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QUALITATIVE, QUANTITATIVE EXPLORATION OF PHYTOCHEMICALS AND DETERMINATION OF ANTIBACTERIAL ACTIVITY OF *SARGASSUM WIGHTII*

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Sargassum wightii, Seaweed, Sulfated polysaccharides, Brown algae, Phytochemicals, Antibacterial activity

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ABSTRACT: Marine carbohydrates play a crucial role in drug development and disease eradication as it is suggested as a promising bioactive material for its various properties. Seaweeds are the marine macroalgal source that contains a large amount of sulfated polysaccharides. *Sargassum wightii* (*S. wightii*) is one of the seaweed which possesses a wide range of pharmacological actions. The aim of the present work is to qualitatively quantitatively analyze the phytochemical substances and screen the antibacterial activity of *S. wightii*. The study is accomplished with chloroform and methanolic extracts of *S. wightii*. Among this, methanolic extract of *S. wightii* shows the presence of more phytochemicals and a substantial antibacterial activity. Quantitative analysis showed the presence of flavonoid content more. Antibacterial activity was more for gram-negative bacteria. Analysis of phytochemical substances and anti-bacterial activity determination reflected significant results. Hence further observations and studies on this macroalgae could bring out promising results in the future for the welfare of mankind.

INTRODUCTION: Marine carbohydrates are the most important organic molecules made by photosynthetic organisms. Carbohydrates are found in various marine environments in different concentrations. Polysaccharides of carbohydrates do have significant roles in various fields such as pharmaceutical, food production, cosmeceutical, and so on. Marine organisms are good resources of nutrients, and they are rich in carbohydrates in the sulfated polysaccharide.

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Seaweeds are microscopic marine algae, which are the rich source of structurally novel and biologically active secondary metabolites that might represent useful leads in the development of new pharmaceutical agents and are used in different pharmaceutical industries, especially in pharmaceutical compound production.

Seaweeds possess a considerable amount of sulfated polysaccharides, which are used in the cosmeceutical industry, besides based on biological applications. Seaweeds that contain sulfated polysaccharides hold potential applications like antiviral activity, antioxidant activity, anticancer activity, immunomodulating activity, antilipidemic activity, in the blood coagulation system, *etc*. Sulfated galactans, like marine organisms, are rich in polysaccharides.

Agar and alginates, like polysaccharides which are extracted from marine organisms, have several applications in food production and cosmeceutical industries. Because of their high health benefits, compound-derived extracts of marine poly-saccharides also possess applications. If much attention is paid to unravel the structural, compositional, and sequential properties of marine carbohydrates in the future, it would become a milestone in the exploration of various health benefits for the upcoming generations [1]. Sargassum is one of the marine macroalgal genera belonging to the family Sargassaceae and is widely distributed in tropical and temperate oceans. It is a large, economically important, and ecologically dominant brown algae present in much of the tropics, and a wide range of bioactive properties have been reported from this species. It is widely distributed in India and many parts of Asia, and it is reported to be used as animal feed, food ingredients, and fertilizer. *Sargassum wightii* shows a good amount of phenols and flavonoids, which support its antimicrobial activity, indicating that this genus is an ideal target *Sargassum wightii* for exploring the presence of biomolecules for various medical and industrial applications [2]. Hence, the present work is to evaluate the phytochemical analysis and the antimicrobial activity of seaweed, *Sargassum wightii* (Brown algae), against different bacterial pathogens.

MATERIALS AND METHODS:

Collection of Plant Material: Fresh samples were collected by handpicking from the Thirumulla-varam coast of Kollam, Kerala, India. The plant was identified and authenticated by Sr. Tessy Joseph, HOD of Department of Pharmacognosy, Nirmala College of Pharmacy, Muvattupuzha, Kerala, India. The collected Seaweeds were cleaned well with seawater to remove all the extraneous matter, then thoroughly washed with distilled water and subjected to shade drying. The shade dried seaweeds were grounded to a fine powder using a mixer grinder. The powdered samples were then stored in an airtight container for further use.

