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Antibacterial Activity and Qualitative Phytochemical Analysis of Selected Seaweeds from Gulf of Mannar Region

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ABSTRACT: The phytochemical analysis of the aqueous extracts of some commonly occurring green seaweed *Cladophora glomerata*, *Ulva lactuca* and *Ulva reticulata*, the red seaweed *Gracilaria corticata* and *Kappaphycus alvarezii* and the brown seaweed *Sargassum wightii* and their evaluated for antibacterial activity by well diffusion assay. Two different solvents namely aqueous and methanol were used for extraction. The phytochemical analysis revealed the presence of Carbohydrates, Alkaloids, Steroids, Tannin & Phenols, Saponins, Fixed oils & Fats, Gums & Mucillage, Proteins, Flavonoids and Volatile oils in varying concentration. The zone of inhibition ranged between 45 – 9mm in aqueous extract and 40 – 6mm in methanolic extract. The maximum activity (45mm) was recorded from 200mg of aqueous extract of *Ulva reticulata* against *Salmonella typhi* and minimum (9mm) by *Ulva lactuca* against *Streptococcus pyogenes* at 50mg level whereas, the methanolic extract showed the maximum activity (40mm) was recorded from 200mg of *U. reticulata* against *Escherichia coli* and *Streptococcus pyogenes* and *Cladophora glomerata* against *Pseudomonas aeruginosa* and minimum (6mm) by 50mg of *Kappaphycus alvarezii* against *Staphylococcus epidermis.*

Key words: Seaweeds, Antibacterial activity, Methanolic extract, Aqueous extract and Phytochemical

Introduction

Seaweeds belong to a group of plants known as algae. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae) depending on their nutrient and chemical composition. Like other plants, seaweeds contain various inorganic and organic substances which can benefit human health (Kuda *et al.*, 2002). Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green algae (Yuan *et al.,* 2005; Bansemir *et al*., 2006; Chew *et al.,* 2008). Algae are the source of amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes and cyclic polysulphides (Mtolera and Semesi, 1996).

The inhibitory substances biosynthesized by the seaweeds were noted as early as in 1917 (Harder and Oppermann, 1953). The first observation regarding antibiotic activities of seaweeds was reported by Pratt *et al.,* 1944. Recent findings evidenced that seaweeds contained antibacterial (Tuney *et al.,* 2006), antiviral (Serkedjieva, 2004, Garg *et al.,* 1992), antifungal (Tang *et al*., 2002, Aliya and Shamaeel, 1999), cytotoxic (Tang *et al*., 2002) and larvicidal potentials (Thangam and Kathiresan 1991). The secondary metabolites synthesized by seaweeds demonstrate a broad spectrum of bioactivity varying from neurologically active in humans to algicidal, nematicidal, insecticidal and ichthyotoxicity in lower form of animals (Smith, 2004). Seaweed's antioxidant properties make it specific for prevention and treatment of cancer, supporting the immune system in eliminating the proliferation of cancer cells, says Tierra. Seaweed is considered a medicinal substance with wet, softening properties, which, according to traditional Chinese medicine, Tierra explains, enables it to dissolve hard nodules and tumors and to reduce swelling of the thyroid and lymph glands. Seaweed helps decongest swollen or inflamed lymph nodes; it can be consumed as a treatment for autoimmune illnesses, including chronic fatigue, HIV, arthritis and chronic allergies (Eleanor and John Lewallen, 2009).

Materials and Methods

Sample collection and preparation

The samples of green seaweed *Cladophora glomerata* (Linn.) Kützing, *Ulva lactuca* Linn. and *Ulva reticulata* Forsskal , the red seaweed *Gracilaria corticata* J. Agardh and *Kappaphycus alvarezii* (Doty) Doty.ex. Silva and the brown seaweed *Sargassum wightii* Greville were collected along the Coast of Mandapam, Gulf of Mannar region during low tides. Then the seaweeds were washed thoroughly with seawater to remove extraneous materials and brought to the laboratory in plastic bags containing water to prevent evaporation. Samples were then shade dried in till constant weight obtained and ground in pulverization to get coarse powder. The powdered samples subsequently stored in refrigerator.

Dried algal material is highly controversial to identify the particular species so we were tried to carry out the qualitative phytochemical behavior of these powder samples. The selected algal species aqueous and methanolic extract were used to screen the antibacterial activity.

