ANTIBACTERIAL ACTIVITY OF SARGASSUM SPECIES SEAWEED EXTRACTS AGAINST *E.COLI*

Running title : Antibacterial activity of *Sargassum* species against the *E.coli*

ABSTRACT:

Introduction: Seaweeds are marine macroscopic algae; they are the raw materials for the production of agar and algin and later they are consumed as foodstuffs. Seaweeds are grouped under three divisions viz., Chlorophyceae, Phaeophyceae, and Rhodophyceae which are found in relatively shallow coastal waters. Sargassum, a genus of brown seaweed, and it is commonly known as gulfweed belonging to the family Sargassaceae. The antibacterial activity of a molecule is completely associated with compounds that kill bacteria or slow down their growth rate, without being highly toxic to nearby tissues. The main aim of the study is to prepare and evaluate the potential antibacterial activity of Sargassum species seaweed extract against *E.coli.*

Materials and method: The fresh seaweed Sargassum sp. was collected from Tuticorin coastal area, Tamilnadu. The extract was prepared using ethanol and stored in a shadowy aluminum container at 4ºC for further analysis. The bacterial suspension was made using Gram-negative Drug-resistant Escherichia coli, Uropathogenic E.coli, and Verotoxin-producing E.coli. The antibacterial activity of seaweed extract was performed with a disc diffusion method. Minimal Inhibitory Concentration of seaweed extract was observed.

Results : The data was collected and tabulated and the bioactivity of the seaweed extracts was expressed as minimum inhibitory concentration (MIC). The antibacterial activity against the selected isolated *E.coli, UPEC* and *VPEC* was more susceptible to the crude extract of the seaweed (*Sargassum sp.*), as the MIC was 20 µg/ml. The extract showed the antibacterial activity.

Conclusion: It can be concluded that *Sargassum sp.* Seaweed has antibacterial potential. Further future work should be done to determine the exact active compounds responsible for activity

Keywords: antibacterial activity, seaweeds, extract, *E.Coli*, natural source

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INTRODUCTION:

Plants are considered as a vital part of the world's natural heritage and nearly 80% of the population relies on plants for healthcare. Some medicinal plants are a more important part of our natural wealth. Some medicinal plants are known for their biological activities such as antimicrobial, anti-inflammatory (1), anticancer(2), (3) antioxidant (4), antidiabetic(5,6), antiviral, antifungal and antibacterial activities. Medicinal plants show abundant supply and with the widespread use and extraction, medicinal plants are used for treating disease.

Seaweeds are marine macroscopic algae; they are the raw materials for the production of agar and algin and later they are consumed as foodstuffs. Seaweeds contain various kinds of organic and inorganic substances and they form one of the important living resources grouped under three divisions viz., Chlorophyceae, Phaeophyceae, and Rhodophyceae which are found in relatively shallow coastal waters (7). Certain biological compounds extracted from some seaweed species, namely Chlorophyceae, Phaeophyceae, and Rhodophyceae where they have potential medicinal activities such as antifungal, antiprotozoal, mosquito, and larva control, antibacterial, antiviral and antitumor (8). Till date, only certain antibacterial activities of brown seaweed species have been studied in detail (9). Seaweeds possess interesting biological activities that contribute to the discovery of natural therapeutic agents and are also used in pharmaceutical and biochemical applications (10). They are sources of vitamins such as A, B1, B12, C, D, and E, riboflavin, niacin etc. In addition to minerals and vitamins, seaweeds are potentially good sources of proteins and fibers. Amino acid, acrylic acid, terpenoids, phlorotannins, steroids, halogenated ketones, and alkanes are some of the bactericidal agents found in algae (11). The compounds with antimicrobial, antiviral, antifungal, and antibacterial activities have been detected in brown, red, and green algae (12).

Sargassum, a genus of brown seaweed and it is commonly known as gulfweed belonging to the family *Sargassaceaec (13).* It is generally attached rocks along tropical and subtropical regions and they produce metabolites of structural classes such as sargaquinoic acids, sargachromenol, plastoquinones, steroids, terpenoids, polysaccharides, polyphenols, glycerides, etc., which possesses several therapeutic activities (14). It has been considered as a medicinal food and research is being done on it to reveal its other pharmacological properties (15). Sargassum inundation events may cause biological and ecological impacts in affected regions. Sargassum

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species decomposition in large amounts along the coastline consumes oxygen, creating a high rate of oxygen-depleted zones resulting in fish kills. Further decomposition of sargassum additionally created hydrogen sulfide gas, which causes a range of health problems in humans (16). The antibacterial activity of a molecule is associated with compounds that kill bacteria which slow down their growth rate, without being highly toxic to nearby tissues. The most recently discovered antimicrobial agents are modified natural compounds and are modified by chemical methods, such as b-lactams (penicillins), Carbapenems, or cephalosporins(17). Antimicrobial agents may be categorised as either bacterial agents that destroy bacteria or bacteriostatic agents that slow down the growth of bacteria. Antibacterial agents are the most effective ones in the war against infectious diseases but, with both extensive use and misuse, the emergence of bacterial resistance to antibacterial agents has become a significant concern for today's pharmaceutical industry. Resistance is most commonly based on developmental processes, such as antibiotic therapy, that lead to inheritable resistance (18). Our team has extensive knowledge and research experience that has translated into high quality publications (19–23) (24) The main aim of the study was to find the potential antibacterial activity of Sargassum species seaweed extract against *E.coli.*

MATERIALS AND METHOD:

Study Settings : Marine Biomedical and Environmental Health Research Lab - Blue Lab Department of Pharmacology, Saveetha Dental College and Hospitals, Chennai, India.

