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PRELIMINARY PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES ON THE CRUDE EXTRACT OF RED ALGAE *GRACILARIA EDULIS* AGAINST CLINICAL ISOLATES

Rajakumar R.* and Allwin Prem Singh Y.

Post graduate and Research Department of Biotechnology, Marudupandiyar College, Thanjavur-613 403, Tamil Nadu, India.

***Corresponding Author: Rajakumar R.**

Post Graduate and Research Department of Biotechnology, Marudupandiyar College, Thanjavur-613 403, Tamil Nadu, India.

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ABSTRACT

Phytochemicals are naturally present in the seaweeds and are biologically significant and plays an essential role in defending themselves against various pathogenic microbes by showing the antimicrobial activity by inhibition or killing mechanisms. In the present study revealed that the preliminary phytochemical, antibacterial and antifungal activities of crude extract of *Gracilaria edulis* were investigated. The methanol and aqueous extracts of *Gracilaria edulis* showed the presence of a number of metabolites such as alkaloids, saponin, phenols, terpenoids, proteins, flavonoids, glycosides, coumarins and tannins. *G.edulis* showed the significant antibacterial activity against in the clinical pathogens of *Pseudomonas aeroginosa, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Streptococcus pyogenes, Salmonell typhi* as well as the fungus *Aspergillus niger, Aspergillus flavus, Rhizopus indicus* and *Candida albicans*. The methanol extracts of *G.edulis* showed maximum activity of 18mm (100µg/ml) of inhibition zone against *Staphylococcus aureus* and followed by the methanolic extracts of *P.aerogenosa* (16mm) and aqueous extract of *S.aureus* (14mm) when it was compared to the positive control Gentamycin. The methanol extract showed the broader spectrum of antibacterial and antifungal activity when compared with aqueous extract.

KEYWORDS: Antimicrobial, phytochemical, *Gracilaria edulis*, aqueous extract.

INTRODUCTION

In recent years, marine resources have attracted attention in the search for bioactive compounds to develop new drugs and healthy foods (Qi, Zhao, Zhang, Li, and Zhao, 2005). In particular, seaweeds are a very important and commercially valuable resource for food, fodder, soil conditioners and pharmaceuticals (Yang *et al*., 2006). Seaweeds of the marine macrophytic algae are an assemblage of the members of Chlorophycease, Phaeophycease and Rhodophycease and, are the common inhabitants of the tidal and inter-tidal environments of the marine ecosystem. Seaweeds have been widely used for human consumption in many parts of the world. In general marine algae contains (i) protein with all the essential amino acids-unlike most plant foods (ii) a high carbohydrate content (iii) an extensive fatty acid profile, including Omega 3 and Omega 6 and (iv) an abundance of vitamins, minerals and trace elements in a naturally-occurring synergistic design. Macroalgae can be classified as red algae (*Rhodophyta*), brownalgae (*Phaeophyta*) or green algae (*Chlorophyta*) depending on their colour, nutrient and chemical composition. Red and brown algae are mainly used as human food sources. Seaweed species are rich in beneficial nutrients, in countries such as China, Japan and Korea, they have been commonly utilized in human alimentation.

Many metabolites isolated from marine algae possess bioactive effects. The discovery of metabolites with biological activities, from macro algae, has increased significantly in the past three decades; on the other hand, seaweeds have recently received significant attention for their potential as natural antioxidants. Marine organisms are a rich source of structurally novel and biologically active metabolites (Perry *et al.,* 1991). Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. Some seaweed has the valuable medicinal value components such as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. In the marine ecosystems seaweeds are directly exposed and are susceptible to ambient microorganisms such as bacteria, fungi and viruses (Ranganayaki *et al.,* 2014).

Gracilaria edulis is exclusively marine red algae. It varies in size and shape. They are either epiphyte, grow

as crust on the rocks or shells as a large fleshy, and branched like thalli. *G.edulis* farming is considered as an alternative livelihood for fisherman around the coastal region. There are several benefits arises from this species such as medical and food wise. In the present study, the phytochemical screening from marine red algae and evaluate their antimicrobial potential.

MATERIALS AND METHODS

Collection and extraction of sample

The fresh Red algae *Gracilaria edulis* was collected by hand picking method at a depth of 1 to 2 meter in low tide Palk bay coastal area of Manora (10.268°N 79.304°E), Tamil Nadu, India and was brought to the laboratory by keeping them in plastic bags with seawater. The sample was washed thoroughly with seawater to remove epiphytes, followed by tap water and distilled water so as to remove the salts and other extraneous materials. The sample was shade dried with sterile paper for 15 to 20 days and ground with the help of electric mixer. The powdered seaweed was extracted with an Methanol in soxhlet extractor for 12hrs. The extracts were then concentrated under reduced pressure using a rotary flash evaporator. In aqueous extraction the sterilized fine powder was mixed with 200ml of Milli Q water and kept in boiling water bath at 60°C for 10 min. The extracts were filtered with Whatman 1 filter paper.

