



Screening of phytochemical constituents and the antimicrobial activity of the macro Algae *sargassum* sp collected from Cape of Camorin

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ABSTRACT

The present study deals with the preliminary analysis for antimicrobial activity of *Sargassum* sp collected from Cape of camorin. The *Sargassum* was extracted with three organic solvents such as Acetone, Aqueous and Ethanol, while antimicrobial activity was studied against 5 pathogens. Results indicated high inhibition of the methanol extract against the pathogens such as *E.coli*(12mm), *staphylococcus aureus*(11mm), *Bacillus* sp(12mm) and *Pseudomonas* (10mm). Further screening for phytochemicals constituents of extracts showed positive results on the presence of alkaloid, Carbohydrates, Flavonoids and Phenolic compounds.

Key words: *Sargassum* sp, Antimicrobial activity, Phytochemistry, Crude extracts.

INTRODUCTION

Marine algal species of seaweed are now in abundant supply on our shores leading to many questions as to the potential for utilization in agriculture and other areas of economic activity.

Internationally seaweed is regarded as an underutilized bio resource. Locally, anecdotal evidence suggests that the application of seaweed enhances the growth of fruit and vegetable crops in a variety of ways.

The term "Seaweed" is not a taxonomic term and is used primarily to describe large, benthic marine algae. Seaweeds are broadly classified into three main groups based on their pigmentation Phaeophyta (brown algae), Rhodophyta (red algae), Chlorophyta (green algae). Brown seaweeds are the second most abundant and comprise the majority of seaweeds that reach our shores. Seaweeds, especially brown seaweeds such as *Sargassum* species, have been used in farming systems in coastal areas of the world since the twelfth century. The term "Seaweed" is not a taxonomic term and is used primarily to describe large, benthic marine algae. Seaweeds are broadly classified into three main groups based on their pigmentation Phaeophyta (brown algae), Rhodophyta (red algae), Chlorophyta (green algae). Brown seaweeds are the second most abundant and comprise the majority of seaweeds that reach our shores. Seaweeds, especially brown seaweeds such as *Sargassum* species, have been used in farming systems in coastal areas of the world since the twelfth century.

In recent years considerable work has been done on natural products for the presence of active molecules that could be used in drug development. Marine algae are being used as food supplement, source of vitamins, and as food additives. Gustafson et al. reported anti-HIV activity of *Lyngbya langerheimii* and *Phormidium* tenure. Furthermore, National Facility for Marine Cyanobacteria has reported its use for treating a number of noxious

effluents containing organophosphorus, pesticides, detergents, antibiotics, and other molecules. However not much work has been done on marine alga, particularly Sargassum sp.

Therefore the present study was undertaken to evaluate the antimicrobial activity taking Sargassum sp. extract, a common seaweed found in Indian ocean.

EXPERIMENTAL SECTION

Sample Collection and Identification :

The algae Sargassum sp was collected from Kanyakumari coastal regions. The algae which attached exclusively on the intertidal rocky substratum was collected. The collected sea weeds was initially washed thoroughly with filtered sea water and again with distilled water to remove extraneous materials. Species was identified using standard books and manuals.

Preparation of sea weed powder :

The fresh algal powder was air dried. The air dried samples are cut into small pieces and then ground in a tissue grinder until reach the fine powder.

Extraction of sea weeds:

The powdered sea weeds are soaked separately in 100ml of organic solvents viz., Acetone, Methanol and Aqueous. The mixture is get incubated for 24 hours to homogenize the sample. Then it was filtered through whatman No:01 filter paper. The filtrate was then concentrated using rotary evaporator. Store the concentrated extract in refrigerator for further use.

Biochemical Characterization :

Test for Carbohydrate:

The presence of carbohydrate in the extracted seaweeds was tested using Molisch's test. Add two drops of Molisch's reagent to 2ml of seaweed extract in a test tube. Mix thoroughly. Add two drops of concentrated sulfuric acid by the side of the test tube slanting the tube. Then erect the test tube slowly. The formation of reddish violet ring at the junction of two liquids indicates the presence of carbohydrate.