Preparation of Plant Extract: Powdered samples then subjected to solvent extraction with chloroform and methanol using a continuous soxhlet extraction process. 10 g powder was initially soaked in 50 ml of each solvent, chloroform & methanol in an airtight conical flask for 2 days, and then it was first filtered through a double-layered muslin cloth and then filtered through Whatman no 1 filter paper and the filtrate was collected into a sterile airtight bottle. The extract was then stored at the refrigerator for further studies.

Chemicals and Reagents: All the materials used were of either AR/LR grade or the best possible grades.

Qualitative Analysis of Phytochemical Substance: [3] Phytochemical analysis of both the extracts was carried out to detect the presence of various biomolecules according to the standard qualitative procedures described by Trease and Evans (1989).

a) **Test for Alkaloids:** 1 ml of 1% HCl was added to 3 ml of extract in a test tube and was treated with a few drops of Meyer's reagent. A creamy white precipitate indicated the presence of alkaloids.

b) **Test for Terpenoids:** 5 ml of the extract was mixed with 2 ml of $CHCl_3$ in a test tube. 3 ml of concentrated H_2SO_4 was then added to the mixture cautiously to form a layer in a slanting position through the sides of the test tubes. An interface with a reddish-brown coloration was formed for the presence of terpenoids.

c) **Test for Saponins:** 5 ml extract was shaken vigorously to obtain a stable, persistent froth. 3 drops of olive oil were then mixed with the frothing and observed for the formation of emulsion, which indicated the presence of saponins.

d) **Test for Flavonoids:** A few drops of 1% NH_3 solution was added to the extract in a test tube. A yellow coloration indicated the presence of flavonoids.

e) **Test for Tannins:** To 0.5 ml of the sample, 1ml of distilled water and 1-2 drops of ferric chloride solution was added. A brownish-green or a blue-black coloration indicated the presence of tannins.

f) **Test for Glycosides:** 10 ml of 50% H_2SO_4 was added to 1 ml of extract in a boiling tube. The mixture was heated in boiling water for 5 min. 10

ml of Fehling's solution (5 ml of each solution A and B) was added and boiled. A brick-red precipitate indicated the presence of glycosides.

Test for Phenols: Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. The formation of bluish-black color indicates the presence of phenol.

Quantitative Analysis of Phytochemicals: [3]

Estimation of Phenols: The total phenolic assay was determined by using the Folin-Ciocalteu assay. A known amount of the sample then ground well with 80% ethanol and subjected to centrifugation at 4000 rpm. An aliquot (1 ml) of extract or standard solution of caffic acid is added to 250 ml of the flask containing 9 ml of distilled water.

About 1 ml of folin-ciocalteu phenol reagent was then added to the mixture and shaken well. After some time about 10 ml of 7% sodium bicarbonate was added to the mixture. The solution was diluted to 25 ml with distilled water, mixed, and incubated for 90 min at room temperature. A reagent blank was prepared with double distilled water. The absorbency is determined by 750 nm with a UV spectrophotometer. Total phenolic content was expressed as mg caffic acid equivalents mg/100 gm dry mass.

Estimation of Flavonoids: The total flavonoid content was measured by the Aluminum chloride calorimetric assay. A known amount of the samples was taken; ground well with 80% ethanol and was centrifuged at 4000 rpm. 1 ml of extract or standard solution of catechin (20, 40, 60, 80, & 100 mg/l) was then added to a 10 ml volumetric flask containing 4 ml of double-distilled water. To the flask was added 0.3 ml 5% sodium nitrate, after 5 minutes 0.3 ml 10% aluminium chloride was added.

At the sixth minute, 2 ml of 1M NaOH was added and the total volume was made up to 10 ml with double distilled water. After mixing the solution well, absorbance was measured against the prepared reagent blank at 510 nm. Total flavonoids content was expressed as mg catechin equivalents (CE)/ 100 g dry mass.

Estimation of Tannin: The vanillin method is applied to yield a red-colored adduct measured spectrophotometrically at 500 nm. The tannins content in extracts examined was calculated using a standard curve drawn up for methanol solutions of catechin x 100 g-1 dried weight.