Sample collection: The fresh seaweed Sargassum sp. was collected from Tuticorin coastal area, Tamilnadu. The sample was washed thoroughly with tap water then shade dried on table tissue paper for 4 weeks and turned into a fine powder.

Preparation of extraction: 10g of dried powdered seaweed sample was mixed with 100ml of Ethanol and allowed to place for 24 hours at ambient temperature. Then the mixture was passing through Whatman filter paper (No.4) then the filtrate was centrifuged at 3000rpm for 10min and further filtered by a 0.45µm syringe micro filter. At last, the solvents are evaporated via a vacuum rotary evaporator until samples are obtained in powder form. Then the sample was stored in a shadowy aluminum container at 4ºC for further analysis.

Bacterial Suspension: Gram-negative Drug-resistant Escherichia coli, Uropathogenic E.coli and Verotoxin-producing E.coli was collected from the Department of Microbiology, Saveetha medical college and hospital, Tamilnadu. The bacterial pathogens were cultured in Muller – Hinton Broth for 24 hr at room temperature. From this bacterial suspension was prepared with saline and the optical density was measured at 600 nm. The concentration of microbial suspension was fixed as 108 CFU/ml. 1ml of suspension was spread over on Muller Hinton agar plate and incubated for 24hrs at ambient temperature.

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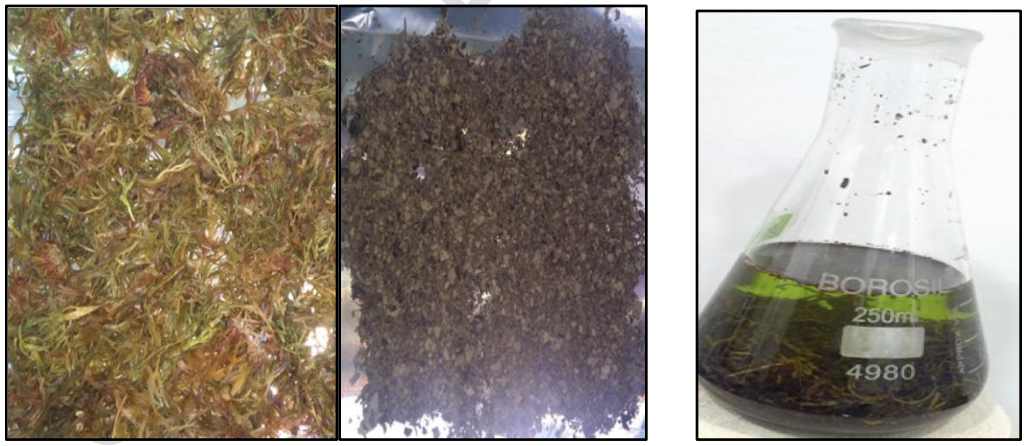
Antibacterial activity: The antibacterial activity of seaweed extract was performed with a disc diffusion method. Whatman filter paper discs (5mm) were impregnated with various concentrations (0.5, 1, 1.5, 2, 2.5 and 3mg/ml) of leaf extract using ethanol and methanol solvent. The inoculated plates were incubated for 24 hr at room temperature and the inhibition zones around the discs were measured. All the results were expressed from an average of three with a standard deviation.

Minimum Inhibitory Concentration: Minimal Inhibitory Concentration of seaweed extract on ethanol and methanol was determined in 10 concentrations (10 -100 µg/ml / 0.001 to 0.1 mg /ml) with blank (extract in Muller Hinton broth). The inoculated bacteria in test tubes are incubated for 24hr in ambient temperature then the optical density was observed.

RESULTS

In the present investigation, the marine brown algae, *Sargassum sp.*, was collected from Tuticorin coastal area, Tamilnadu. The collected sample seaweed was dried on table tissue paper for 4 weeks and turned into a fine powder. The extract was prepared using ethanol (figure 1 & figure 2). As for the solvent extract, methanol exhibited higher antibacterial activity against tested human pathogenic bacteria. The antibacterial activity was done using a disc diffusion method. The disc diffusion test was done and the results are mentioned in Table 1 for different concentrations for the selected clinical isolates (*E.coli, UPEC, VPEC*). Followed by Minimum Inhibitory Concentration (MIC) of seaweed extract on ethanol and methanol was determined in 10 concentrations and the results are mentioned in Table 2.



