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PHYTOCHEMICAL PROFILING AND ANTIMICROBIAL ACTIVITY SCREENING OF SEAWEEDS COLLECTED FROM RAMESWARAM SEA COAST, TAMIL NADU, INDIA

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ABSTRACT

Objective: The present study was undertaken to screen the phytochemical constituents and antimicrobial potential of selected seaweed collected from Rameswaram, Southern coast of India.

Methods: The present study was carried out to investigate the presence of phytochemical constituents and antibacterial activity of selected marine (brown, red, and green) macroalgae collected from Mandapam Coast. The antibacterial activity of the seaweeds was examined by the agar well diffusion method.

Results: Among the three seaweeds, *T.conoides* showed the maximum number of phytochemical constituents in the aqueous extract. The aqueous extract of the seaweeds *H.musciformis*, *C. racemosa, and T.conoides*tested against *P.aeruginosa* and *S.aureus*in which better antibacterial activity showed in the following order: *T.conoides>>H.musciformis>>C. racemosa*.

Conclusion: The aqueous extracts of algal species used in the present investigation showed better antibacterial activity against pathogens. Thus, we conclude that seaweeds may be an answer to theunsolved and growing problem of resistant bacteria and a novel untapped source to combatdeadlydangerous diseases.

Keywords: Seaweed, Phytochemicals, Antibacterial, Well diffusion method, Mandapam Coast.

INTRODUCTION

The Indian coastline, with its distinct coastal habitats, facilitates the luxuriant development of varied seaweed species of significant economic significance. Nevertheless, the most recent floristic work, compiled by the Botanical Survey of India, enumerated the occurrence of 865 taxa from Indian waters of which 212 species belong to Chlorophyta, 211 to Ochrophyta, and 442 to Rhodophyta. The most common are Rhodophyta (434 species), followed by Chlorophyta (216 species), Phaeophyta (191 species), and Xanthophyta (3 species). Among these, the highest number of species reported was in Tamil Nadu (302), followed by Gujarat (202), Maharashtra (159), Lakshadweep (89), Andhra Pradesh (79), and Goa (75). (1)

The most important marine algae in India in terms of ubiquitous composition are Ulva and Caulerpa between greens, Hypnea, and Kappaphycus between reds, and Sargassum and Turbinaria between browns. The coasts of Gujarat, Kerala, and Tamil Nadu have most of the algae mentioned in India (2). Seaweeds are noticed to be luxuriant in the Gulf of Mannar, Coastal Zone, which is located between India and Sri Lanka. It runs along its Indian side from Pamban Islands, the Southern Coastline, which includes the popular Rameshwaram pilgrim center to Kanyakumari (3).

'Seaweed' is a misnomer; it is not a weed at all, but a plant with a multitude of uses. Etymology suggests that the word was in use since the 1570s, in times when the world had little knowledge of their myriad applications. Seaweeds are plant-like ocean organisms that are botanically classified as microphysics marine algae. Maybe a better alternative is 'sea-plant' or 'sea vegetable. Marine algae are one of the largest producers of biomass in the marine environment (4).

We are now at a crucial stage where it is essential to explore new strategies to combat infectious diseases. The oceans are practically untapped resources from which new bioactive compounds can be identified. The aquatic ecosystem, which accounts for about half of global biodiversity, is an immense opportunity for new compounds (5). Like other seeds, seaweed contains a number of inorganic and organic compounds that can support human health (6). Seaweeds are known to be a source of bioactive compounds since they are capable of producing a wide range of secondary metabolites distinguished by a wide range of biological activities. Antioxidant, antiviral, antifungal, and antimicrobial compounds have been found in brown, red, and green algae (7) (8).

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Seaweed has great value in providing low-cost, wholesome nutrition and therapeutic protection. They contain more than 60 trace elements, minerals, protein, iodine, bromine, vitamins, and several bioactive substances of economic value. They have been screened extensively to isolate lifesaving drugs or biologically active substances all over the world (9). Still, now, there is scanty information regarding the pharmaceutical potentiality of these species. That is why the objectives of the present study were to evaluate antimicrobial activity and phytochemical patterns of three widely available seaweed species *Hypneamusciformis, Caulerpa racemose*, and*Turbinariaconiodes*.

RESEARCH METHODS

Collection of macroalgae

Seaweeds were collected during the lowest tide of chart datum from the seaweed infested locations along the Southeast coast of India, Mandapam, Rameswaram District, Tamilnadu, India. The macroalgae which infested exclusively on the intertidal rocky and other substratum was selected for the collection to avoid other microalgal contamination.

