drugs commonly employed in the treatment of infectious diseases. The undesirable side effects of certain antibiotics and the emergence of previously uncommon infections have forced scientists into looking for new antibiotic substances from various sources like marine macroalgae. Macroalgae serve as an important source of bioactive natural substances (Smit, 2004). Many metabolites isolates isolated from macroalgae have been shown to possess bioactive effects (Faulkner, 2002).

Natural products from marine algae have attracted the attention of biologists and chemists the world over for the last five decades. Many of these compounds are used to treat diseases like cancer, acquired immune-deficiency syndrome, inflammation, pain, arthritis, as well as viral, bacterial, and fungal infections. The marine red alga showed the phytochemical constituents like phenols, alkaloids, saponins, flavonoids and steroids. Red algae and their extracts have been studied as novel sources of variety of compounds and reported for their biological activity for potential medicinal use. Red algae are the source of amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes, cyclic polysulphides, fatty acids, acrylic acid (Taskin E et al., 2007). These compounds probably have diverse simultaneous functions for the seaweeds and can act as allelopathic, antimicrobial, antifouling, and herbivore deterrents, or as ultraviolet-screening agents. They are also used by the pharmaceutical industry in drug development to treat diseases like cancer, acquired immune-deficiency syndrome (AIDS), inflammation, pain, arthritis, infection for virus, bacteria and fungus (Deig E F et al., 1974).

Phenolic compounds are commonly found in brown, green, red seaweeds, whose antioxidant properties have been correlated to their phenolic contents. Recently, a number of studies have been reported on the phytochemistry of plants across the world. Saponins are considered as a key ingredient in traditional Chinese medicine and are responsible for most of the observed biological effects. Saponins are known to produce inhibitory effect on inflammation. Flavonoids in human diet may reduce the risk of various cancers, as well as preventing menopausal symptoms. Its potent water-soluble antioxidants and free radical scavengers prevent oxidative cell damage and have strong anti-cancer activity. They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity (Cushnie and lamp, 2005; De Sousa et al., 2007; Yadhav et al., 2011). Flavonoids are largest group of polyphenolic compounds and

are known to contain a broad spectrum of chemicals and biological activities including antioxidant and free radical scavenging properties. They are remarkable group of plant metabolites. Flavonoids are perhaps best known to enhance the effects of ascorbic acid (Yasantha A et al., 2007). The secondary metabolites of seaweeds have always attracted the interest of biochemists because of their diversity as compared with those present in the leaves of higher plants. Isoprenoids (terpenes, carotenoids, steroids), polyketides (Phlorotannins), amino-acid-derived natural products (alkaloids), and shikimates (flavonoids) are the major groups of secondary metabolites found in algae (Mendis and Kim, 2011).

Antioxidant compounds play an important role in various fields such as medical field (to treat cancers, cardiovascular disorders, and chronic inflammations), cosmetics (anti- ageing process), food industries (food preservative) and others (Kohen R; Nyska A 2002). Over the years, the search for new antioxidant compounds from natural products has mounted. This is due to health concerns regarding the potential toxic and side effects generated from synthetic antioxidants, as well as changes in consumer preferences for natural products (Safer AM, 1999). As algae are photosynthetic organisms, they produced free radicals and other oxidative reagents when they are exposed to high oxygen concentrations and light. It is considered because of absence of structural damage that these organisms are able to generate the necessary compounds to protect themselves against oxidation. Hence, algae is a potent antioxidant compounds that could also be suitable for protecting our bodies against the damaging effect of reactive oxygen species produced as a result of normal metabolism of the body.

Many commercialized synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used under strict regulation in certain countries because of their potential health hazards. Thus, the search for alternative antioxidants from natural products has increased and among them, aquatic plants have gained the focus. Seaweeds or marine macroalgae have been known as rich sources of various natural antioxidants. Compounds such as polyphenols, catechin, flavonols, flavonol glycosides, and phlorotannins have been discovered from methanol extract of red and brown algae. The uniqueness of their molecular skeleton and structures has contributed to the strong antioxidant activity. Polyphenols for instant uses its phenol rings as electron traps for free radicals.

Much previous research has studied the biological effects of natural marine products, including their antioxidant, antibacterial, anti-malarial, antiviral, anti-inflammatory and cytotoxic activities (Skropeta D, 2008). However, only a few studies have examined the biological

potential of the marine algae (seaweeds) (Jung W.K. et al., 2009). Diverse antioxidants found in seaweeds such as polysaccharides, dietary fibres, minerals, proteins, amino acids, vitamins, polyphenols, and carotenoids have been recorded by several scientists (Burtin, 2003). Seaweeds contain a remarkable range of potential novel antioxidants that help in counteracting the environmental pressures (Lesser, 2006).Natural antioxidants are superior to synthetic antioxidants since they are free of environmental contaminants and perform a wide range of beneficial functions. Such additives are secure to be used in medicines, nutritional supplements, nutraceuticals, including cosmetics to enhance consumer wellbeing, lessen the impact of infectious diseases, besides other wider forms of immune system function.

Natural antioxidants are superior to synthetic antioxidants because they do never include chemical pollutants and have a wide range of benefits. These are safe for use as ingredients in medicine, dietary supplements, nutraceuticals, and cosmetics with the objective of improving customer health, reducing the effects of harmful diseases, and other broader aspects of immune system function (Shahidi, 2009). Several countries have reported the antioxidant result of input seaweeds such Matsukava, 1997), Indonesia (Santos' et al., 2004), Korea (Heo et al., 2005), India (Chandini et al., 2008) and Malaysia (Matanjun et al., 2008). Notably, there is a great deal of interest in monitoring the potential targeted therapies through natural products, with a particular focus on marine entities. Thus, many marine organisms develop biochemical pathways in relation to environmental stresses such as space competition, maintaining unfolded surfaces, avoiding predation, and the ability to reproduce successfully (Konig et al., 1994). Seaweeds are a powerful group of secondary metabolites with a wide range of structures. These bioactive compounds provide resistance to herbivores, fouling species, and pathogens as well as reproduction, UV defence and allopathic agent resistance (Hay, 1996). Antioxidants play prominent role in the later stages of cancer development. The most powerful water-soluble antioxidants found in algae are polyphenols, phycobiliproteins and vitamins (Plaza M et al., 2008). Oxidative processes promote carcinogenesis. The antioxidants may be able to cause the regression of premalignant lesions and inhibit their development into cancer. It is found that, several algal species have prevented oxidative damage by scavenging free radicals and active oxygen and hence able to prevent the occurrence of cancer cell formation (Richardson JS,1993), these antioxidants are considered key compounds to fight against various diseases (e.g. Cancer, chronic inflammation, atherosclerosis and cardiovascular disorder) and ageing processes (Kohen R and Nyska A, 2002).

