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ORIGINAL ARTICLE

In Vitro Study Antibacterial, Anti - Hemolytic and Antioxidant Activity of Green Seaweed *Halimeda gracilis* (Harvey Ex L.Agardh, 1887)

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ABSTRACT
Seaweed is a marine macroalgae found globally in the ocean having potent applications. Seaweed based industries are blooming nowadays due to their versatile applications in agriculture, pharmaceuticals and nutraceuticals. Our objective of the study was to determine the phytochemicals, Antibacterial, Antioxidant and Anti-hemolytic activity of the extracts Butanol and Methanol of H. gracilis. The phytoconstituents like alkaloids, flavonoids, phenols, saponins, sterols, terpenoids, glycosides, Quinone's and tannin were qualitatively investigated in both the extracts of H.gracilis species. Antibacterial efficacy of Butanol and Methanol extract of H.gracilis were determined against the human pathogens (Klebsiellasp, Salmonellasp, Vibriosp and Bacillussp). The highest activity was observed in the butanol extract for Klebsiellasp strain (24 mm) and the minimum activity in Bacillus sp (18mm) whereas Methanol extract showed maximum activity against Salmonella sp (24 mm) and minimum activity in Bacillus sp (20mm). Thin layer chromatography (TLC) profiling reveals the presence of amino acids and peptides. The hemolytic activity was recorded high in Methanolic extract 46 HU/mg whereas butanol shows less activity of 32 HU/mg. The radical scavenging activity of H. gracilis recorded maximum activity in Methanolic extract.
Key words: *Antibacterial activity, Anti-hemolytic, DPPH and phytochemicals.*

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INTRODUCTION

Seaweed are marine non flowering plants which are found attached to the underneath of the shallow coastal waters. Based on the pigments, seaweeds are classified into three major categorizes viz., Green (*Chlorophyceae*), Brown (*Phaeophyceae*), and Red (*Rhodophyceae*). Seaweeds are important renewable resources as a part of human civilization from ancient times as early as 2500 years ago in the Chinese literature [1].India is a tropical south Asian country with the coastline of more than 7000 km, which harbors a great diversity of marine algae species. Southwest coast of India has a unique marine habitat flooded with diverse seaweeds. Many of the rocky beaches, mudflats, estuaries, coral reef and lagoons alongside the Indian coast provide model habitats for the growth of seaweed [5].Seaweeds are rich in minerals [2], high amount of potassium, magnesium and calcium which helps in maintaining mineral balance and blood pressure [3], enriched with fatty acids [4]. Histidine and Taurine amino acids which are abundantly present in seaweed species having wide pharmacological importance.

Antioxidant activity of macro algae is appreciable, since these species are believed to develop a very efficient antioxidant defense system owing to the strong UV radiation in the tropical environment. In fact, earlier studies have proved that UV radiation stimulates the promotion of antioxidant defense in macro algae [6, 7]. Antioxidant compounds play an important role against different types of diseases (e.g.,

chronic inflammation, atherosclerosis, cancer and cardiovascular disorders) and ageing processes [8], they have considerable commercial potential in food production, medicine and the cosmetic industry.
More than 300 secondary metabolites have been identified in green algae and less than half of the green algae are under the order Bryopsidales [9]. Green algae belonging to the Order Udoteaceae, Caulerpaceae, and Halimedaceae produce more than 85% of bioactive compounds than Bryopsidales. Seaweed belonging to the order Halimedaceae has the potential of antioxidant and anticancer agents[10]. The present study was aimed to determine its antibacterial, anti-hemolytic, phytochemical and antioxidant activity properties of the solvent extract (Butanol and Methanol) *Halimeda gracilis.*

MATERIAL AND METHODS

Collection of Seaweed

Seaweed *Halimeda gracilis* were handpicked and collected wild in mass quantityfrom the Mandapam coastal area (9.2770ºN and 79.1252 º E) on the Southeast coast of India. Collected Seaweeds were transferred to the laboratory in a plastic container**.** They were washed completely with running tap water to remove animal castings, epiphytes, sand particles and attached debris. The final washings were done using distilled water and then they were shade dried.

Preparation of Seaweed extract

Shaded dried seaweed were powdered using mechanical blender. It was stored in airtight containers. The seaweed extract were done by following the method of Sreenivasa-Rao and Parekh [11]. The seaweed dried powder was extracted in soxhlet apparatus using Methanol and Butanol (200ml) solvent for using. Extract were stored at 4^0C until used.