Preservation of seaweeds

Immediately after the collection of algal samples, the surface of the samples was washed in fresh seawater to avoid further algal contamination, to eliminate epiphytes, extraneous material sand, and other calcareous impurities. Later, the obtained macroalgae samples were shifted to the laboratory in sterile polythene bags under ice at 20^{o} C to prevent decomposition and degradation of metabolites for detection and potential comparison.

Macroalgae Identification

The algae thus collected was described with the aid of seaweed taxonomists at the Salt and Marine Chemical Research Institute (CSMCRI) Mandapam Camp, Tamil Nadu, India.

Preparation of algal powder

The species identified were thoroughly sterilized with tap water until undesired impurities, adhering sand particles and extraneous matter such as epiphytes, pebbles, surface salty mature shells were removed. Then the seaweeds were rinsed with sterile distilled water three times to remove extra sand and dust. Later, they were scattered over filter paper and left for a few hours to absorb extra water. Then the washed algae were cut into small pieces, shade dried for two weeks, and then the samples were turned into coarse powder by grinding them in an electric mixer grinder. The powdered samples were properly packed in zip lock bags and placed in the refrigerator at 4^{o} C. They were then tested for their phytochemicals, nanoparticles, antibacterial activity.

Preparation of Pure Algal Extract (PAE)

The pure algal extract was prepared by adding 5g algal powder into 100 mL of distilled water in a conical flask and placed in a hot plate with the magnetic stirred for 15 minutes. The extract was purified using the Buchner funnel and the Whatman No. 1 filter paper and sterile cotton wool, and the supernatant was used and processed at 4^{o}C for further process.

Phytochemical screening

The sample was subjected to a qualitative test for the number of phytochemicals present in the collected algal samples for the identification of phytochemical constituents according to standard procedures (10).

Antibacterial assay

The seaweed extracts were screened against the selected human pathogen. The antibacterial bioassay of the seaweed extract was carried out using the agar well diffusion method (11). For testing antibacterial activity, the following (gram+ve: *Staphylococcus aureus*) (gram-ve:*Pseudomonas aeruginosa*) bacterial strain was selected. The antibacterial

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assay using gram (+ve), gram (-ve) bacteria was carried out using the agar plate method. At first, 6mm hole wells were punched in nutrient agar medium (Hi-Media Laboratories Pvt. Ltd) using a cork borer in nutrient agar plates inoculated with the test microorganisms. Petri dishes were left 15minutes until bacteria absorbed to the medium. The Water extracts of the collected test samples were tested in five dose levels of 20µl, 40µl, 60µl, 80µl, 100µl respectively. To prevent drying all plates were covered with sterile plastic bags. The Petri dishes were incubated under 37ºC for 24hrs. The assay was run in triplicate. After incubation, the inhibition zones around the wells were measured on the underside of Petri dishes and expressed in the nearest millimeter. The pure solvent was used as a negative control and Ciprofloxacin antibiotics disc as the positive control(12) (13).

RESULTS

Qualitative phytochemical analysis

Phytochemical profiling of seaweeds is the basis for drug designing and development against many clinical complications. The phytochemistry of aquatic organisms is gaining importance in recent years across the world. Seaweeds are comprised of major secondary metabolites such as alkaloids, glycosides, flavonoids, saponins, tannins, steroids, and related active metabolites with potential pharmaceutical applications (14).The present investigation brings out adequate data on the phytochemical constituents present in all the selected *Hypneamusciformis; Caulreparacemosa; Turbinaria*conoides red, green, and brown algae which are presented in Table.1 respectively.

Table:1 Phytochemical screening of aqueous extracted seaweeds collected from Mandapam, Rameswaram coast, Tamil Nadu.

S.No	Phytochemical	*Hypneamusciformis*	*Caulreparacemosa*	*Turbinariaconoides*
1	Alkaloids	+++	++	+++
2	Phenols	++	+	+
3	Flavonoids	++	+	++
4	Anthraquinones	-	-	-
5	Tannins	++	++	++
6	Saponins	++	++	++
7	Coumarins	-	-	-
8	Carbohydrate	-	+	+
9	Proteins	-	+	+
10	Quinines	-	-	-
11	Glycosides	-	-	-
12	Terpenoids	+	+	++
Total		**6**	**8**	**8**

(+++):HigherPresence;(++):Moderatepresence;(+)-Presence

Aqueous extract of *Caulerpa racemosa* showed the presence of eight bioactive compounds like alkaloids, phenols, flavonoids, tannins, saponins, carbohydrates, proteins, and terpenoids. While anthraquinones, quinines, glycosides, and coumarins, did not show any positive results for their presence in aqueous extract of *Caulerpa racemosa*. In