Preparation of the extracts
Extraction of algal materials

Various extracts of the study algae was prepared according to the methodology of Indian Pharmacopoeia (Anonymous, 1966). The fresh materials were dried in shade conditions and the dried materials were subjected to pulverization to get coarse powder. The coarse powder material was subjected to Soxhlet extraction separately and successively with methanol and distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50^{0}C). Both the extracts were stored in a refrigerator in air tight containers. Among the extracts the aqueous extract was analyzed for phytochemical screening of compounds and both the extracts were examined antibacterial activity.

Qualitative phytochemical studies

Qualitative phytochemical were analyzed by using the procedures of Kokatae (1994). Alkaloids, carbohydrates, tannins, phenols, flavonoids, gums and mucilages, phytosterol, proteins and amino acids, fixed oils, fats, volatile oil and saponins were qualitatively analyzed.

Test pathogens

Antibacterial activity of six different seaweed extracts were investigated against five bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus epidermis* and *Streptococcus pyogenes* used for the present study were obtained from the Microbial Type Culture Collection (MTCC), The Institute of microbial technology, Sector 39-4, Chandigarh, India.

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Antibacterial activity of the algae extract

The aqueous and methanolic extracts of different seaweeds were used throughout the study. The condensed extracts were dissolved in 4% $DMSO_4$ (Dimethyl Sulphoxide).The methanolic and aqueous extract of 50, 100 & 200mg were tested against different bacterial test pathogens for their antibacterial activity.

Antibacterial activity of the seaweed extracts was tested using well diffusion method (Bauer *et al.,* 1996). The prepared culture plates were inoculated with different selected bacterial strains using streak plate method. Wells were made on the agar surface with 6mm cork borer. The extracts were poured into the well using sterile syringe. The plates were incubated at 37±2^{o}C for 24 hours for bacterial activity. The plates were observed for the zone formation around the wells.

The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average values were tabulated.

Result and Discussion

Phytochemical evaluation

Phytochemical analysis of all the aqueous extract revealed that carbohydrates, Saponins and Proteins are generally present in all the seaweeds. Gums & Mucillage were found in all the seaweeds except *Cladophora glomerata*. Other metabolites such as Alkaloids, Steroids, Fixed oils & Fats, and Volatile oils were absent in all the extracts (Table 1).

Table 1: Qualitative phytochemical studies of seaweed powder

S. No	Name of the compounds	Name of the test	Name of the seaweeds					
			Cladophora glomerata	***Ulva lactuca***	***Ulva reticulata***	***Gracilaria corticata***	***Kappaphycus alvarezii***	***Sargassum wightii***
1	Carbohydrates	Fehling's	+ +	+ + +	+ + +	+ +	+ + + +	+ +
		Benedicts	+ + + +	+ + +	+ + +	+	+ + + +	+ + + +
2	Alkaloids	Mayer's	-	-	-	-	-	-
		Hager's	-	-	-	-	-	-
		Wagner's	-	-	-	-	-	-
		Dragen Dorfff's	-	-	-	-	-	-
3	Steroids	Chloroform + acetic acid + H_2SO_4	-	-	-	-	-	-
4	Tannin & Phenols	10% Lead acetate	+ +	-	-	+ +	+ + + +	+ + +
		5% Ferric Chloride	+ +	-	-	-	+ +	+ +
		1% gelatin	-	-	-	+	+ +	+ + +
		10% Sodium chloride	+ + +	-	-	+ + +	+ + +	+ + + +
5	Saponins	Foam test	+ + +	+ + +	+ +	+	+ + +	+ + +
6	Fixed oils & Fats	Spot test	-	-	-	-	-	-
7	Gums & Mucillage	Alcoholic precipitation	-	+ + +	+ +	+ +	+ +	+ +
8	Proteins	Biuret test	+ +	+ +	+ +	+ +	+ + +	+ + +
9	Flavonoids	Na OH / HCL	-	-	-	-	-	-
10	Volatile oils	Hydro distillation method	-	-	-	-	-	-

+ = 20 % of measure; + + = 40% of measure; + + + = 60% of measure; + + + + = 80% of measure; - = absent

Antibacterial activity of seaweed extracts

Antibacterial activities of crude extracts of six marine algae from along the Coast of Mandapam, Gulf of Mannar region were determined by well diffusion assay and the results are summarized in Table 2 and Table 3. Both crude methanolic and aqueous forms of the extracts of all the seaweeds exhibited varying degree of antibacterial activities against the test organisms. On a general note, methanolic extracts exhibited higher degree of antibacterial activities than the aqueous extracts. Methanol extracts obtained from the brown algae such as *Laminaria angustata*, *Undaria pinnatifida*, *Rhodomela larix* and *Sargassum gracilis* found along the coast of Japan inhibited the several kinds of pathogenic bacteria. The extract prepared from *Sargassum gracilis* strongly inhibited the growth of *Bacillus mesentericus* (Saito and Nakamura, 1951).Crude methanolic extracts of all tested algae showed inhibition against all test pathogens and the extract of *Ulva reticulata* was the most effective. Whereas, the aqueous extracts of *Ulva reticulata* not showed inhibition against *Escherichia coli* and *Pseudomonas aeruginosa* and *Sargassum wightii* against *Staphylococcus epidermis*.