Phytochemical Analysis

Phytochemical screening was carried out to assess the qualitative chemical composition of crude extracts using commonly employed precipitation and coloration to identify the major natural chemical groups such as alkaloids, saponin, phenols, terpenoids, proteins, carbohydrates, flavonoids, glycosides, coumarins, steroids and tannins (Raman, 2006).

Antibacterial Activity

Antibacterial activity was carried out against six selected Bacterial pathogens (such as *Pseudomonas aeroginosa, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Streptococcus pyogenes, Salmonell typhi*). The strains used for the present study were obtained from Department of Microbiology, Marudupandiyar College, Thanjavur. In order to access the biological significance and ability of the plant part, the minimal inhibitory activity was determined by Agar well diffusion method. Petri plates containing 20ml of Muller Hinton medium were seeded each with 24hr old culture of bacterial strains. Wells of approximately 10mm diameter was bored using a well cutter and 50µl and 100µl of the extracts were added to the well from a stock concentration of 0.1g/1ml. The plates were then incubated at 37°C for 24 hours. Antibacterial activity was assayed by measuring the diameter of the inhibition zone in millimetres formed around the wells (NCCLS, 1993). Gentamycin (standard antibacterial agent, concentration: 20mg/ml) was used as a positive control.

Antifungal Activity

Antifungal activity was also determined by Agar well diffusion method. Potato Dextrose agar plates were prepared and overnight grown isolates of fungi such as *Aspergillus niger, Aspergillus flavus, Rhizopus indicus* and *Candida albicans* were swabbed. Wells of approximately 10mm diameter was bored using a well cutter and extracts of 50µl and 100µl concentrations were added and the zones of inhibition were measured after overnight incubation which was then compared with that of standard antibiotics. Clotrimazole was used as a positive control.

RESULTS AND DISCUSSION

Phytochemical analysis

Phytochemical screening revealed that the presence of alkaloids, saponin, phenols, terpenoids, proteins, carbohydrates, flavonoids, coumarins, steroids and tannins in the and absence of glycosides in the methanolic extract and the presence of saponin, phenols, proteins, carbohydrates, flavonoids, glycosides, tannins and absence of alkaloids, terpenoids, coumarins and steroids in the aqueous extract of *Gracilaria edulis* (Table.1).

Antimicrobial activity

The antimicrobial activity methanol and aqueous extracts of *G.edulis* against bacterial and fungal pathogens and the result presented in the Table 2 and 3. The antibacterial activities of *G.edulis* extracts against 6 human pathogens are presents in the Table.2. The methanol extracts of *G.edulis* showed maximum activity of 18mm (100µg/ml) of inhibition zone against *Staphylococcus aureus* and followed by the methanolic extracts of *P.aerogenosa* (16mm) and aqueous extract of *S.aureus* (14mm) when it was compared to the positive control Gentamycin. The lowest activity was showed in the aqueous extract against *S. typhi* (1mm in 50µg/ml). The antifungal activities of the methanolic and aqueous extracts of *G.edulis* were studies for strains of 4 fungal pathogens. The results were analyzed with that regular antibiotic Clotrimazole. The maximum inhibition zone was observed the methanolic extracts against *A. niger* (20mm), *A. flavus* (17mm) and *C. albicans* (17mm) in the concentration of 100µg/ml (Table.3). The minimum zone was observed in the aqueous extract of *G.edulis* against *R.indicus* in the concentration of 50µg/ml.

DISCUSSION

Marine halophytes provide good source of medicinal as well as natural health products or compounds by synthesis or secretion. These groups of plants provides large amount of antioxidants, phenol compounds, enzymes, biomolecules like carbohydrate content and some other biochemical compounds like free amino acids, phytochemical set in terms of reducing the saline stress. Phenolics compounds are synthesized via phenyl propanoid pathway and play a defence mechanism against biotic and abiotic stress.