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Hypneamusciformis presence of six bioactive compounds like alkaloids, phenols, flavonoids, tannins, saponins, and terpenoids were observed while carbohydrate, protein, anthraquinones, quinines, glycosides, and coumarins were not present in the aqueous extract of *Hypneamusciformis*. Aqueous extract of *Turbanariaconoides* showed the presence of eight bioactive compounds like alkaloids, phenols, flavonoids, tannins, saponins, carbohydrates, proteins, and terpenoids. While anthraquinones, quinines, glycosides, and coumarins, did not show any positive results for their presence in aqueous extract of *Turbanariaconoides*. Thus, the present investigation brings out adequate data on the phytochemical constituents present in *Turbanariaconoides* seaweeds.

Antibacterial activity

The antimicrobial compounds derived from the marine flora consist of diverse groups of chemical compounds. The cell extracts and active constituents of various algae have been shown to have antibacterial activity against Gram positive and Gram negative bacteria. With the above idea on pharmacological benefits of macroalga in the present study marine algae, *Hypneamusciformis; Caulreparacemosa; Turbinariaconoides* were analyzed for their efficiency towards the multidrug-resistant human pathogen. The results of the antibacterial activity against the tested pathogen were tabulated and presented in form of Table.2

Table. 2 Antibacterial Zone of inhibition of aqueous extract of selected seaweeds against selected gram positive and gram negative multidrug-resistant clinical bacterial strain

Concentration(µl)	Zoneofinhibition(mm)					
	Hypneamusciformis		*Caulreparacemosa*		*Turbinariaconoides*	
	+ve	-ve	+ve	-ve	+ve	-ve
20	20.93±0.50	23.12±0.11	7.0±0.80	8.5±0.15	19.20±0.43	21.10±0.36
40	22.00±0.10	25.3±0.21	9.0±0.49	10.5±0.22	21.06±0.20	24.16±0.56
60	25.20±0.50	28.5±0.27	12.0±0.20	13.6±0.18	24.13±0.51	27.83±0.37
80	29.16±0.47	32.2±0.19	14.1±0.32	15.5±0.21	25.80±0.62	30.06±0.30
100	32.93±0.30	34.1±0.30	17.3±1.25	19.6±0.19	27.83±0.37	33.10±0.36
PC	**15.13±0.32**	**19.06±0.20**	**5.9±0.10**	**9.01±0.11**	**18.11±0.10**	**19.06±0.20**

* (+ve): ***Staphylococcusaureus*** **(-ve):*Pseudomonas aeruginosa*;**

*****PC-positivecontrol-AntibioticCiprofloxacin

* Meanofthreeassays;±Standard deviation

20,40, 60, 80, 100 µl concentration of aqueous extract of *Hypneamusciformis* was taken and analyzed for antibacterial activity against Gram positive and Gram negative human pathogen. Among the treatments, the minimum inhibition was noted in 20 µl concentration against *S. aureus* (20.93±0.50 mm) and *P. aeruginosa* (23.12±0.11mm). The maximum inhibition noted in 100 µl concentration against *S. aureus* (32.93±0.30mm) and *P. aeruginosa* (34.1±0.30) followed by 80µl and 60µl concentration against *S. aureus* (29.16±0.47mm), (25.20±0.50mm) and against *P. aeruginosa*

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(32.2±0.19mm), (28.5±0.27mm). A moderate zone of inhibition was observed at 40µl concentration against *S. aureus* (22.00±0.10mm)and

P. aeruginosa (25.3 ±0.21mm).

From Table. 2 Among the 20, 40, 60, 80, 100 µl concentration of aqueous extract of *Caulerpa racemosa* taken and analyzed for antibacterial activity against Gram positive and Gram negative human pathogen the treatment of 20 µl concentration, showing the minimum inhibition (7.0±0.80) against *S. aureus* and (8.5±0.15) against *P. aeruginosa*. The maximum inhibition noted in 100 µl concentration against *S. aureus* (17.3±1.25mm) and *P. aeruginosa* (19.6±0.19mm) followed by 80µl and 60µl concentration against S. aureus (14.1±0.32mm), (12.0±0.20mm) and against *P. aeruginosa* (15.5±0.21mm), (13.6±0.18mm). A moderate zone of inhibition was observed at 40µl concentration against *S. aureus* (9.0±0.49) and *P. aeruginosa* (10.5±0.22mm).