Table 2: Inhibition zone of aqueous seaweed extracts against test pathogens

Seaweeds	Concentrations (mg)	Fungal pathogens showing zone of inhibition (mm)				
		Escherichia coli	***Pseudomonas aeruginosa***	***Salmonella typhi***	***Staphylococcus epidermis***	***Streptococcus pyogenes***
Cladophora glomerata	50	15 ± 1.7	16 ± 2.4	13 ± 3.7	19 ± 1.4	15 ± 2.8
	100	26 ± 1.7	21 ± 2.4	26 ± 3.7	27 ± 2.4	23 ± 3.7
	200	32 ± 2.0	30 ± 2.8	35 ± 3.7	34 ± 2.8	30 ± 2.8
Ulva lactuca	50	11 ± 1.4	15 ± 1.4	18 ± 3.7	10 ± 2.8	09 ± 1.4
	100	17 ± 2.4	23 ± 3.7	22 ± 2.4	15 ± 1.4	15 ± 2.4
	200	30 ± 3.7	30 ± 3.7	30 ± 2.4	22 ± 1.4	26 ± 2.8
Ulva reticulata	50	-	-	23 ± 3.7	20 ± 3.7	14 ± 3.7
	100	-	-	33 ± 2.8	30 ± 2.8	16 ± 1.4
	200	-	-	45 ± 3.7	42 ± 2.4	42 ± 2.4
Gracilaria corticata	50	19 ± 2.4	22 ± 2.4	14 ± 1.4	20 ± 2.4	21 ± 1.4
	100	29 ± 1.4	27 ± 3.7	26 ± 1.4	26 ± 1.4	27 ± 2.8
	200	38 ± 2.4	36 ± 2.4	36 ± 2.4	32 ± 2.4	35 ± 2.4
Kappaphycus alvarezii	50	13 ± 3.7	25 ± 2.8	27 ± 2.4	23 ± 2.4	14 ± 2.4
	100	19 ± 2.4	32 ± 2.8	30 ± 2.8	28 ± 2.8	24 ± 1.4
	200	32 ± 2.8	40 ± 2.8	35 ± 3.7	40 ± 2.8	35 ± 2.8
Sargassum wightii	50	15 ± 2.4	10 ± 2.4	12 ± 2.4	-	15 ± 3.7
	100	20 ± 4.9	16 ± 1.4	14 ± 2.4	-	20 ± 4.9
	200	26 ± 2.4	18 ± 2.8	22 ± 2.8	-	22 ± 2.4

Table 3: Inhibition zone of methanolic extracts against test pathogens

Seaweeds	Concentrations (mg)	Fungal pathogens showing zone of inhibition (mm)				
		Escherichia coli	***Pseudomonas aeruginosa***	***Salmonella typhi***	***Staphylococcus epidermis***	***Streptococcus pyogenes***
Cladophora glomerata	50	08 ± 1.7	14 ± 1.4	15 ± 1.4	-	-
	100	10 ± 1.7	27 ± 2.8	20 ± 1.4	18 ± 2.4	07 ± 2.8
	200	28 ± 1.7	40 ± 3.7	35 ± 6.2	27 ± 2.4	25 ± 2.4
Ulva lactuca	50	08 ± 1.4	16 ± 1.4	12 ± 2.8	-	10 ± 1.4
	100	15 ± 2.8	27 ± 2.4	26 ± 3.7	18 ± 2.8	20 ± 2.4
	200	26 ± 2.4	30 ± 3.7	28 ± 2.4	31 ± 1.4	27 ± 3.7
Ulva reticulata	50	10 ± 3.7	12 ± 2.4	20 ± 3.7	23 ± 3.7	07 ± 2.4
	100	30 ± 2.4	18 ± 3.7	28 ± 2.4	30 ± 2.8	24 ±2 .4
	200	40 ± 2.8	26 ± 1.4	38 ± 2.4	36 ± 2.4	40 ± 2.4
Gracilaria corticata	50	-	16 ± 2.4	10 ± 2.4	16 ± 2.4	-
	100	25 ± 1.4	20 ± 2.4	20 ± 4.9	20 ± 2.4	12 ± 2.4
	200	30 ± 3.7	29 ± 1.4	31 ± 2.8	26 ± 2.8	22 ± 1.4
Kappaphycus alvarezii	50	08 ± 2.4	10 ± 2.8	10 ± 2.8	06 ± 1.4	-
	100	12 ± 1.4	15 ± 5.1	20 ± 2.4	15 ± 3.7	13 ± 2.8
	200	22 ± 2.4	28 ± 2.8	32 ± 2.8	29 ± 1.4	23 ± 2.8
Sargassum wightii	50	10 ± 2.4	10 ± 2.4	08 ± 1.4	10 ± 1.4	07 ± 1.4
	100	19 ± 2.4	20 ± 2.8	22 ± 3.7	18 ± 2.8	27 ± 2.8
	200	32 ± 3.7	38 ± 2.4	34 ± 4.9	30 ± 2.4	39 ± 3.7