Test Organisms: Eight different bacterial strains of *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholera, Klebsiella pneumonia* and *Escherichia coli* were obtained from department of Pharmacognosy, Nirmala College of Pharmacy, Kerala.

Screening of Antimicrobial Activity:

Bacterial Inoculum Preparation: Bacterial inoculum was prepared at first using a loopful of test organisms in 5 ml of Nutrient broth and incubated at 37 ºC for 3-5 h till moderate turbidity was developed. The turbidity was then compared with 0.5 Mc Farland standards and then used for the determination of antibacterial activity.

Agar Plate Diffusion Assay: [4] The antibacterial activity of *Sargassum wightii* was performed by the agar well diffusion method. Petri plates containing about 20 mL Muller-Hinton's agar medium was prepared by adding 0.26 g agar broth and 0.312 g agar as a solidifying agent in 20 mL sterilized water and allowed to solidify. Then plates were dried, and 0.1 ml of test organisms were taken from the stock broth and swabbed on agar medium by using sterilized buds.

Then the wells of 6 mm diameter were made on the agar plates by using a cork borer. 25 µl extract and positive control tetracycline each added to the respective wells with the help of a sterilized pipette.

Then the plates were incubated at 37 ºC for 48 h. The antibacterial activity of the test and reference were observed and compared through the zone of inhibition (in mm) on the plates.

RESULTS:

Qualitative Analysis of Phytochemical Substance Screening in Brown Macroalgae, *S. wightii*: Phytochemicals are non-nutritive plant chemicals that reflect protective or disease preventive properties [5]. Here, phytochemical screening of both the extracts showed the presence or absence of various compounds.

From which methanol extract showed the presence of a maximum number of compounds.

TABLE 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF CHLOROFORM & METHANOLIC EXTRACTS OF *S. WIGHTII*

Test	Chloroform Extract	Methanol Extract
Carbohydrates	+	+
Proteins and Amino acids	+	+
Steroids	-	+
Glycosides	-	+
Alkaloids	+	+
Phenolic compounds	+	+
Flavonoids	+	+
Tannins	-	+
Saponins	-	+
Terpenoids	-	-
Mucilage	-	-

(+) = Present, (-) = Absent

Quantitative Analysis of the Phytochemical Substance in Seaweed: Quantitative phytochemical analysis showed the presence of bioactive compounds like.

TABLE 2: QUANTITATIVE ANALYSIS OF PHYTO-CHEMICAL SUBSTANCE *S. WIGHTII*

Phenol Content	190.39 ± 15.27
Flavonoids	358.50 ± 13.28
Tannins	30.35 ± 0.54

Screening of Antibacterial Activity of *S. wightii:* Antibacterial activity of two different extracts of *S. wightii* has been checked against various gram +ve and –ve bacteria, and the result is shown in **Table 3** below.

TABLE 3: DIAMETER OF ZONE OF INHIBITION (IN MM) PRODUCED BY THE SOLVENT EXTRACTS OF *S. WIGHTII* AGAINST VARIOUS MICROORGANISMS

Bacteria	Chloroform Extract 25µl	Methanol Extract 25µl
Staphylococcus aureus	8.2	9 ± 0.45
Bacillus subtilis	Trace	11 ± 0.51
Pseudomonas aeruginosa	12.3 ± 0.11	12 ± 0.48
Vibrio cholera	7	10
Klebsiella pneumonia	2	10 ± 0.38
Salmonella typhi	8.9 ± 0.16	10.7 ± 0.32
Escherichia coli	7.5	18 ± 0.21

