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Antibacterial Activity of the Crude Extract of the Seaweed (*Ulva* Species) Using Clinical Isolates

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ABSTRACT

Introduction: Most of the bioactive substances isolated from marine seagrass are chemically classified as brominated, aromatics, nitrogen-heterocyclic, nitro sulphuric-heterocyclic, sterols, dibutanoids, proteins, peptides, and sulfated polysaccharides. Seagrass can be used as a foodstuff, animal fodder, fertilizer, and industrial material. 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. The aim of the study was to find the antibacterial activity of the seaweed (Ulva sps) against clinically selected isolates (*Klebsiella pneumonia, Salmonella typhi* and *Streptococcus* sp)

Materials and Methods: The fresh seaweed *Ulva* sp. was collected and shade dried then the crude extract was prepared. The pathogenic *Klebsiella pneumonia, Salmonella typhi*, and *Streptococcus* sp., was collected. The disc diffusion test and Minimum inhibitory concentration test was done.

Results and Discussion: 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 *Klebsiella pneumonia, Salmonella typhi*, and *Streptococcus* sp. *Salmonella typhi* was more susceptible for the crude extract of the seaweed (*Ulva* sps) as the MIC = 30 µg/mL. The extract shows potential bactericidal activities against the different pathogens.

Conclusion: The study concluded that the seaweed (*Ulva* sp) has very good antibacterial activity against the selected isolates (*Klebsiella, Streptococcus, Salmonella*) and *Streptococcus* sp had more MIC when compared to other isolates.

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INTRODUCTION

Plants have been used in various fields through the ages. The term medicinal plants include various types of plants used in herbalism and some of these plants have medicinal activities. These medicinal plants are considered as a rich resource of ingredients which can be used in drug development and synthesis.[1] Plants have antibacterial, antifungal, anti inflammatory,[2] anti diabetic,[3,4] anti cholesterol, anti oxidant,[5] anti cancer,[6,4,7] and enzyme inhibitory effect.[8] Indeed, even so a large portion of these pills which we take and use during our day to day life came from plants. Therapeutic plants are often utilized as crude materials for extraction of dynamic fixings which are utilized in the amalgamation of various medications.[9]

Seaweed are marine algae, they are the crude materials for the preparation of agar and algin and later they are devoured as groceries and many other useful substances.[10] Ocean growth contains different sorts of both organic and inorganic substances and the significant living assets are gathered under three divisions viz., Chlorophyceae, Phaeophyceae, and Rhodophyceae which are found in shallow seaside waters. Certain natural mixtures extricated from some ocean growth species, to be specific Chlorophyceae, Phaeophyceae, and Rhodophyceae have potential properties like antifungal, antiprotozoal, mosquito, antibacterial, antiviral and antitumor.

Ulva lactuca is a thin flat green algae that grows out of discoid fastness. The margin is a little blurred and often torn. It can reach 7.1 inch or more in length, although generally much less, and up to 12 inches

KEYWORDS:
Antibacterial activity, Natural source, Pathogens, Seaweed, Ulva lactuca.

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across. The membrane is two cells thick, soft and translucent, and grows attached to rocks or other algae by a small disk-shaped holding fast. The high sugar part incorporates a huge assortment of effectively dissolvable polysaccharides, for example, laminarin, alginate, mannitol, or fucoidan in earthy colored sorts; starch, mannans and sulfated galactans in red kinds, and Ulvan in green sorts. Alginate, one of the primary underlying polymers of earthy colored kelp, gives both solidness and adaptability to the examples presented to streaming water and is one of the modernly pertinent starch intensifies found in ocean growth biomass, as are different hydrocolloids, for example, agar and carrageenans, which are generally utilized as thickeners, gelling specialists or emulsifiers. Different other non-carb items obtained from kelp incorporate protein, lipids, phenols and terpenoids, and minerals, for example, iodine, potash, and phosphorus—valuable for human and creature nourishment.[11] The reaping of macroalgae—a significant crude material for food—before their seashore could well be formed into a viable arrangement. The interest of macroalgae in human sustenance is because of their high mineral focus, (for example, calcium, magnesium, and potassium) and glutamic corrosiveness, which makes them additionally helpful as taste enhancers. Green growth could assist with tending to probably the greatest test right now looked at by the food business, which is the always developing human populace.