Objectives of the study:

- To give a taxonomic description of *Gracilaria corticata*.
- Preliminary phytochemical assessment of the alga.
- Assessment of anti-bacterial potential of the alga in its dried form extracted in two different solvents – ethanol and chloroform.
- Evaluate the difference in anti-bacterial potential shown by the alga in the two different solvents against gram-positive *Staphylococcus* and gram-negative *E. coli*.
- Assessment of anti-oxidant potential of *G. corticata* extracted in ethanol and chloroform using the Ferric Reducing Antioxidant Power assay.
- Estimate the extractive value of the plant, in both Ethanol and Chloroform Solvents.

REVIEW OF LITERATURE

In a study conducted by Inci Tuney (2006), antibacterial activity of extracts or components from various algae has been demonstrated in vitro against both gram-positive and gram-negative bacteria. The antibacterial susceptibility test was performed using the agar disc diffusion method, with 6 mm discs impregnated with 20 µl of extracts and placed in infected agar. *Gracilaria* chloroform extract was tested for antibacterial properties against *Staphylococcus aureus* bacterial strains. *Gracilaria* extract showed action in *S. aureus* extract. Ethanol extracts from *G. domigensis* and *G. sjoestedii* showed antibacterial activity against *E. coli* and *S. aureus*. (TÜney, İnci, et al., 2006)

Krishnapriya et al., (2013) conducted an antibacterial activity on the seaweed extracts, carried out by agar disc diffusion assay. The Muller Hinton agar (MHA) medium was used for this study using bacterial pathogens. Among the solvent extracts, methanol extract showed best results for both positive and negative strains. Chloroform extract of G. *verrucossa* gave the highest zone of inhibition measuring 21±1.0 mm. Ethanol extract of G. *acerosa* also showed a zone of inhibition of 12±1.0 mm. Ethanol and chloroform extracts of G. *verrucossa* gave clearly distinct zone of inhibition measuring 8±1.0 and 9±1.0 mm, with respect to control (25±1.0 mm) against *Staphylococcus*. (Varier, Krishnapriya Madhu, et al., 2013)

Saranraj, P. (2013) conducted a study and the methanol extract of *Gracilaria folifera* (5.0mg/ml) showed highest mean zone of inhibition (18±0.4mm) against the Gram positive cocci *Streptococcus pyogenes* followed by *Bacillus subtilis* (17±0.5mm), *Staphylococcus aureus* (17±0.3mm), *Streptococcus epidermis* (16±0.6mm)and B*acillus cereus* (16±0.2mm) .For Gram negative bacterium, the maximum zone of inhibition was recorded in methanol extract of *Gracilaria folifera* against *Klebsiella pneumoniae* (17±0.5mm) followed by *Salmonella typhi* (16±0.6mm), *Pseudomonas aeruginosa* (16±0.5mm), *Escherichia coli* (16±0.3mm). The zone of inhibition obtained from the Hexane extract of seaweed *Gracilaria*

folifera against bacterial pathogens was comparatively very less when compared to the other solvent extracts. No zone of inhibition was seen in DMSO control and the positive control Ampicillin showed zone of inhibition ranging from13±0.8 mm to 20±0.8mm against the test bacterial pathogens. (Saranraj, P., 2013)

The antibacterial properties of eight crude extracts of local *Acanthophora spicifera* obtained by two distinct extraction methods were investigated by Zakaria (2010) using soxhlet extraction and solvent partitioning. By using the Disc diffusion method, these extracts were evaluated in vitro against 18 bacteria, 3 yeasts, and 6 fungal strains. The results demonstrated that the solvent partitioning extracts of methanol and ethyl acetate had a greater spectrum of action against the tested bacterial strains. *Bacillus cereus* ATCC 10876, *Bacillus licheniformis* ATCC 12759, Menthicilin Resistance *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* ATCC 27853, *Yersinia* sp., and *Citrobacter freundii* displayed inhibitory zones against these two extracts. While methanol extracts from Soxhlet extraction and butanol from solvent partitioning had no antibacterial activity against *P. aeruginosa* ATCC 27853, the other six extracts did. (Zakaria, et al., 2010)

In a study done by Ibraheem et al.; 2017; simplex extracts of *Acanthophora* showed potent inhibitory growth activities against three Gram positive bacteria [*Streptococcus agalactiae, pyogenes* and *Streptococcus sanguis* of inhibition ranging from [23.1±0.58 to 20.6±0.63 mm] and showed moderate activities with [*Corynebacterium diphtheriae, Bacillus subtilis* and *Staphylococcus aureus*] with inhibition zones ranging from [20.1±1.5 to 16.3±2.1 mm].

Also the crude extracts were found to be more active than the positive control Ampicillin, (22.3±1.5 mm), against *Streptococcus agalactiae* which showing inhibition zone. The hydro alcoholic extracts of the selected species were investigated for their antimicrobial activities using Agar well diffusion and Muller Henton against gram positive and gram negative bacteria. (Ibraheem, Ibraheem BM, et al., 2017)

In a study by Nurul Aili Zakaria et al.; (2011), the antimicrobial activities of the hexane extract were evaluated using disc diffusion method against 8 Gram-negative and 10 Gram-positive bacterial strains. Out of all bacterial tested, only a Gram-positive bacterium and a Gram-negative bacterium were susceptible to the extracts. The hexane extract showed antibacterial activity against both Gram-positive bacterium and Gram-negative bacterium (P. aeruginosa ATCC 27853). While, chloroform and ethyl acetate extract only showed inhibitory effect on

P. aeruginosa ATCC 27853 with inhibition zone of 9.0 mm. No inhibitory effect was showed by methanol extract on bacteria tested. (Zakaria, Nurul Aili, et al., 2011).

