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In-Vitro Antibacterial activity and Preliminary Phytochemical Screening of five Seaweeds from Mandapam Coast, Tamil Nadu, India

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Abstract

In-vitro antibacterial activity and phytochemical screening of ethanol extracts of five seaweeds (*Acanthophora spicifera, Hypnea valentiae, Padina gymnospora, Ulva reticulate* and *Caulerpa racemosa*) from Mandapam coastal region Ramanathapuram were investigated. The seaweeds which were used for present study belongs to red algae, brown algae and green algae. The tested microorganisms for antimicrobial activity were gram positive and gram negative bacteria and fungal pathogen. The tested five seaweeds for phytochemical screening showed positive results for biological active compound such as alkaloids, terpenoids, saponins and phenolic. The antibacterial activity in *Acanthophora spicifera* ethonal extracts reveled the maximum zone of inhibition against *Escherichia coli* (20 mm) and minimum zone of inhibition observed in *Staphylococcus aureus* (14 mm). Our findings provide the evidence that the ethonal extract of five seaweeds indicates the good antimicrobial activity and hence the seaweeds proved to be an effective therapeutic agent.

Keywords: Phytochemical analysis, antibacterial activity, Seaweeds extract, bacteria.

Introduction

The marine environment contains over 80% of world's plant species and with more than 150,000 marine algae found in the intertidal zones and tropical waters of the ocean, it is a primary source of natural products (Cynthia Layse *et al*. 2011).

Seaweeds contain many different varieties active compound which has a wide spectrum of biological activities. It was observed, the presence of antiviral, antibacterial and antifungal actives compounds in Rhodophta (red algae), Phaeophyta (brown algae) and Chlorophyta (green algae) against antiviral, antifungal and antibacterial activities (Newman *et al*. 2003 and Chakraborthy *et al*. 2010). Wide range of biological activities due to the presences of biological active compound in Seaweeds the biological activities such as antibiotics, antioxidant and antiinflammatory (Tuney *et al*. 2006; Patra *et al*. 2008). Some macro algae have biologically active components which affected the germination of some pathogenic bacteria and break the cell wall of bacteria (Kolanjinathan *et al*. 2009). Hornsey and Hide (1985) found that many of the marine seaweeds crude extracts contain inhibition activity against pathogenic microorganisms. Some of the seaweeds contain different bioactive substances which incorporated medicine and pharmacotherapy, whereas some of the isolated compounds have bacteriostatic and bactericidal properties. Different kinds of diseases were treated with antibiotics, extracted from terrestrial plants that were used as therapeutic agents; new compounds were reported in oceans and have commercial value (Shimaa *et al*. 2016).

In Indian coast regions 841 species of marine algae were found in both inter-tidal and deep water. They contain infinite inorganic and organic compound valuable for human health (Nupur *et al*. 2017). In India, large numbers of ethnomedicinal plants have been used to treat different kinds of diseases. Screening of bioactive compounds from marine algae may offer an idea for the increase of new novel drugs. Seaweeds have been screened widely to isolate drugs or biologically active compound from all over the world (Caccamese *et al*. 1980). Of all the natural sources for antibiotic, the ocean environment is clearly the last clear frontier and it was know that ocean sources possess significant potential to produce new novel drugs (Fenical, 1997). The present study was undertaken to screen the preliminary phytochemical analysis and to determine the antimicrobial activity of different seaweeds.

Material and Methods

Preliminary Phytochemical Screening

Five seaweeds species, *Acanthophora spicifera, Hypnea valentiae, Padina gymnospora, Ulva reticulate* and *Caulerpa racemosa* (Figure-1) were collected from Mandapam, Ramanathapuram. Different species of collected algae were cleaned with seawater to remove impurities. The seaweeds were transported to the laboratory in sterile polythene bags. In the laboratory, samples were rinsed with tap water and were shade dried, cut into small pieces and powdered in a mixer grinder.

Organic solvent ethanol was used for extraction. Five grams of each powdered sample were soaked in 40 ml of the solvent for three days. Remain extracts were filtered and concentrated in a rotatory evaporator at 35^0C. The residual water was removed with a vacuum pump. All the solvent extracts were subjected to preliminary qualitative tests for the identification of different phytochemical compound as per the standard method (Trease and Evans, 1985; Parekh and Chanda, 2007).