Test for Protein :

The presence of protein in the seaweed extract is determined by using Biuret test method. To 2ml of seaweed extract add 1ml of 40% NaOH solution and 1 or 2 drops 1%

CuSO₄ solution. The formation of violet color indicates the presence of peptide linkage of the extract.

Phytochemical characterization:

Tests for Alkaloids

0.5g of extract was stirred with 5ml of 1percent aqueous hydrochloric acid on a water bath from that 1ml portion was treated with Dragendorff's reagent. Turbidity of precipitation showed the presence of alkaloids in the extract.

Test for Tannin

A few ml of the sample was taken in a boiling tube and vanillin reagent was added to it. Lack of color change showed the absence of tannin.

Test for Flavonoid

A few ml of sample was taken in a boiling tube and treated with magnesium turnings and few drops of concentrated hydrochloric acid. The lack of appearance of blue colour confirms the absence of flavonoids in the extract.

Test for Phenol

The sample was taken in small quantity that was treated with alcoholic ferric chloride. The yellow color formation indicates the presence of phenol.

Test for Sugar/Glucosides

A few ml of sample was taken in a boiling tube and mixed with equal quantity of anthrone reagent. Then it was treated with 2 drops of concentrated sulfuric acid and heated in a boiling water bath gently. The green color formation indicates the presence of sugar.

Evaluation of antimicrobial activity studies :**Agar Well Diffusion Method**

Antibacterial activity was determined against the bacteria such as E.coli, Staphylococcus aureus, Bacillus sp, Pseudomonas and Candida albicans using the well bore assay method. The sterile bore was impregnated with different extracts (50 mg/ml). Agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. The impregnated extracts were placed on the Muller Hinton medium suitably spaced apart and the plates were incubated at 37°C for 24h. The diameter of the growth inhibition halos caused by the organic extracts against the organisms was measured by a ruler and expressed in millimeter.

RESULTS AND DISCUSSION

The organic extracts of the seaweed were assayed for its Phytochemistry and its antimicrobial activity by using agar diffusion method. The phytochemistry results was shown in Table no :01 and the Antimicrobial activity of these extracts is shown in the following table:02.

The table :01 clearly indicates the presence of Biochemical and phytochemical compounds present in the three different extracts.

Table : 1

<i>Sl. No</i>	<i>Phytochemistry</i>	<i>Aqueous</i>	<i>Acetone</i>	<i>Ethanol</i>
1	Saponin	Absence	Absence	Absence
2	Terpenoids	Absence	Presence	Absence
3	Tannins	Presence	Presence	Presence
4	Steroids	Absence	Presence	Presence
5	Glycosides	Absence	Absence	Absence
6	Alkaloids	Presence	Presence	Presence
7	Flavanoids	Absence	Presence	Presence
8	Anthraquinones	Presence	Absence	Absence
9	Phenol	Presence	Presence	Presence

The Aqueous extract of the sargassam showed positive results for the presence of Tannins, Alkaloids, Anthraquinones and Phenols. The Acetone extracts showed positive results for the presence of Terpenoids, Tannins, Steroids, Alkaloids, Flavanoids and Phenols. Mean while the Ethanol extracts showed positive results for the presence of Tannins, Steroids, alkaloids, Flavanoids and Phenols.

Table : 2 The activity of the organic extracts against the pathogens .All the three extracts showed a good controlling effect on both the gram negative organisms and Gram positive organisms

Compounds	Microorganisms			
	<i>E.coli</i>	<i>Staph.aureus</i>	<i>Bacillus</i>	<i>Pseudomonas</i>
Aqueous extract	10mm	12mm	11mm	12mm
Replicate	10mm	12mm	10mm	11mm
Acetone extract	12mm	14mm	12mm	12mm
Replicate	11mm	12mm	12mm	14mm
Ethanol extract	12mm	13mm	14mm	14mm
Replicate	11mm	14mm	12mm	12mm

CONCLUSION

The seaweeds extracts that were tested for its antimicrobial effect successfully showed the maximum zone of inhibition against most of the tested microorganisms. All the four solvent extracts were found to be effective against the pathogens. The scopes of using seaweeds in the development of new pharmaceutical agents are having a new hope in the present study.

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