Marine macroalgae are an important renewable resource of bioactive compounds useful for healthy food and alternative medications capable of regulator diseases or multi- resistant strains of pathogenic microorganisms (Pérez et al., 2016).

Phytochemicals are chemicals produced by plants through secondary metabolism. They generally have biological activities in the plant host and play a role in plant growth or defense against predators, pathogens or competitors (Molyneux RJ et al., 2007). They are commonly found in fruits, vegetables, nuts, legumes, and grains. Phytochemicals include all plant compounds both plant chemicals that are beneficial and those that are toxic. Some phytochemicals possess incredible health benefits while others are toxic to health (Hennemen K, 2016). Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignins, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides (Hahn NI, 1998). Phenolics have been reported to be the most abundant and structurally diverse plant phytochemicals (Yoshie Y et al., 2001). Marine algae are a rich source of bioactive secondary metabolites, including phenols and polyphenols (Andrade et al., 2013; Maharana et al., 2015; Fernando et al., 2016). Flavonoids are perhaps best known to enhance the effects of ascorbic acid (Yasantha A et al., 2007).

Isaiah Nirmal Kumar et al., (2014) determined total phenolic assay by using Folin Ciocalteu assay. Zhishen J et al., (1999) and Isaiah Nirmal Kumar et al., (2014) used aluminum chloride calorimetric assay for measuring total flavonoid content of selected seaweeds from Okha coast.

Harder (1917) was the first to note seaweed's antimicrobial properties. Bactericidal compounds have been discovered in a variety of algal organisms (Glombitza, 1979; Banerjee et al., 2009). In a study conducted by Inci Tuney (2006), antibacterial activity of extracts or components from various algae has been demonstrated in vitro against both gram-positive and gram-negative bacteria. The antibacterial susceptibility test was performed using the agar disc diffusion method, with 6 mm discs impregnated with 20 µl of extracts and placed in infected agar (Inci Tuney 2006). The antimicrobial activity of seaweeds, including Gracilaria corticata, Acanthophora spicifera extracts was studied by ethanolic solvents against antimicrobial activity of Staphylococcus aureus, Bacillus thuringiensis, Pseudomonas and Klebsiella human pathogens (Ahilya Vitthal Waghmode et al., 2021). Antimicrobial activity was measured using ELISA microplate reader.

A number of antimicrobial compounds have been identified In microalgae as well as macroalgae (De Marsac and Houmard, 1993). Antimicrobial activity was evaluated using the disc diffusion technique in petri dishes (NCCLS, 1993). Briefly, sterile filter paper discs, 6 mm in diameter were loaded with 25 µL of the different antibacterial compound extracts and were air dried. Discs containing standard concentration of ciprofloxacin for bacteria and amphotericin B for fungi were used as control. The discs were placed on Muller Hinton agar plates (Chowdhury et al., 2015). Ethanolic and methanolic extracts of Acanthaphora spicifera performed a good antimicrobial activity but chloroform extract was not good enough (Chowdhury et al., 2015).

The antibacterial activity of five gracilaria species was determined in both gram positive and gram-negative bacteria. In the preliminary assay ten different organic solvents like Acetone, Butanol, Ethanol, Ethyl acetate, Isoamyl alcohol, Methanol and Propanol (polar) and Benzene, Chloroform and Hexane (non-polar) were evaluated (Prasad M. P., ShekharSushant, Rindhe Ganesh, 2012).

Antioxidants are compounds that protect human, animal and plant cells against the damaging effects of free radicals. Free radicals can be defined as any species containing one or more unpaired electrons in atomic or molecular orbitals and capable of independent existence (Halliwell, 2011). In recent times, marine algae have been gaining importance as sources of pharmacologically active constituents possessing antioxidant, antiproliferative, antimutagenic, antidiabetic, anticoagulant, antibacterial and antitumor activities (Smit AJ, 2004; Folmer F et al., 2010). Exploration for bioactive compounds led to the screening of selected marine algae from the Tamil Nadu coast for antiproliferative and antioxidant activities (Murugan K & Iyer VV, 2013).

Ahilya Vitthal Waghmode et al., (2021) determined antioxidant potential of various seaweed extracts using the DPPH and FRAP assay. The FRAP assay showed a highest antioxidant activity in methanolic extract of the green seaweed Ulva. The most FRAP activity was observed in U. lactuca (81.80%) followed by C. peltata and Batrachospermum, while less activity was detected in S. ilicifolium (48.70%). The antioxidant activity pattern of methanolic solvents varied due to the presence of various compounds with different species. A high value of astaxanthin has been recorded in green alga, Ulva intestinalis (Banerjee et al., 2009).

MATERIALS AND METHODS

SPECIMEN COLLECTION

The specimen was collected by hand picking from Thikkodi beach, Calicut. The collected samples were washed immediately in seawater and then washed with fresh water and transported to the laboratory. It was again washed thoroughly to remove impurities and sand and rinsed with distilled water. The sample was identified taxonomically as *Gracilaria corticata.* Collected sample was taxonomically evaluated using the standard literature.

PHYTOCHEMICAL ANALYSIS

Qualitative Analysis

Extract Preparation

2g of shade dried plant material is taken in clean dry conical flask to it 20ml of the extracting solvent, ethanol, methanol and water was added and kept in the mechanical shaker for 24 hours. Then it was filtered using Whatman No 1 filter paper and made up to 50ml.

The following standard protocols were used for qualitative analysis of samples to check for the presence of Alkaloids, Cardiac glycosides, Flavonoids, Phenols, Saponins, Tannins and Terpenoids.

Test for Flavonoids:

2 ml of each extract was added with few drops of 20% sodium hydroxide, formation of intense yellow colour is observed. To this, few drops of 70% dilute hydrochloric acid were added and yellow colour was disappeared. Formation and disappearance of yellow colour indicates the presence of flavonoids in the sample extract.

Test for Alkaloids:

To 1ml of the extract 1ml of any one of the below reagent is added.

Mayer’s reagent:

1.36g of $HgCl_2$ was dissolved in 60ml distilled water (solution A) and 5g of potassium iodide was dissolved in 10ml distilled water (solution B) .Both solutions A and B were mixed and made upto 100ml.