The concentration of 20, 40, 60, 80, 100 µl aqueous extract of *Turbinariaconoides* was taken and analyzed for antibacterial activity against *S.aureus* -Gram positive and *P. aeruginosa* - Gram negative human pathogen. The maximum inhibition noted in 100 µl concentration against *S. aureus* (27.83±0.37mm) and *P. aeruginosa* (30.06±0.30mm) followed by 80µl and 60µl concentration against *S. aureus* (25.80±0.62mm), (24.13±0.51mm) and against P. aeruginosa (30.06±0.30), (27.83±0.37). A moderate zone of inhibition was observed at 40µl concentration against S. aureus (21.06±0.20mm) and *P. aeruginosa* (24.16±0.56mm). Among the treatment, the minimum inhibition was noted in 20 µl concentration against S. aureus (19.20±0.43mm) and P. aeruginosa (21.10±0.36mm).

On comparing to selected elution of seaweeds the control treatment of selected antibiotic ciprofloxacin inhibited the tested pathogen by showing a clear zone of inhibition diameter of (15.13±0.32mm; 5.9±0.10mm and 18.11±0.10mm) against different elution of selected red, brown, and green algae aqueous extracts and 19.06±0.20; 9.01±0.11and 19.06±0.20 mm of inhibition zone against gram negative selected bacterial strain which was less. All the selected elution concentrations showed the best zone of inhibition than control disc treatment bacteria.

DISCUSSIONS

Qualitative phytochemical analysis

Phytochemical profiling of the seaweed samples revealed the presence of different phytochemicals like phenol, tannin, and flavonoids which were found in greater amount in all three seaweeds *Hypneamusciformis* (Red) and *Caulerpa racemosa* (Green) and *Turbinariaconoides* (Brown) extracts (15). These phytochemicals could exhibit antimicrobial activity for the presence of the phytochemical constituency. Phytochemical screening provides important ideas for the development of new drugs against deadly diseases. Recently a number of studies have been reported on the phytochemistry of plants across the world (16) (17).

So, from the above profilingresults, we conclude that the presence of secondary bioactive metabolites of commercialimportance in the selected marine algal seaweed *Hypneamusciformis*(Red) and *Caulerparacemosa*(Green)and*Turbanariaconoides*(Brown)extracts will actas a precursor forisolation of selective secondary metabolites compounds to act against multi-resistant humanpathogensinpharmaceuticalindustriesinfuture.

Antibacterial activity

The results also indicated that the aqueous extracts of macroalgae successfully inhibited Pseudomonas aeruginosa which is in agreement with the earlier findings of Jose et al. (2008) (18). These differences among the algal concentration inhibition nature could be due to the different solubility behavior of secondary metabolites which could be influenced by theseasonal and geographical distribution of the species as indicated by Padmakumar (2002)(19). Yuvaraj et al., 2011(20) stated that among marine organisms, macroalgae are rich sources of structurally diverse bioactive compounds with different bioactivity spectra and biomedical value. Pramitha and Lipton, 2012 (21) study proved the antifouling, anticoagulant, glucosidase inhibitory properties, antimicrobial activities of aqueous extract of macroalgae, growth responses of microalgae, and selective cytotoxic activities of macroalgae from the Indian coast.

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Veeragurunathan and Geetha (2009)(22)showed evidence from the observation that the aqueous extracts of the brown algae effectively inhibited most of the pathogens with the maximum zone of 15 mm produced against *S. aureus*. Preliminary pharmacological investigation of the algae belonging to the genus Dictyota revealed its content of considerable antibacterial, antifungal, antiviral (Nizmuddin, and Campbell, 1995) (23).

In the present study *Hypneamusciformis* (Red) and *Caulerpa racemosa* (Green) and *Turbanariaconoides* (Brown), extracts showed the presence of the phenolic compound in water extracts. Arunkumar et al., 2010 (24) stated that general phenolic compounds possessed specific physical, chemical, and biological activities that make them useful as drugs. phenolic compounds especially polyphenols and tannin have been reported to have antimicrobial, anti-carcinogenic, and antioxidant properties. Thus, research and utilization of the marine algal community have increased markedly that directly offers an enormous untapped reservoir of novel drug leads endowed with ingenious structures and potential biological activities(25).

CONCLUSION

The aqueous extracts of algal species used in the present investigation showed better antibacterial activity against pathogens used. They are potential sources of bioactive compounds and should be investigated for natural antibiotics. Thus, the present anti-microbial results prove that applications of these eco-friendly nanoparticles with bactericidal and other medical applications will have high potentiality for large-scale synthesis in the future. But further research should be made to identify and purify these antibacterial substances. Thus we conclude that seaweeds may be an answer to the unsolved and growing problem of resistant bacteria and a novel untapped source to combat deadly dangerous diseases.

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