Phytochemical Analysis

The seaweed samples were exposed to a qualitative test for the investigation of phytochemical content withthe standard procedures [12].The seaweed powder extracts were tested for alkaloids, flavonoids,Quinones,saponins, steroids, tannins, and terpenoids.

Thin layer chromatography

The seaweed extraction used in phytochemical analysis, and qualitative analysis of secondary metabolites by TLC method [12].

Test microorganisms

Test pathogens Gram negative - *Klebsiella*sp, *Salmonella*sp, *Vibrio*spand Gram positive - *Bacillus* sp.were collected from Raja Muthiya Medical College and Hospital, Annamalai University, Chidambaram, Tamil Nadu.

Preparation of inoculum

The bacterial isolates were inoculated in nutrient broth and incubated at 37ºC for 4 hours in a shaker (Orbitech, Scigenics, India) and were used for anti - bacterial activity effect and to examine its MIC of various extracts and fractions.

Preparation of discs

Extract of known quantity was dissolved in DMSO. It was then filter sterilized by using sortorious syringe filter of pore size 0.22 µm. sterile discs of 6 mm diameter (Hi-Media) were loaded with various concentrations of extracts and they were dried. Dried discs were then stored in sterile containers for further use. Solvent loaded discs were used as negative control. Chloramphenicol Loaded Hi-Media discs were used as positive control.

Antibacterial properties

Antibacterial activity was determined by the following agar diffusion method [13]. The inoculated bacterial strains (0.1 ml) were culture in Mueller Hinton Agar medium (20ml) and kept for 24 hours at 37ºC. Following this, paper disc were impregnated on the shallow of the plate by using sterilized forceps and then loaded with different concentration on 50µg/ml, 75µg/ml and 100 µg/ml of each integumentary extract. These prepared plates were then incubated for 24 hours at 37ºC. Finally, the antibacterial activity was assessed by observing the zone of inhibition. The antibiotic Ampicillin (30µg/ml) was used as positive control.

Anti-hemolytic assay

The hemolytic activity of crude extracts of seaweed (*Halimedagracilis*) were assayed on chick erythrocytes the method described by Paniprasad and venkateswaran[14].

Anti-oxidant assay

DPPH free radical scavenging assay

The antioxidant potency of the different algal extracts was determined using 2,2-diphenyl-1-picrlyhydrazyl (DPPH) free radicals scavenging technique. 150 µl DPPH solution (4.3 mg dissolved in 3.3 ml methanol) was added to 3 ml methanol and the absorbance was taken immediately at 517 nm for

control reading. Different volumes of test sample (20, 40, 60, 80 and100 µl) were taken and diluted with methanol up to 3 ml to be screened with 100 µl DPPH solution added to each test tube. The mixture was vortexes and kept at room temperature for5 min in the dark place. Absorbance was taken at 517 nm spectrophotometrically using methanol as a blank. The percentage of DPPH free radicals scavenging activity was calculated with the formula:

% Inhibition= [(Absorbance of the control – Absorbance of the test sample) / Absorbance of the control] x 100

Entirely reactions were carried out in duplicates and the points of purple color develop and decolorization indicates the free radial scavenging activity of the extracts [15]. The antioxidant effects of the tested extracts will then be compared to that produced by ascorbic acid as standard antioxidant.

RESULTS

Phytochemical test

The qualitative phytochemical screening was done in the Butanol and Methanol extracts of *H. gracilis* and it was carried out to assess the presence of bioactive compounds which might have anti-bacterial potency. Alkaloids, saponins were identified from two different extracts of *H. gracilis*. Flavonoids, Tannin, Glycosides were identified from methanol extract and Terpenoids, steroids; Quinones were identified from Butanol extract (Table 1).

Thin layer chromatography

Thin layer chromatography (TLC) profiling was done for the seaweed extracts in solvent system of Butanol and Methanol in proportions of 6:4. The plates developed light pink spot in both the solvent systems when the TLC plate is sprayed with ninhydrin.Ninhydrin is the chemical compound that is used widely in detection of finger markers on porous surfaces such as paper and cardboard. This compound was reacting with amino acid.The pink spot obtained, indicated the presence of amino acids and peptides.