Dragendroff's reagent :

8g Bismuth Sub Nitrite was dissolved in 20ml con.HNO_3 to form solution A. 27g of potassium iodide was dissolved in 50ml of distilled water to form solution B. Both solution A and B were mixed and allowed to stand when KNO_3 precipitates out, supernatant was discarded and made up to 100ml with distilled water.

Wagner's reagent:

1027g of iodine and 2g potassium iodide were dissolved in 5ml distilled water. It was then made up to 100ml with distilled water.

Presence of precipitate in all three reagents confirms the presence of alkaloids

Test for Saponins:

To 2 ml of each extract, 6 ml of distilled water were added and shaken vigorously; formation of bubbles or persistent foam indicates the presence of saponins.

Test for Tannins:

To 2 ml of each extract, 10% of alcoholic ferric chloride was added; formation of brownish blue or black colour indicates the presence of tannins.

Test for Phenols:

To 2 ml of each extract, 2 ml of 5% aqueous ferric chloride were added; formation of blue colour indicates the presence of phenols in the sample extract.

Test for Cardiac Glycosides:

To 1 ml of each extract, 0.5ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution were added, formation of brown ring at the interface indicates the presence of cardiac glycosides in the sample extract.

Test for Terpenoids:

Take 1 ml of extract of each solvent and add 0.5 ml of chloroform followed by a few drops of concentrated sulphuric acid, formation of reddish brown precipitate indicates the presence of terpenoids in the extract.

Preparation of algal extract for antimicrobial and antioxidant studies

The cleaned samples were shade dried, cut into small pieces and powdered in a mixer grinder. The organic solvents chloroform and ethanol were used for the extraction process due to its higher efficiency using Soxhlet extraction method. 20g of samples were packed in a thimble and placed in the extractor. 200ml of the solvent was added into the flask and heated. The temperature was maintained at 80^0C to 85^0C throughout the extraction. The soluble active constituents of the extract remained in the flask and the process was repeated until the compounds were completely extracted. The liquid extract was then cooled and concentrated by using an evaporator.

The beaker with dried extract was weighed and noted. DMSO was used to dissolve the extracts from the beaker. Later the weight of the beaker alone was noted. Hence, the actual weight of the dried extract was obtained. Similarly, the weight of dried extract of *Gracilaria*, in ethanol and chloroform was 0.46g and 10mg respectively. From this the extractive value was calculated using the formula

Extractive value (%) = (Weight of dried extract/ Weight of plant material) X 100

PREPARATION OF EXTRACT IN VARIOUS CONCENTRATIONS

From the stock extract, concentrations of 10%, 20%, 40%, 60% (v/v) was made. The stock concentration of *Gracilaria* in ethanol and chloroform was 10mg/ml and 10mg/ml respectively. From the stock the appropriate amounts was pipetted out and made up to the required concentrations using DMSO.

ANTIBACTERIAL ACTIVITY IN *GRACILARIA*

PREPARATION OF BACTERIAL CULTURE

In the present study, the extracts were evaluated for antimicrobial activity against *Staphylococcus* strain and *E. coli*, a Gram positive and a Gram negative bacteria respectively. 3g of nutrient broth was dissolved in 100ml of distilled water in a conical flask. The broth is sterilized by autoclaving for 15 minutes. Both of the obtained bacterial stains were inoculated in the nutrient broth in laminar air flow and incubated in appropriate conditions for 24hrs.

PREPARATION OF PETRI PLATES

The selected two species of seaweeds were analysed for the antimicrobial activity for gram negative *Escherichia coli* and gram positive *Staphylococcus* by disc diffusion methods. Agar medium was prepared by dissolving 4g agar and 2.6g of nutrient broth in 100ml distilled water. The mixture is sterilized in an autoclave for 15 minutes. Just after sterilization the mixture was poured into petri plates in laminar air flow. The petri plates were allowed to solidify under aseptic conditions.

ANTIMICROBIAL TEST BY DISC DIFFUSION METHOD

Bacteria were inoculated onto the prepared agar petri plates using sterilized cotton swabs. Sterilized 6mm discs were taken from filter paper and autoclaved and is used for the method. The disc was then dipped in different concentrations of stock (10, 20, 40, 60) and placed on the agar plate using sterile forceps. Tetracycline was used as positive control and DMSO was used as negative control. This was done for both extracts of *Gracilaria* against the two strains of bacteria. The petri plates were incubated at 37^{o}C for 24 hours and results were recorded.

ANTIOXIDANT ASSAY

Ferric reducing power assay

The total antioxidant activity can be measured by the ferric reducing antioxidant power assay(FRAP).The flavonoids and phenolic acids present in the medicinal plant exhibit strong

antioxidant activity which is depending on their potential to form the complex with metal atoms , particularly iron and copper. This method is based on the principle of, increase in the absorbance of the reaction mixture, indicates an increase in antioxidant activity.

The antioxidant compound present in the samples forms a coloured complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700nm by UV-Spectrophotometer.

Reagent preparation

0.2M Phosphate buffer : Dissolve 27.218g of KH2PO4 in 1000ml distilled H20

Potassium ferricyanide (1%) : 1g of K3Fe(CN)6 was dissolved in 100ml distilled H2O

Trichloroacetic acid (10%) : Dissolve 10ml of TCA was dissolved in 90ml distilled H2O

Ferric chloride (0.1%) : 0.1g of FeCl3 was dissolved in 100ml distilled H2O

Ascorbic acid (0.1%) : 1mg of Ascorbic acid was dissolved in 1ml of distilled H2O

Working procedure

0.02g of the algal residue was dissolved in 30ml ethanol and used as the stock. Various concentrations of the extract was prepared by taking 2ml, 4ml, 6 ml, 8ml and 10ml of the stock and making up to 10ml using distilled H2O. Then 1 ml from each dilution was pipetted out into separate test tubes. To this 2.5 ml of 0.2M sodium phosphate buffer and 2.5ml of 1% K3Fe(CN)6 solutions were added and the reaction mixture was incubated in a boiling water bath at 50o C for 20 minutes. Following this 2.5 ml of 10% trichloroacetic acid was added to the mixture and mixed. Then 2.5ml of solution was pipetted out from each reaction mix into separate test tubes, to which 2.5 ml of distilled water and 0.5ml of 0.1% FeCl3 were added. Solvent solution along with the above stated reagents, was used as control. The coloured solutions including control were read at 700nm with reference to standard using UV-Spectrophotometer to find the absorbance. Here, Ascorbic acid was used as a reference standard, the reducing power of the samples were comparable with the reference standard.