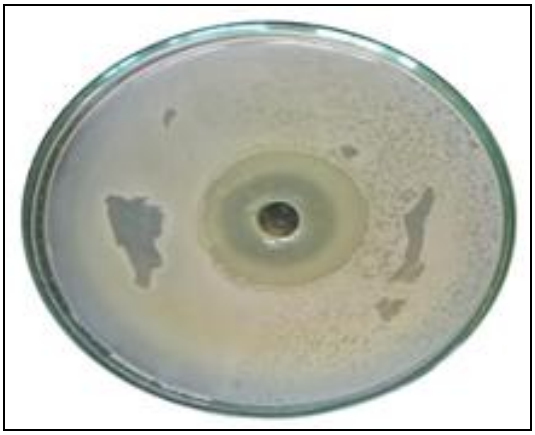

FIG. 1: ZONE OF INHIBITION (18 ± 0.21 MM) PRODUCED BY THE METHANOL EXTRACT OF *S. WIGHTII* AGAINST *ESCHERICHIA COLI*

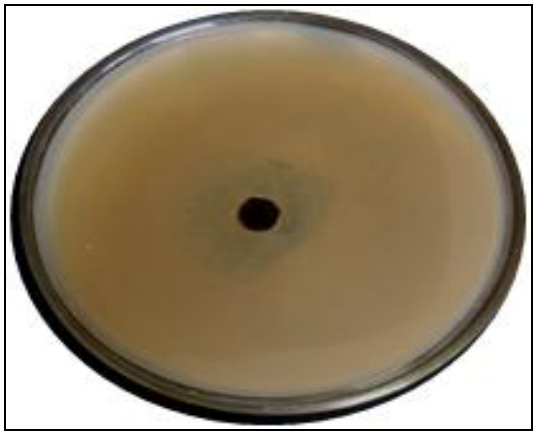

FIG. 2: ZONE OF INHIBITION (12 ± 0.48 MM) PRODUCED BY THE METHANOL EXTRACT OF *S. WIGHTII* AGAINST *PSEUDOMONAS AERUGINOSA*

FIG. 3: ZONE OF INHIBITION (12.3 ± 0.11 MM) PRODUCED BY THE CHLOROFORM EXTRACT OF *S. WIGHTII* AGAINST *PSEUDOMONAS AERUGINOSA*

DISCUSSION: *Sargassum wightii* is one of the marine brown seaweed species, with immense biological applications and is known to be rich in sulfated polysaccharides content, and these sulfated polysaccharides were found to possess a wide range pharmacological and biomedical properties [6].

The important phytochemicals viz. Proteins and Amino acids Steroids Glycosides Alkaloids Phenolic compounds Flavonoids Carbohydrates Tannins Saponins Terpenoids and Mucilage was screened for their presence and presented in **Table 1**. And the presence of activity might be due to the presence of phytochemicals, alkaloids, phenols, and sugars [7].

Quantitative phytochemical analysis of chloroform & methanolic extracts of *S. wightii* is presented in **Table 2**. Flavonoids content was more, and it was found to be 358.50 ± 13.28. Some studies also reveal that flavonoid content could contribute to this effect [8]. Phenolic compounds exert a significant effect on antibacterial activity [9]. And the results of the quantitative analysis of *S. wightii* extracts reveals that more zone of inhibition obtained maybe because of the presence of these phytochemicals could inhibit the growth of bacteria more profoundly.

The antibacterial activity of *S. wightii* extracts on seven human pathogens is presented in **Table 3**. Antibacterial activity of the solvent extracts was performed against different bacterial pathogens by the agar well diffusion method. Both the seaweed extract has shown moderate antibacterial activity. Out of which methanolic extract has shown significant activity against various Gram (+)ve and Gram (-)ve human pathogenic microbes. The zone of inhibition was in the range of 2 to 18 mm. The maximum antibacterial activity of 18.0 ± 0.21 mm was found to be exhibited by the crude methanol extract against gram-negative bacteria, *E. coli.* Extracts showed a better antimicrobial effect for gram-negative bacteria rather than gram-positive bacteria. The phytochemicals which were present in the extracts might be responsible for this activity, and the solvents could extract out maximum phytochemicals to elicit the antibacterial activity. And the results also indicate that methanol will be the desirable solvent for *Sargassum wightii* to claim more antibacterial activity [10-13].

CONCLUSION: The present investigation brings out fruitful results on the phytochemical composition and antibacterial potential of the seaweed extract *S. wightii* [14]. This work reflects the vital role of the presence of phytochemicals in the methanolic extract of *S. wightii*, its antibacterial activity, and the aptness of the solvent to elicit the required pharmacological action mentioned above. Hence proper researches on this seaweed could be a promising factor for the various pharma contributors in the future for the welfare of mankind [15].

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