Test for Alkaloids

About 1 ml of each solvent extract was boiled in 2 ml of 1 % Hydrochloric acid in a water bath for 5 minutes. The mixture was allowed to cool and filtered. The filtrate was shared in equal proportion into 5 test tubes and named as A, B, C, D and E. 1 ml portion of the filtrate was treated with drops of the following reagents. With Dragendroff's reagent, a red precipitate was shown. With Mayer's reagent, a creamy white colour precipitate formed which indicates the presence of alkaloids in the sample (Harborne, 1973).

Test for Steroids

To 0.5 ml of the ethanolic extract of each sample, 2ml of acetic anhydride was added. Two ml of sulphuric acid was mixed into the test tube and the colour change was observed from violet to blue or green which indicated the presence of steroidsin the sample.

Test for Terpenoids

To 5 ml of test extract, 2 ml of chloroform and 3 ml of concentrated sulphuric acid was added. Reddish brown colour formation indicated the presence of terpenoids in the sample.

Test for Tannins

About 2 ml of the solvent extract was boiled with 5 ml of 45 % ethanol for 5 minutes. The mixture was cooled and filtered. 1 ml of the filtrate was added to 3 drops of lead sub-acetate solution. A gelatinous precipitate was observed which indicates the presence of Tannins. Another 1 ml of the filtrate was diluted with distilled water and 2 drops of ferric chloride was added. A transient green colour indicates the presence of Tannins.

Test for Saponins

Small amount of the test extract was boiled with 5 ml of distilled water for 5 minutes. Mixture was filtered while still hot and the filtrate was used for the following tests. To 1 ml of the filtrate, 2 drops of olive oil was added. The mixture was shaken and observed for the formation of emulsion. 1 ml of the filtrate was diluted with 5 ml of distilled water. The mixture was vigorously shaken and observed for frothing.

Test for Phenolic Compounds

To the solvent extracts, 2 ml of ethanol was added to which a few drops of ferric chloride were incorporated. Formation of blue colour represented the presence of phenolic compounds.

Antibacterial Activity

Two Gram negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*, and two Gram positive cultures of *Staphylococcus aureus*, *Bacillus subtilis* were obtained from the Guna Laboratoryin in Ramanathapuram, Tamil Nadu. They were stored at 4^0C at refrigerator. The bactericidal activity of the crude extract was determined in accordance with the agar well diffusion method (Irobi *et al*. 1994) 100 µl of cell suspension was spread on Mueller Hinton agar medium. Wells were bored onto the agar using a sterile 6 mm diameter cork borer. 70 µL /ml of the crude extract was added into the wells and incubated at 37^0C for 24h.The plates were

observed for zone of inhibition after 24h. These effects were compared with Gentamycin at a concentration of 10µg/ml respectively.

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*, and *Candida albicans* were swabbed. Wells of approximately 6mm diameter was bored using a well cutter and extracts of 70µl 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

Successive isolation of bioactive compounds from algae materials is largely dependent on type of solvent used in the extraction procedure. A preliminary qualitative phytochemical screening of the crude extracts of five seaweeds viz., *Acanthophora spicifera, Hypnea valentiae, Padina gymnospora, Ulva reticulate* and *Caulerpa racemosa* was carried out to assess the presence of bioactive compounds.

In the present study *Acanthophora spicifera* and *Caulerpa racemosa* ethanol extract revealed that alkaloids, terpenoids, saponins and phenolic. Alkaloids, tannins, saponins, and phenolic were detected in the ethanol extracts of three algae such as *Hypnea valentiae, Padina gymnospora* and *Ulva reticulate*. The phytochemical constituents such as alkaloids, steroids, terpenoids, tannins, saponin and phenols are the secondary metabolites of seaweeds serve as production against predation by many other microorganisms. The phytochemical constituents of the selected algae were investigated and are summarized in Table -1.

Table -1: Preliminary phytochemical screening of the samples.