OBSERVATION AND RESULTS

The current study aimed at the taxonomic description of the red seaweed *Gracilaria corticata* and the estimation of its extractive value and antimicrobial potential in two solvents, ethanol and chloroform. The antimicrobial potential activity was studied against Gram positive *Staphylococcus* and Gram negative *E. coli,* two non-pathogenic bacteria. The results obtained are described below.

TAXONOMIC DESCRIPTION

Kingdom: Plantae

Phylum: Rhodophyta

Class: Florideophyceae

Order: Gracilariales

Family: Gracilariaceae

Genus: *Gracilaria*

Species: *corticata*

Gracilaria corticata (J. Agardh) J. Agardh

Thallus erect, up to 14cm in length, arising singly from a discoid holdfast. Stipe very short, terete, up to 5mm long, often inconspicuous. Branching frequently, becoming denser in upper parts of the plant; mostly dichotomous, and producing a bushy appearance. Axes compressed, almost cartilaginous; constricted at the base in basal branches. Blades linear, up to 15cm long, up to 4mm wide; apices generally obtuse, acute in finer branches. Blade surface and margins smooth. Fresh specimens purple to green and firm but pliable (Iyer et al, 2004).

Gracilaria corticata (J. Agardh) J. Agardh

EXTRACTIVE VALUE

Extractive values of plant materials are used to evaluate extracts of the sample, in order to get an idea about the nature of chemical constituents present in it. It can also be used to assess quality, purity and detect adulteration of the extract.

In the present study, polar and non-polar solvents were used for eluting the valuable phyto-compounds present in the sample. Extractive values of ethanol and chloroform extracts of *Gracilaria corticata* used in the antibacterial study, are estimated in the table 1 given below;

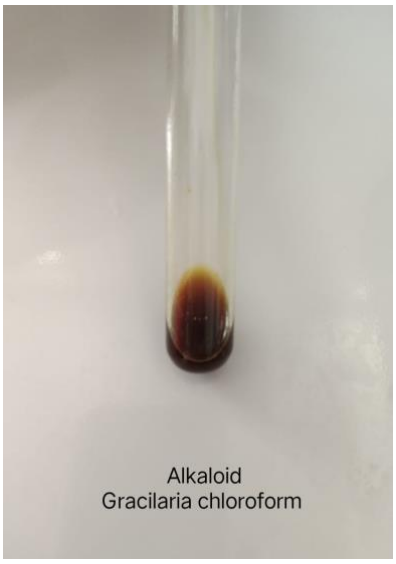
Table 1: Extractive value of solvents administered for *Gracilaria corticata*

Solvent	Extractive value of the sample (%)
Ethanol	2.3
Chloroform	0.5

The extractive value was greater for the ethanolic extract than for chloroform suggesting that polar solvent was more efficient in extracting the phytochemicals from the algae.

PHYTOCHEMICAL ANALYSIS

	Ethanol	Chloroform
Alkaloid	+	+
Flavonoid	+	+
Phenol	-	-
Tannin	+	+
Saponin	+	+
Terpenoid	-	-
Cardiac glycosides	+	+




Tannin
Gracilaria
Ethanol


Tannin
Gracilaria chloroform


Saponin


Terpenoid
Gracilaria
(chloroform)


Terpenoid
gracilaria (ethanol)


Gracilaria (ethanol
and chloroform)

ANTIBACTERIAL ACTIVITY

The extracts of the algae exhibited moderate to mild antibacterial activity against the two microorganisms. The activity observed can be described as being bacteriostatic showing very mild zones of inhibition. The ethanol extract of the algae showed mild antibacterial activity against both the test organisms. *Gracilaria* shows mild action against gram negative bacteria at all concentrations of ethanol extract used in the current study against both test organisms. Table 2; Fig. 2

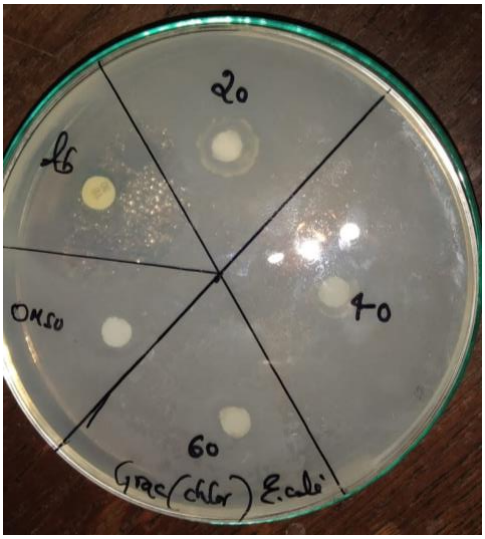
Table 2: Antibacterial activity of ethanolic extract of *Gracilaria corticata* against *E. coli* and *Staphylococcus* bacteria*:*

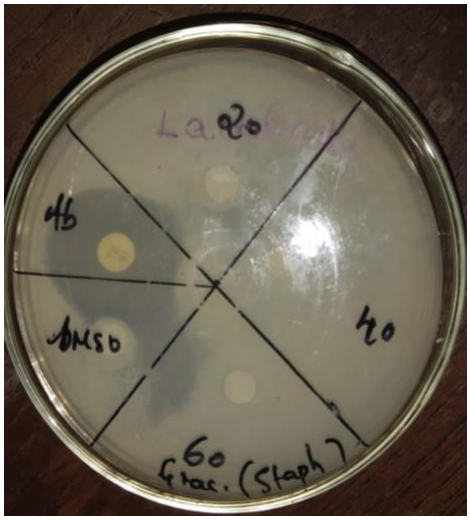
Concentration (%)	***E. coli***	***Staphylococcus***
20	Mild action	Mild action
40	Mild action	Mild action
60	Mild action	Mild action

Table 3: Antibacterial activity of chloroform extract of *Gracilaria corticata* against *E. coli* and *Staphylococcus* bacteria

Concentration (%)	***E. coli***	***Staphylococcus***
20	No action	No action
40	No action	No action
60	No action	Mild action