(A) **(B** (C)

Figure 1: The image (A) depicts that *Sargassum sp.* are collected, (B) depicts the dried *Sargassum sp.* and (C) depicts the crude extract taken from the seaweed (*Sargassum sp.*).

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Table 1: This table depicts the zone of inhibition for different concentrations for the clinical isolated species (*E.coli, UPEC, VPEC)* with positive control as tetracycline and negative control as DMSO.
UPEC - *Uropathogenic E.Coli*; VPEC - *Verotoxin producing E.coli*

Zone of inhibition			
µg/ml	E.coli	UPEC	VPEC
0	0	0	0
50	7±1.2	3±1.2	2±1.4
100	10±1.2	5±1.2	4±1.2
150	12±1.6	7±1.3	5±1.6
200	16±1.4	10±1.6	8±2.3
250	19±1.2	11±1.4	10±1.8
300	23±1.8	12±1.2	11±2.5

Table 2: This table depicts the Minimum Inhibitory Concentration (MIC) for the selected clinical isolates (*E.coli, UPEC, VPEC)* with positive control as tetracycline.

Minimum Inhibitory Concentration							
	0	10	20	30	40	50	MIC (µg/ml)
E.coli	+	+	-	-	-	-	20
Tetracycline	+	-	-	-	-	-	10
UPEC	+	+	+	+	-	-	40
Tetracycline	+	+	-	-	-	-	20

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VPEC	+	+	+	+	-	-	40
Tetracycline	+	+	-	-	-	-	20

DISCUSSION:

The antibacterial activity of the extract was analysed using two types of assays, namely disc diffusion method and Minimum Inhibitory Concentration (MIC) assay. The disc diffusion test was done and the results are mentioned in Table 1 for different concentrations for the selected clinical isolates (*E.coli, UPEC, VPEC).* For one gram of E.coli, for 0 µg/ml concentration of the crude extract the zone of inhibition is 0, for 50 µg/ml concentration the zone of inhibition was 7 with standard error of ±1.2, for 100 µg/ml concentration the zone of inhibition is 10±1.2, for 150 µg/ml the zone of inhibition is 12±1.6 and for 200 µg/ml the zone of inhibition is found to be 16±1.4 , and for 250 µg/ml the zone of inhibition seen is 19±1.2 and for 300 µg/ml the inhibition was 23±1.8.

The same was done for *UPEC*, for 50 µg/ml the zone of inhibition is 3±1.2, for 100 µg/ml the inhibition seen was 5±1.2, and for 150 µg/ml the zone of inhibition seen is 7±1.3, for 200 µg/ml of the crude extract the zone of inhibition seen is 10±1.6, for 250 µg/ml the zone of inhibition is 11±1.4 and for 300 µg/ml the inhibition is 12±1.2. For one gram of *VPEC*, for 50 µg/ml of the crude extract, the zone of inhibition seen is 2±1.4, for 100 µg/ml the zone of inhibition seen is 4±1.2, for 150 µg/ml the zone of inhibition seen is around 5±1.6 and for 200 µg/ml of the crude extract it shows the zone of inhibition of 8±2.3, for 250 µg/ml the zone of inhibition seen is 10±1.8 and for 300 µg/ml the inhibition seen is 11±2.5. From the disc diffusion susceptibility test done for the highest concentration of 300 µg/ml of the crude extract, *E.coli* showed the highest zone of inhibition of the selected clinical isolates and *UPEC* showed the second highest zone of inhibition followed by *VPEC.* Many studies have been done by our team of researchers (25) - (26).

The Minimum Inhibitory Concentration (MIC) test was also done on the selected extracts with positive control as tetracycline and the results are mentioned in Table 2. For *E.coli* the MIC was found to be 20 µg/ml of the crude extract of the seaweed and for tetracycline the MIC for *E.coli* is seen to be 10 µg/ml. For *UPEC* the MIC was found to be 40 µg/ml of the crude extract of the seaweed and for tetracycline the MIC for *E.coli* is seen to be 20 µg/ml. For *VPEC* the MIC was

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found to be 40 µg/ml of the crude extract of the seaweed and for tetracycline the MIC for *E.coli* is seen to be 20 µg/ml.

Many similar previous studies were done on the antibacterial activity of many species of seaweed. A supporting study was done by Chong Chico-Wei (27) on seaweed species on antibacterial activity on various isolates with similar results observed and similar antibacterial activity is seen. Similar to the present investigation Rosaline et al (28) reported that the methanol extract of *Sargassum sp.* showed antibacterial activity of several Gram-negative bacteria. The limitation of the study was carried out only on E.coli species. Further research should be explored in different types of species and observe the changes.

CONCLUSION:

In this study, the antibacterial activity of the *Sargassum sp.* Seaweed varies according to the selected isolates (*E.coli, UPEC, VPEC)* and extraction solvent(29-38). *E.coli* showed the highest zone of inhibition when compared to the other selected clinical isolates of *E.coli.* The Minimum Inhibitory Concentration (MIC) for *Uropathogenic E.Coli* and *Verotoxin producing E.coli* was found to be similar and higher than the other selected clinical isolates of *E.coli.*

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