Antibacterial properties

The antibacterial activity of Butanol and Methanol extracts of the *H. gracilis*was experimented. The results suggested that they show strong inhibition over growth of bacterial strains. The maximum zone of inhibition was observed in Butanol extract in *Klebsiella* sp. (24mm) and minimum zone in *Bacillus* sp. (18mm). Methanol extract showed maximum zone on *Salmonella* sp. (24mm) and minimum zone in *Bacillus* sp. (20mm). The comparative antibacterial effects of the extracts of *H. gracilis* were done by using standard drug Ampicillin.

Hemolytic assay

The seaweed extract were shown to produce prominent hemolytic activity on chicken blood. Hemolytic factors were present in the samples. The chicken blood showed 32HU/mg of Butanol extract in H.*gracilis*and the highest hemolytic activity of 46HU/mg were observed in the Methanolic extract of *H. gracilis.*

Antioxidant assay

The scavenging activity of the sample on DPPH radical was found to be strongly dependent on concentration. In general, the scavenging effects on the DPPH radical increased sharply with increasing concentration of all the samples and standards to a certain range and then slowly increased. The antioxidant components extracted from the seaweeds material. This study was found to be seaweed extracts of the intentional samples to show variable degrees of free radical scavenging activity. Table 4 and fig 3 combined methanol extraction of H. *gracilis* showed the maximum activity of more than (57.65921 %). The DPPH activity of standard ascorbic acid showed higher free radical scavenging activity (66.966 %) than methanol extract of H. *gracilis* at each different point.

Table.1- Phytochemical screening of seaweeds extract

Test	*H. gracilis*	
	Butanol	*Methanol*
Alkaloids	+	+
Steroids	+	-
Terpernoid	-	-
Tannin	-	+
Saponin	+	+
Flavonoid	-	-
Quinones	+	-
Glycosides	-	+

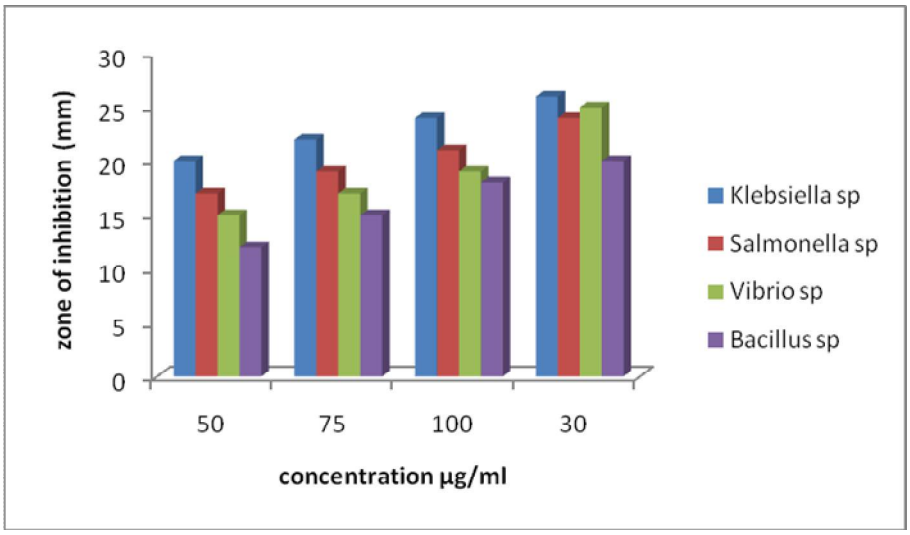

Fig.1- Antibacterial activity of Butanol extract in H. gracilis against bacterial pathogens