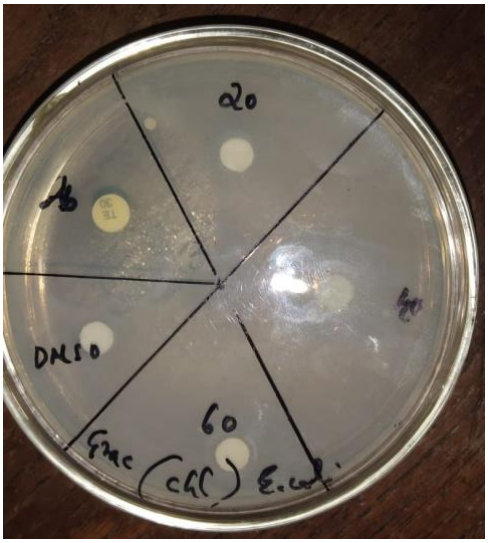
The chloroform extract of *Gracilaria* has no significant effect on the bacterial growth. Mild bacteriaostatic activity is observed at higher extract concentrations on *Staphylococcus*. No potential activity could be observed on the growth of *E. coli* in any of the concentrations used for the current study Table 3: Fig. 3

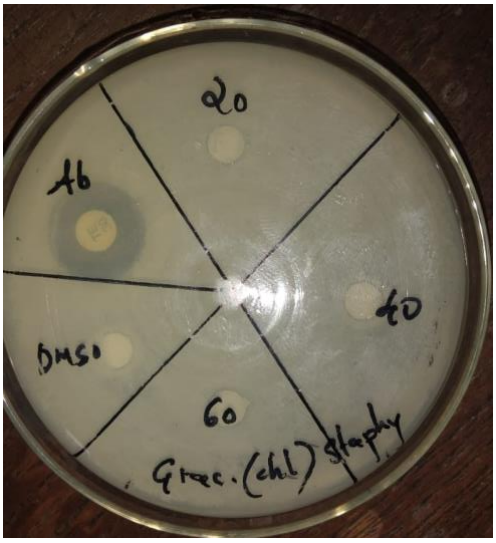




E. coli *Staphylococci*

Fig 2: Antimicrobial activity of Ethanol extract of *Gracilaria*





E. coli *Staphylococci*

Fig 3: Antimicrobial activity of Chloroform extract of *Gracilaria*

Ferric Reducing Antioxidant Power (FRAP) Assay

The total antioxidant activity can be measured by the ferric reducing antioxidant power assay(FRAP).The flavonoids and phenolic acids present in the medicinal plant exhibit strong antioxidant activity which is depending on their potential to form the complex with metal atoms, particularly iron and copper. This method is based on the principle of, increase in the absorbance of the reaction mixture, indicates an increase in antioxidant activity.

The antioxidant compound present in the samples forms a coloured complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700nm by UV-Spectrophotometer.

Table 4: Absorbance of various concentration of standard, ascorbic acid in FRAP assay

Solvent/Std/Conc	Absorbance
2ml	0.51
4ml	1.45
6ml	1.62
8ml	2.03
10ml	2.31

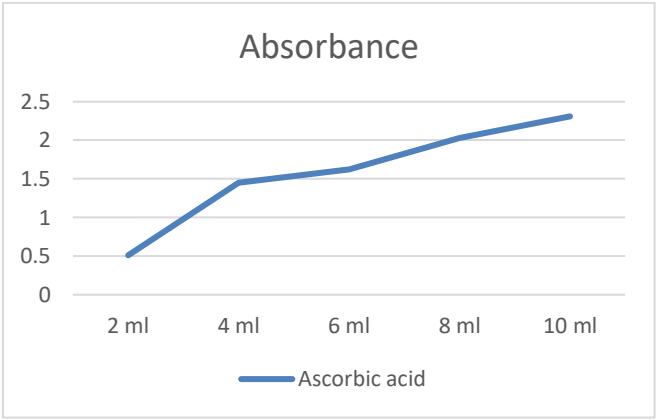


Table 5: Absorbance of various concentrations of ethanol and chloroform extracts of *G. corticata* in FRAP assay

Extract Conc	Absorbance of ethanol extract	Absorbance of chloroform extract
2 ml	0.030	0.023
4 ml	0.034	0.034
6 ml	0.065	0.045
8 ml	0.067	0.048
10 ml	0.070	0.064

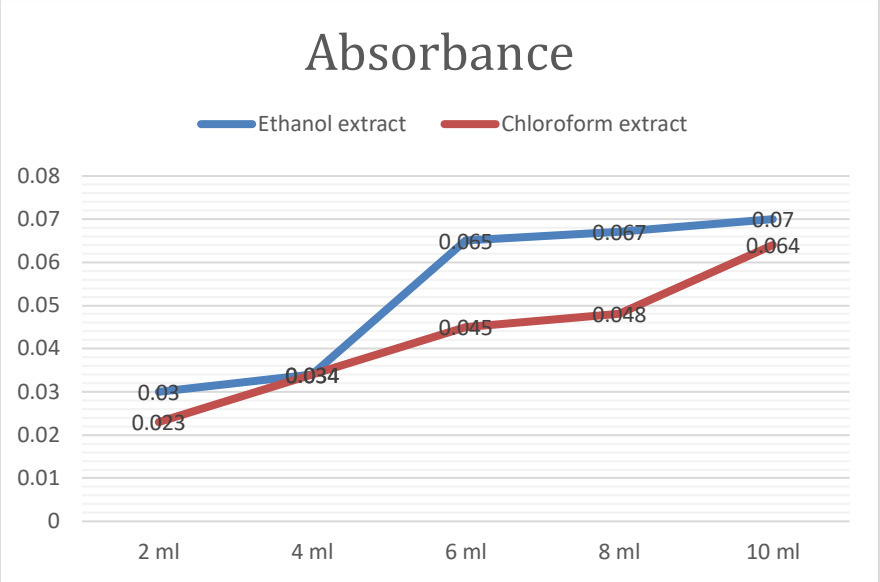
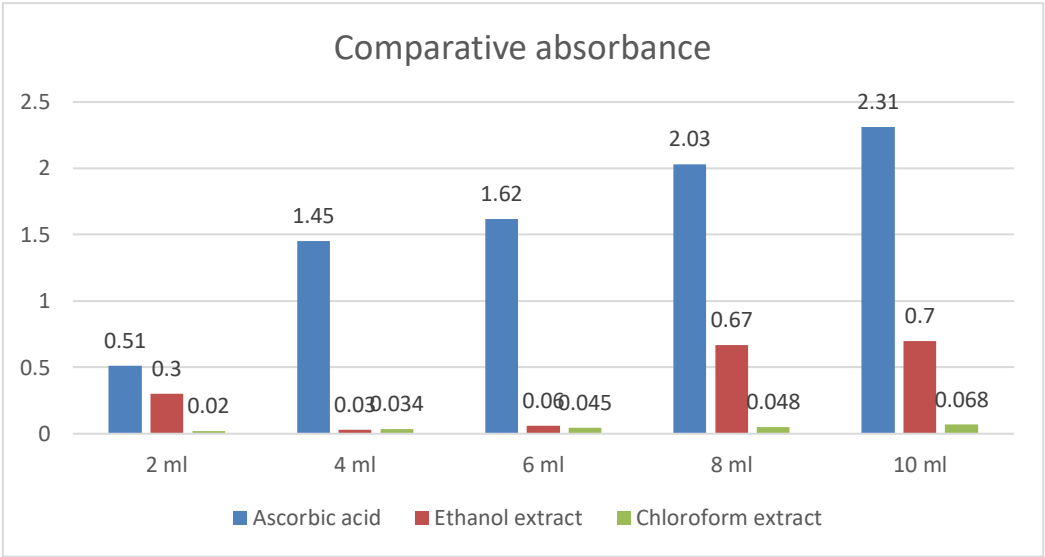