Antibacterial as well as antiviral activity of a molecule is completely associated with compounds that kill bacteria and viruses in the province or 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 mode, such as b-lactams (penicillins) Carbapenems, or cephalosporins. Pure natural drugs, such as aminoglycosides and other fully synthetic antibiotics, such as sulfonamides, are also widely used. Antimicrobial agents (seaweed,extract,enzymes) may be categorized as either bacterial agents that destroy bacteria or bacteriostatic agents that slow down the growth of bacteria.[12] 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 resistanceOur team has extensive knowledge and research experience that has translated into high quality publications.[13-18] The aim of the present study was to evaluate the antibacterial activity of *Ulva* sp. against selected clinical isolates.

MATERIALS AND METHODS

Study setting: The study was conducted in the Marine Biomedical and Environmental Health Research Lab - Blue Lab, Saveetha Dental College.

Sample collection: The fresh seaweed *Ulva* sp. was collected from Rameshwaram coast, 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 extract: 10g of dried powdered seaweed samples was mixed with 100ml of ethanol and allowed to place

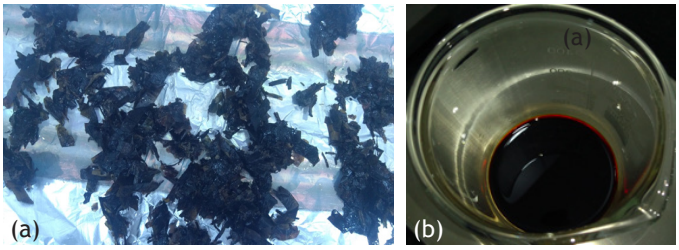

FIGURE 1: (a) The image depicts the crude extract taken from the seaweed (*Ulva* sp.). (b) The image depicts the collected seaweed (*Ulva* sp.) from the Rameshwaram coast, Tamil Nadu and dried under shade

for 24 hours at ambient temperature. Then the mixture was passed through Whatman filter paper (No.4) then the filtrate was centrifuged at 3000 rpm for 10 min and further filtered by a 0.45µm syringe microfilter. At last, the solvents are evaporated via a vacuum rotary evaporator until samples obtain in powder form. Then the sample was stored in a shadowy aluminum container at 4°C for further analysis. (Figure1)

Bacterial Suspension: The pathogenic *Klebsiella pneumonia*, *Salmonella typhi*, and *Streptococcus* sp., was collected from 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 10^8 CFU/mL. 1mL of suspension was spread over on Muller Hinton agar plate and incubated for 24hrs at ambient temperature.

Antibacterial activity: The antibacterial activity of seaweed extract was performed with the disc diffusion method. Whatman filter paper discs (5 mm) were impregnated with various concentrations (0.5, 1, 1.5, 2, 2.5, and 3mg/mL) of leaf extract using ethanol solvent. The inoculated plates were incubated for 24 hours 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 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 were incubated for 24hr at ambient temperature then the optical density was observed.

RESULTS

Antibacterial activity of the seaweed (*Ulva* sp.) was analyzed with two types of assay, namely disc diffusion susceptibility testing and Minimum Inhibitory Concentration (MIC). The disc diffusion screening testing can only give the qualitative but not the quantitative assessment. The disc diffusion test was done and the results are mentioned in Table 1 for different concentrations for the selected clinical isolates (*Klebsiella* sp., *Streptococcus* sp., *Salmonella* sp.). For one gram of *Klebsiella* sp., 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 4 with standard error of ±1.08, for 100 µg/mL concentration the zone of inhibition is 9±2.1, for 150 µg/mL the zone of inhibition is 13±1.9 and for 200 µg/mL the zone of

Table 1: Depicts the zone of inhibition for different concentrations for the clinical isolated species (*Klebsiella* sp., *Streptococcus* sp., *Salmonella* sp.) with positive control as tetracycline and negative control as DMSO