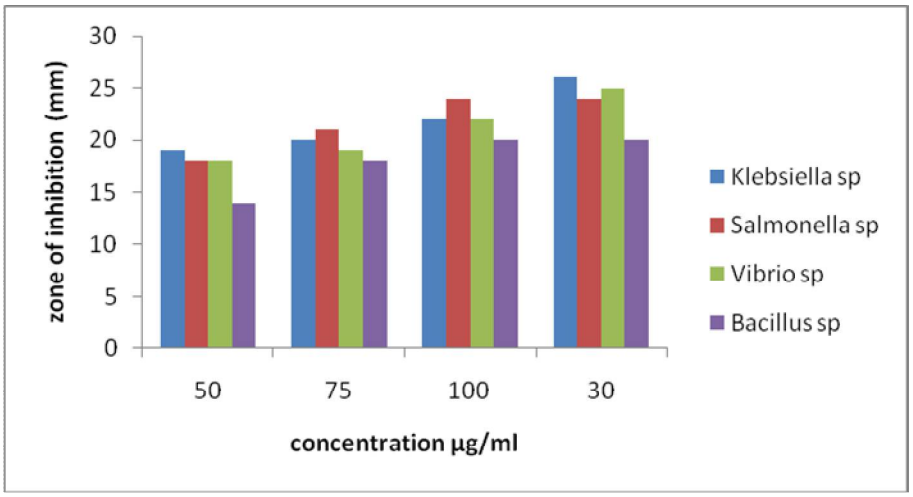

Fig.2- Antibacterial activity of the Methanol extract in H. gracilis against bacterial pathogens

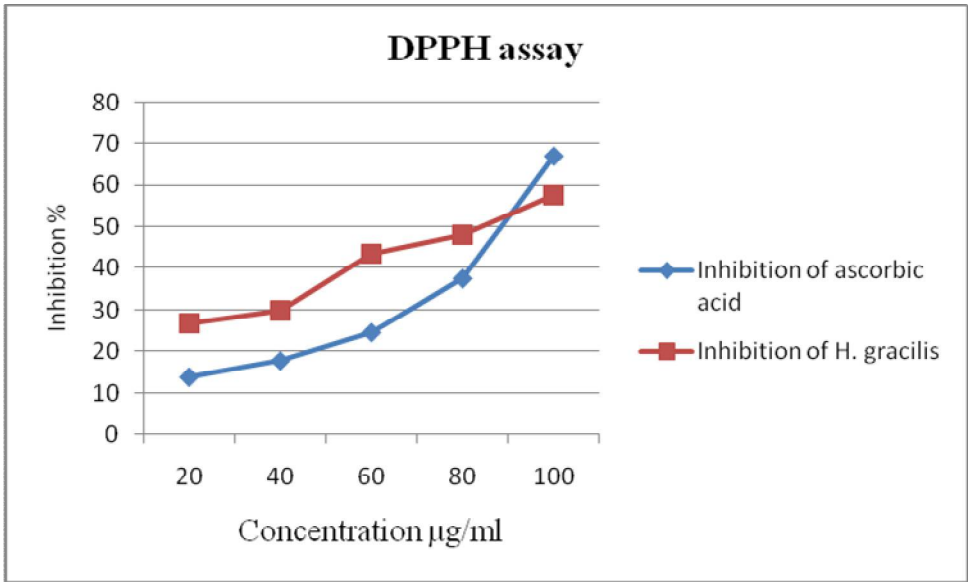

Fig 3 – Antioxidant activity by DPPH method

DISCUSSION

Marine organisms are a rich source of novel and biologically active metabolites, producing the pharmaceutical industry with potential bioactive compounds of interest. It has been shown that both solvent extracts and constituents of different algae have in vitro antibacterial activity against gram-positive and gram-negative bacteria [16,17]. In the present analysis, we noticed that several major phytochemical compounds, antibacterial activities, hemolytic activities, and antioxidant activity were established in the solvent extraction of *Halimedagracilis*.

In the present study, the solvent extraction of *Halimeda gracilis* identified several most important phytochemical compounds, Antibacterial activities, Hemolytic activities, and antioxidant activity. The bioactive component is present in the seaweed to effect of antibacterial and antioxidant activity. To reports have identified seaweeds as being rich in natural antioxidant compounds [18].

In this study 8 most important phytochemicals namely Alkaloids, Steroids, Terpenoids, Tannin, Saponin, Flavonoid, Quinones and Glycosides tested for two different extraction solvent was using in the *Halimeda gracilis* sea weed species.

The preliminary phytochemical screening suggests that different compounds are present. Seaweeds are abundant in secondary metabolites, including alkaloids, glycosides, flavonoids, saponins, tannins, steroids, and associated active metabolites, which have been widely used in the drug and pharmaceutical industries and are of great medicinal value. Phenols and flavonoids are antioxidants and play a key role in seaweed bioactivity. The antimicrobial, anti-inflammatory and haemolytic effects of some of these bioactivities are [19]alkaloids, tannins, saponins, steroids, Quinone's and glycosides were contained in the present analysis of the methanol and butanol extract of *Halimedagracilis* seaweed. Similar findings from four separate H extracts have been described. Gracilis includes terpenoids, hormones, tannins, saponins, alkaloids, quinones and glycosides [20].