In this study, the antibacterial activity of aqueous and methanolic extract of all selected algal species were screened through the well diffusion method against *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typhi , Staphylococcus epidermis* and *Streptococcus pyogenes*. The analogous manner the most of the workers screened the activity of seaweeds exhibited antibacterial activity: *C. ashmeadii*, *C. paspaloides* and *C. prolifera* (Freile-Pelegrin and Morales, 2004*)*.

Antibacterial potential of seaweeds such as *Gracilaria foloferia*, *Padina tetrastomatica*, *Caurelrpa recemosa* and *Ulva lactuca* was evaluated against both Gram negative and Gram positive human pathogenic bacteria. Methanol extracts of all seaweeds test exhibited broad spectrum of antibacterial activity. Green algal members showed higher antibacterial activity than red. *Escherchia coli* alone resistant to all the seaweed extracts except *Sargassum tencerium* (Kandhasamy and Arunachalam, 2008). In present study the zone of inhibition ranged between 45 – 9 mm in aqueous extract and 40 – 6 mm in methanolic extract. The maximum activity (45mm) was recorded from 200mg of aqueous extract of *Ulva reticulata* against *Salmonella typhi* and minimum (9mm) by *Ulva lactuca* against *Streptococcus pyogenes* at 50 mg level whereas, the methanolic extract showed the maximum activity (40mm) was recorded from 200mg of *U. reticulata* against *Escherichia coli* and *Streptococcus pyogenes* and *Cladophora glomerata* against *Pseudomonas aeruginosa* and minimum (6mm) by 50 mg of *Kappaphycus alvarezii* against *Staphylococcus epidermis.* Different concentration of the fractions isolated from the seaweeds showed antibacterial activity against the test bacteria *Staphylococcus aureus* and *Escherichia coli* (Parekh, 1985). Studies conducted on 30 species of seaweeds collected along the Coast of Mandapam, Tamil Nadu for their hemolytic and antimicrobial potential. Results indicated that extracts obtained from the seaweeds such as *Enteromorpha compressa*, *Cladophoropsis zoolingeri*, *Padina gymnospora*, *Sargassum wightii* and *Gracilaria corticata* showed antibacterial activity against the Gram negative bacteria and Gram positive cultures of *Bacillus* (Rao *et al.*, 1991).

Our results were direct evidence of these algal containing antibacterial activities which is due to its bearing secondary metabolic compounds. Most of the researchers reported that many bioactive and pharmacologically important compounds such as

alginate, carrageen and agar as phycocolloids have been obtained from seaweeds and used in medicine and pharmacy (Siddhanta *et al.*, 1997). Fatty acids are isolated from micro algae that exhibited antibacterial activity (Kellam *et al.*, 1988). Many workers revealed that the crude extracts of Indian seaweeds are active against Gram-positive bacteria (Rao and Parekh, 1981). Methanolic extracts of fifty-six seaweeds collected from South African coast, belonging to Chlorophyceae, Phaeophyceae and Rhodophyceae showed antibacterial activity. Among them, Phaeophyceae members showed highest antibacterial activity (Vlachos *et al.*, 1997). The present investigation brings out adequate data on the antibacterial potential of Methanolic and aqueous extracts of six seaweeds. Further research studies are being carried out on the other species of seaweeds from the same habitat in order to provide complete data of the antimicrobial potential seaweeds along the Coast of Mandapam, Gulf of Mannar region. It is also necessary for successful separation, purification and characterization of biologically active compounds using chromatographic and spectroscopic techniques for the synthesis novel antibiotics.

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