Fig: 4: The absorbance of ethanol and chloroform extracts of *G. corticata* in FRAP assay

The inhibition of ferric radicals by ethanol and chloroform extract of *Gracilaria corticata* was in a concentration dependant manner. The ethanol extract exhibits slightly better antioxidant activity than chloroform extract as shown in Table 5 and fig 4.



A comparative chart of the absorbance of Ascorbic acid, ethanol and chloroform extracts of *G. corticata* in FRAP assay

DISCUSSION

Algae have attracted great importance in the recent years due to the large number and amounts of bioactive components in them. More than 600 trace elements are found in high concentration in the seaweeds compared to the terrestrial plants, because of which it has various pharmacological activities. The seaweeds offer greatest wealth in terms of biomass and Rhodophytes show the largest representation among them. The red sea weed *Gracilaria* is amongst the largest group with over 150 species world wide and nearly 28 species in India (Sahoo *et al*., 2001).

Gracilaria has been identified as a rich source of various bioactive compounds as assessed by the studies on its different species such as *G. corticata, G. dentata, G. edulis, G. opuntia, G. pygmaea* and *G. verrucosa* carried out by various researchers. In a study carried out by Balakrishnan *et al.* in 2013, phytochemical screening of *G. corticata* was done using different solvents like methanol, ethanol, petroleum ether and acetone revealing the presence of most of the bioactive components of which alkaloids, phenols, quinones and steroids are most abundant. In a similar investigation led by Gnanaprakasam *et al*, (2017), the hexane, chloroform, ethyl acetate, acetone and methanol extracts of *G. corticata* were used to analyse the phytochemicals, and results revealed that the terpenoids, tannins and phenolic compounds were present in the all the extracts and alkaloids were present only in the chloroform and ethyl acetate extracts.

Antibacterial activity refers to the process of killing or inhibiting the disease-causing bacteria. Several plants have been traditionally used for their antibacterial activity. Like plants some algae also exhibit antibacterial properties due to the presence of terpenoids, steroids, saponins, tannins, and flavonoids. There are numerous reports regarding the inhibitory activities of macroalgae against human pathogens, fungi and yeasts. So, the use of algae as an alternative for prevention and treatment of infectious diseases has been suggested by Abirami and Kowsalya (2012). In the present study ethanolic and chloroform extract were evaluated for activity against Gram positive *Staphylococci* and Gram-negative *E. coli.* It was found that ethanolic extract had bacteriostatic activity against both the bacteria at all concentrations treated and the effect was dose dependent. Sanaraj P., 2013, in his study on *G. edulis* also reported maximum activity against Gram positive bacteria in ethanol extracts. Rashida et al

(2019) also report ethanol extract of *Gracilaria* to have higher antibacterial activity than other solvents.

In the preliminary assay conducted to evaluate antibacterial activity of *Gracilaria* species against human pathogens by Sushanth (2012), ten different organic solvents were considered. This study also reported that the extracts of ethanol and chloroform were the most potent of all.

Johnsi et al (2011), studied the antibacterial activity of aqueous extract of four seaweeds against ten pathogenic bacteria. This study reports the aqueous extract of *Gracilaria corticata* as having the highest potency against the pathogen *Proteus mirabilis.* In the current study however extracts in both solvents show bacteriostatic activity against E. coli and Staphylococci. Neither extracts are bactericidal and show a mild inhibitory zone of 7 - 8 mm.

Different solvent systems were used to extract bioactive principles from macroalgae with concomitant changes in the antibacterial activities. The solvents such as acetone, benzene, butanol (Vanitha et al., 2003; Prakash et al., 2005), ethanol (Selvi et al., 2001) were used to extract antimicrobial compounds from macroalgae. The aqueous extracts prepared from seven macroalgal samples showed varying degrees of activity against tested pathogens, including the Gram positive and Gram negative bacteria. (Johnsi et al, 2011). Padmakumar (2002) is of the opinion that these differences are due to the different solubility behaviour of secondary metabolites which could be influenced by seasonal and geographical distribution of the species.

Antioxidants are compounds that protect human, animal and plant cells against the damaging effects of free radicals. Free radicals can be defined as any species containing one or more unpaired electrons in atomic or molecular orbitals and capable of independent existence (Halliwell, 2011). In recent times, marine algae have been gaining importance as sources of pharmacologically active constituents possessing antioxidant, antiproliferative, antimutagenic, antidiabetic, anticoagulant, antibacterial and antitumor activities (Smit AJ, 2004; Folmer F et al., 2010). Exploration for bioactive compounds led to the screening of selected marine algae from the Tamil Nadu coast for antiproliferative and antioxidant activities (Murugan K & Iyer VV, 2013).