The results of phytochemical screening of extracts the strength of active principle depend on the use of a suitable solvent besides the type of the plant species to achieve positive results. Hence extracts of *G.edulis* is highly recommended for the herbal preparations to the traditional medicinal parishioners. The results also show that more separation of compound is extracted by methanolic extract of *G.edulis.* Less separation of compound is recorded from aqueous extracts (Table.1). This was correlated with the previous work done by Harold Peter, 2011, reported that the whole plant extracts of *Canthium parviflorum* revealed the presence of phytochemicals such as alkaloids, oils, flavanoids, gums, phenols, saponins, steroids, tannins and terpenoids. Flavonoids possess anti-allergic, anti-inflammatory, antiviral and antioxidant activities (Bbosa, 2010). The results obtained in this study thus suggest that the identified phytochemical compounds may be the bioactive constituents of the marine red algae *G.edulis* is proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

The antibacterial activity of seaweeds may be influenced by some factors such as the habitat and the season of algal collection, different growth stages of plants, experimental methods etc. But variation in antibacterial activity may be due to the method of extraction and solvent used in the extraction preparation (Kandhasamy and Arunachalam, 2008). The present study reported that the methanol extracts of *G.edulis* showed maximum activity of 18mm (100µg/ml) of inhibition zone against *Staphylococcus aureus* and followed by the methanolic extracts of *P.aerogenosa* (16mm) and aqueous extract of *S.aureus* (14mm) when it was compared to the positive control Gentamycin. Kumar *et al.*, (2009) reported that the antimicrobial compounds from marine halophytes (*Salicornia brachiata, Suaeda maritima* and *Sesuvium portulacastrum*) revealed that antimicrobial activity were due to the presence of bioactive components such as sulfated polysaccharides. In addition the presence of sulfated galactose unit of the phycocolloid, 2-N-palmitoyl, 1-4-5, dihydro, 1,3,4,5 tetrahydroxyl spingosine and halogenated compounds were also identified as significant antimicrobial activity (De Nys *et al.*, 1995).

Ethanolic extracts of some coastal plants also possess activity against the pathogens. Nair and Chanda (2007) reported that the ethanolic extracts of nine medicinal plants showed significant antibacterial activity against the human pathogens than the other extracts. Boopathy (2003) studied the biology and antimicrobial activities of salt marsh and coastal plants. He examined the ethanolic extracts of *Suaeda monoica* and *Suaeda maritima* salt marsh plant showed effective antimicrobial activities towards dreadful pathogens. Anti-microbial activity of salt marsh differs with the solvent extracts and against the pathogen; it may be due to the habitat and the season of the salt marsh collection (Majak *et al.*, 1980). The present investigations also provide the effective activity and an alternative source of antibacterial compounds for human pathogens. Sheela and Kannan (2003) were reported that the antimicrobial activities of plants may vary from species to species. The efficiency of the antimicrobial activity of plants should be determined by the physiological and biochemical synthesis of antimicrobial principles.

CONCLUSION

The present study provides valuable information regarding the potential of seaweed as natural sources for Phytochemical constituents and Antimicrobial activities. The results emerged from the present study validate the potential use of renewable sources like these candidate seaweed to offer excellent nutritional and health package for use in food supplements in nutraceutical formulation and as health food for human consumption. Qualitative analysis of *G.edulis* showed that the presence of biologically important phytoconstituents viz., Flavonoids, Terpenoids, Tannin, Phenol and alkaloids. The results of the present study demonstrated that the *G.edulis* respectively possessed antimicrobial activities against the clinically isolated species of both bacteria and fungi. The antimicrobial activities suggest the possibility of therapeutic value of seaweed against the bacterial infection. The present study concluded that the marine red algae *G.edulis* has variety of biologically active molecules which can be used as a source of antibiotics. Further study need the purification of active compounds and structural elucidation can be used for drug discovery.

Table: 1 Preliminary Phytochemical screening of *Gracilaria edulis*

S.No	Phytochemical constituents	Methanol extract	Aqueous extract
1	Alkaloids	+	-
2	Saponin	+	+
3	Phenols	+	+
4	Terpenoids	+	-
6	Proteins	+	+
7	Carbohydrates	+	+
8	Flavonoids	+	+
9	Glycoside	-	+
10	Coumarins	+	-
11	Steroids	+	-
12	Tannins	+	+

Table: 2 Antibacterial activities of *Gracilaria edulis*

S.No	Test Organisms	Zone of inhibition (mm)				
		Methanol extract		Aqueous extract		Positive control
		50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	
1	*Pseudomonas aeroginosa,*	10	16	8	12	25
2	*Bacillus subtilis,*	3	7	3	3	24
3	*Bacillus cereus,*	5	9	2	6	22
4	*Streptococcus pyogenes*	9	13	4	7	21
5	*Salmonell typhi*	4	7	1	3	16
6	*Staphylococcus aureus*	12	18	8	14	17

Table: 3 Antifungal activities of *Gracilaria edulis*

S.No	Test Organisms	Zone of inhibition (mm)				
		Methanol extract		Aqueous extract		Positive control
		50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	
1	*Aspergillus niger,*	12	20	8	15	25
2	*Aspergillus flavus,*	9	17	5	12	25
3	*Rhizopus indicus*	3	8	1	4	22
4	*Candida albicans*	8	17	3	12	21

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