In addition, the presence of amino acids and peptides in the methanol and butanol extract of *Halimeda gracilis* was suggested by our present TLC plate method showing a pink spot. The comparable findings exposed in the *U. Fasciata* Seaweed Extract [21].

Seaweeds are known as a source of bioactive compounds which have been found in green, brown and red algae with antibacterial, anticoagulant, antifungal, anti-inflammatory antiviral activities [22]. Owing to their widespread existence in the aquatic environment, the selected bacterial strains (*Klebsiella*sp, *Salmonella*sp, *Vibri*o sp and *Bacillus*sp) are of considerable significance in terms of causing economic losses. Seaweed *H.gracilis*has antibacterial potential against Gram negative and Gram positive human pathogenic bacteria. There was a wide range of antibacterial activity in the methanol and butanol extract of the *H.gracilis* seaweed test. The maximum zone (22mm) activity was reported in *Salmonella* sp. in the current study. In an extract of methanolic in *Klebsiella* sp., low activity (18mm) was reported in an extract of butanol. Similar findings have also been obtained, showing the highest inhibition zone against *Klebsiella pneumoniae* (18 mm), *Salmonella typhi* (15 mm), *Vibrio cholera* (14 mm) and the minimum inhibition zone against *E.coli* (11 mm)[23] and *Halimeda* sp.No antibacterial activity was demonstrated, while the antibacterial activity of these species was widely documented in *Halimeda* sp. [24,25,26].Respectively Seaweed methanol extracts have also been reported to contain phenolics, alkaloids, and amino acids that may be responsible for antimicrobial activity [27,28].

The seaweed extract as well as the produced hemolytic activity on chicken erythrocytes. Hemolytic factors were present in the seaweed sample. In our present study, high hemolytic activity was present in the methanolic solvent seaweed extract. Similarly, the crude extract of hemolytic activity on chicken and go at blood the most vulnerable to lysis provoked by the *U. fasciata*. The extract of chicken blood showed a maximum of 64 HU/mg for *U. fasciata* and the goat blood showed a maximum of 32 HU/ mg[22].

The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. In the present study, DPPH scavenging activity was compared to the highest scavenging activity was similarly found in the Ethanolic extract from *S. wightii* [29].

The effect of antioxidants on radical scavenging of DPPH is thought to be due to their capacity to donate hydrogen. Seaweed extracts of methanol and butanol demonstrated antioxidant activity to varying degrees during the study. At concentrations of 20, 40, 60, 80 and 100 mg, the scavenging effect of the checked extracts on the DPPH radical decreased in the order of *Halimeda gracilis* Methanol and butanol extract, respectively. Several studies have shown a very important link between the phenolic content and the efficacy of antioxidants in seaweed extracts [30]. In accordance with our results, higher radical scavenging activity was found in *Sargassum wightii* (brown algae) with the largest phenolic constituent [31, 32], where higher phenolic content and increased antioxidant activity were observed in *Halimeda gracilis* methanol extract.

The major role of antioxidants in the health of humans has been demonstrated hence increasing its interest in such products and their need by consumers. Marine algae function as important resources for bioactive natural products [33]. Algae have traditionally been used as a diet in different countries; they have been hardly used as phytopharmaceutical or traditional medicine [34, 35]. The importance of the study of seaweeds as sources of antioxidants compounds has increased in the previous years. Seaweed extracts have the ability to constrain lipid peroxidation or to scavenge free radicals [36].

CONCLUSION

This study showed the antimicrobial, free radical scavenging activity and hemolytic activity of the methanol and butanol extract of *Halimeda gracilis* seaweed. The presence of phytochemical substances present in the extract may be an activity of the methanol extract of *Halimeda gracilis* seaweed. The research provides opportunities for new types of bioactive compounds to be produced; however, there is still no clear mechanism of inhibition and stability of the extracts and further studies should be involved. Seaweed is one of the most important marine living properties. These plant are using in food, feed and medicine fertilizer and as source of medicinal drugs preparation and are used for industrial production. They are consumed on a large scale for several purposes using in industries production. The seaweed has many industrial uses for present in multi-functional properties in the form of food, energy, medicine and cosmetics and also very useful to feed for cattle and birds.

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