The standard protocols were used for qualitative analysis of samples to check for the presence of alkaloids,cardiac glycosides, flavonoids,phenols,tannins, saponins and terpenoids.It is

concluded that Gracilaria corticata has alkaloids, flavonoids,tannins,saponins and cardiac glycosides. Alkaloids are reported to be biologically and therapeutically active (e.g. morphine, atropine and quinine) and have numerous medical applications.Flavonoids are reported to possess antioxidant, free radical scavenger, antileukemic, vasodilator and antibacterial properties and are reported to be useful for improving blood circulation in brain of Alzheimeric patients.Saponins have a wide range of medicinal properties including hypo-cholesterolemic, anticarcinogenic, anti-inflammatory, anti-microbial and antioxidant. The cardiac glycosides are basically steroids with an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle when administered through injection into man or animal. Cardiac glycosides and catecchloamine are agents of choice in treatment of congestive cardiac failure.

The total antioxidant activity can be measured by the ferric reducing antioxidant power assay(FRAP).The flavonoids and phenolic acids present in the medicinal plant exhibit strong antioxidant activity which is depending on their potential to form the complex with metal atoms, particularly iron and copper. This method is based on the principle of, increase in the absorbance of the reaction mixture, indicates an increase in antioxidant activity. From the results it is concluded that the inhibition of ferric radicals by ethanol and chloroform extract of *Gracilaria corticata* was in a concentration dependant manner. The ethanol extract exhibits slightly better antioxidant activity than chloroform extract.

Marine algal extracts have been demonstrated to possess strong antioxidant properties (Fernando et al., 2016). The present study indicates that the seaweed possesses strong antioxidant activity. The reducing capacity of a compound may serve as a vital recorder of its potential anti-oxidant activity. For the estimation of reductive ability, transformation of Fe3+-Fe2+was investigated. The change in the optical density of the mixture was measured at 700 nm (Polterait, 1997). An increase in optical density indicates higher reductive ability (Repetto M.G et al., 2002). The study also evaluated the difference in antioxidant potential shown by the algae in two different solvents; Ethanol and Chloroform.

SUMMARY AND CONCLUSION

Algae are an important constituent of the aquatic ecosystems and can be seen in water bodies like oceans, seas, lakes, estuaries and so on. They can be of different types and in different colours. Mostly seen seaweeds are macroalgae. They can be used as food and are a storehouse of bioactive components like vitamins, phenolics, terpenoids and other secondary metabolites. They also possess antibacterial, antioxidant, antifungal properties. Red algae is used for the extraction of agar (*Gracilaria*). It also shows few antibacterial properties.

The present study was done to estimate the difference in extractive yield, phytochemical analysis , antioxidant and antimicrobial potential of the dried form of, *Gracilaria corticata* in polar and non-polar solvents, ethanol and chloroform respectively. The whole plant body was taken for the study. The cleaned, dried and powdered sample was extracted using the sohxlet apparatus. Extractive values of plant materials are often used to evaluate extracts of the sample, in order to get an idea about the nature of chemical constituents present in it. It can also be used to assess quality, purity and detect adulteration of the extract. *G. corticata* showed a better elution for polar solvent than non-polar solvent. The extractive yield obtained was more for ethanolic extract (2.3%) as compared to chloroform extract (0.5%).

The standard protocols were used for qualitative analysis of samples to check for the presence of alkaloids,cardiac glycosides, flavonoids,phenols,tannins, saponins and terpenoids.It is concluded that *Gracilaria corticata* has alkaloids, flavonoids,tannins,saponins and cardiac glycosides.

In the present project, antibacterial potential of *Gracilaria corticata* was tested against two non pathogenic bacteria, the Gram negative *E. coli* and the Gram negative *Staphylococcus* by the disc diffusion method. It is concluded that the organic solvent extraction by ethanol and chloroform was suitable to verify the antimicrobial properties of *Gracilaria corticata* and they were supported by many investigations.

The current investigation showed that *G corticata* has antimicrobial potential. The ethanol extract has better antimicrobial activity when compared to chloroform extracts. Ethanol extract

was bacteriostatic for both gram negative (*E. coli*) and gram positive (*Staphylococcus*) bacteria at all concentrations studied. Whereas the extract in chloroform showed no significant activity except in the higher concentration (60%) and only against the Gram positive *Staphylococcus*.

The total antioxidant activity is measured by the ferric reducing antioxidant power assay(FRAP).The flavonoids and phenolic acids present in the medicinal plant exhibit strong antioxidant activity which is depending on their potential to form the complex with metal atoms, particularly iron and copper. This method is based on the principle of, increase in the absorbance of the reaction mixture, indicates an increase in antioxidant activity. From the results it is concluded that the inhibition of ferric radicals by ethanol and chloroform extract of Gracilaria corticata was in a concentration dependant manner. The ethanol extract exhibits slightly better antioxidant activity than chloroform extract.

The present study justifies the claimed uses of *G. corticata* in the traditional system of medicine to treat various infectious diseases caused by the microbes. These results suggest the possibility of using marine algae extracts in therapy as natural alternatives to antibiotics currently in the market, and clearly show that seaweeds are a valuable source of biologically active compounds.

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. To balance the oxidative stress, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase), produced internally, or the dietary antioxidants vitamin C and vitamin E. Algae possess potent antioxidant compounds that are suitable for protecting human bodies against damaging effects of reactive oxygen species produced as a result of normal metabolism of the body. Thus, it reduces the risk of various cancers, as well as prevents menopausal symptoms.

The total antioxidant activity of ethanol and chloroform extracts of *Gracilaria corticata* was measured using Ferric Reducing Antioxidant Power (FRAP) Assay. The assay measures the antioxidant potential in the sample through the reduction of ferric ion (Fe3+) to ferrous ion (Fe2+). Following the reduction of ferric ion, a colored complex develops which is read using spectrophotometer at 700nm. Thus, the colored complex formed by ethanolic and chloroform extract of *Gracilaria corticata* signifies its antioxidant potential. Both the extracts administered in the Ferric Reducing Antioxidant Power Assay, were found to possess good antioxidant effect

which was concentration dependent. Hence, this potential can be further utilized and can be studied further.

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