Phytochemical Constituent	***Acanthophora spicifera***	***Hypnea valentiae***	***Padina gymnospora***	***Ulva reticulata***	***Caulerpa racemosa***
Alkaloids	+	+	+	+	+
Steroids	-	-	-	-	
Terpenoids	+	-	-	-	+
Tannins	-	+	+	+	-
Saponins	+	+	+	+	+
Phenolic	+	+	+	+	+

(+ Present, - Absent)

Antimicrobial efficacy of the solvent extracts namely, ethanol has been investigated against few human pathogenic bacteria. Antimicrobial activity of these test algae is shown in Table-2. The activity of these extracts has been compared with that of standard drug Gentamycin (10µg/ml). Results of the present study reveal that all the five tested seaweed extracts possess potential antimicrobial activity against gram negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae* and two Gram positive cultures of *Staphylococcus aureus* and *Bacillus subtilis.*

Among the five seaweeds the *Acanthophora spicifera* ethonal extracts revealed the maximum zone inhibition against *Escherichia coli* (20 mm) and minimum zone of inhibition observed in *Staphylococcus aureus* (14 mm). The antibacterial activity of *Hypnea valentiae* ethanol extract, showed a maximum zone inhibition against *Klebsiella pneumoniae* (18 mm) and minimum of (16 mm) against *Escherichia coli* and *Bacillus subtilis*. The ethanol extract of the *Padina gymnospora* possessed the highest zone of inhibition against *Escherichia coli* of about 19 mm and least activity observed in *Bacillus subtilis* (16 mm). The ethonal extract of *Ulva reticulata* showed a relatively high of inhibition (19 mm) against the gram negative bacteria such as *Escherichia coli* and lowest inhibition was observed in gram positive bacteria of *Staphylococcus aureus* (16 mm). The methanol extracts of *Caulerpa racemosa* showed maximum activity of 19 mm of inhibition zone against *Escherichia coli* (Table-2).

The antifungal activities of the ethanolic extracts of five seaweeds were studies for strains of two fungal pathogens. The results were examined with that regular antibiotic Clotrimazole. The maximum inhibition zone was observed the ethanolic extracts against *Candida albicans* (19 mm) in the concentration of 70µg/ml (Table.3). The minimum zone was observed in the ethanolic extract of *Caulerpa racemosa* against *Candida albicans*.

Table 2: Antibacterial activity of five different seaweeds

Test Organisms	**Zone of inhibition (mm)**				
	S. aureus	***E.coli***	***K. pneumonia***	***B.subtilis***	**Gentamycin**
Acanthophora spicifera	14	20	18	15	23
Hypnea valentiae	17	16	18	16	26
Padina gymnospora	18	19	18	16	24
Ulva reticulate	15	19	18	16	23
Caulerpa racemosa	18	19	17	17	22

Table 3: Antibacterial activity of five different seaweeds

Test Organisms	**Zone of inhibition (mm)**	
	Aspergillus niger	***Candida albicans***
Acanthophora spicifera	18	19
Hypnea valentiae	15	14
Padina gymnospora	14	15
Ulva reticulata	16	17
Caulerpa racemosa	14	13

Figure-1.Collection of seaweeds from Ramanathapuram District

Acanthophora spicifera

Hypnea valentiae

Padina gymnospora

Ulva reticulata

Caulerpa racemosa

The different seaweeds of extracts revealed a significant antibacterial activity against Gram negative as well as Gram positive bacteria. The highest zone of inhibition observed in *Acanthophora spicifera* ethanolic extract against Gram negative bacteria of *Escherichia coli* (20

mm). And lowest zone of inhibition showed in *Acanthophora spicifera* against *Staphylococcus aureus* (14 mm). The seaweeds of ethanolic extracts revealed different degrees of antimicrobial activities against different bacteria. The seaweeds contain various secondary metabolites such as alkaloids, sterols, and phenolic these compound show bioactivity against bacteria, fungi and viruses (Hornsey and Hide, 1985; Nirmal Kumar *et al* 2010). The presents of phenolics and alkaloids sterols affect the growth and metabolism of microorganisms (Jayashree *et al.* 2013).

Conclusion

Results revealed that the marine algae have several chemical constituents such as phenols, flavonoids, alkaloids, tannins and terpenoids of high therapeutic efficacy. Further studies are required to investigate the extracts of for phytoconstituents and potential pharmacological properties.

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