	ZONE OF INHIBITION (mm)		
Concentration of crude extract (µg/mL)	*Klebsiella sp.*	*Streptococcus sp.*	*Salmonella sp.*
0	0	0	0
50	4±1.08	3±1.2	3±1.2
100	9±2.1	9±2.6	7±1.9
150	13±1.9	14±2.2	13±2.2
200	17±2.4	19±1.4	18±1.4
250	20±1.5	22±1.7	22±2.5
300	24±2.3	28±2.4	25±1.8

Table 2: This table depicts the Minimum Inhibitory Concentration (MIC) for the selected clinical isolates (*Klebsiella* sp., *Streptococcus* sp., *Salmonella* sp.) with positive control as tetracycline

	MINIMUM INHIBITORY CONCENTRATION						
MIC	*0*	*10*	*20*	*30*	*40*	*50*	*MIC µg/mL*
Klebsiella	+	+	+	+	+	-	50
Tetracycline	+	+	-	-	-	-	20
Streptococcus	+	+	+	+	-	-	40
Tetracycline	+	+	-	-	-	-	20
Salmonella	+	+	+	-	-	-	30
Tetracycline	+	+	-	-	-	-	20

inhibition is found to be 17±2.4, and for 250 µg/mL the zone of inhibition seen is 20±1.5 and for 300 µg/mL the inhibition was 24±2.3.

The same was done for *Streptococcus* sp., for 50 µg/mL the zone of inhibition is 3±1.2, for 100 µg/mL the inhibition seen was 9±2.6, and for 150 µg/mL the zone of inhibition seen is 14±2.2, for 200 µg/mL of the crude extract the zone of inhibition seen is 19±1.4, for 250 µg/mL the zone of inhibition is 22±1.7 and for 300 µg/mL the inhibition is 28±2.4. For one gram of *Salmonella typhi,* for 50 µg/mL of the crude extract, the zone of inhibition seen is 3±1.2, for 100 µg/mL the zone of inhibition seen is 7±1.9, for 150 µg/mL the zone of inhibition seen is around 13±2.2 and for 200 µg/mL of the crude extract it shows the zone of inhibition of 18±1.4, for 250 µg/mL the zone of inhibition seen is 22±2.5 and for 300 µg/mL the inhibition seen is 25±1.8.

From the disc diffusion susceptibility test done for the highest concentration of 300 µg/mL of the crude extract, *Streptococcus* sp. showed the highest zone of inhibition of the selected clinical isolates and *Salmonella* showed the second highest zone of inhibition and followed by *Klebsiella* sp. The minimum inhibition concentration test was also done on the selected isolates with positive control as tetracycline the results are mentioned in Table 2. For *Klebsiella* sp. the MIC was seen at 40 µg/mL of the crude extract of the seaweed (*Ulva* sp.) and for tetracycline the MIC for *Klebsiella* sp. is seen to be 20 µg/mL. For *Streptococcus* sp. the MIC was seen at 30 µg/mL of the crude extract and for tetracycline as positive control is seen at 20 µg/mL. For *Salmonella typhi,* the MIC was seen at 30 µg/mL and for tetracycline the MIC is 20 µg/mL.

DISCUSSION

Many similar previous studies have been done on antibacterial activity of many species of seaweeds. A supporting similar similar study was done by Ravikumar S[19] on seaweed species on antibacterial activity on various clinical isolates and the results were similar to that of our study and marine macroalgae showed similar antibacterial activity against the selected clinical isolates and another study done by M K Hasamy[20] on antibacterial activity of seaweed and the results of his study shows that of similarity of our study the zone of inhibition for *Klebsiella* in our study is 13±1.9 and in his study the zone of inhibition seen is 12±1.08 which is similar to our study.

Another study was done by Chandrasekaran N[21] on antibacterial activity of seaweed and in this study the authors studied the MIC of the crude extract against selective pathogens and one of the being *Salmonella typhi,* the results of the study was similar to that of ou t study and the crude extract of that study and out study showed very low MIC against *Salmonella typhi* and in another study done by Shafay SIM[22] which was a similar study and agenda, the results of the study were opposing the findings of our study in which *Salmonella typhi* showed high MIC unlike our study in which it showed the lowest MIC.[2, 23-38]

A similar study done by Rao P(39) on antibacterial activity of Green Seaweed Caulerpha sertularioides and in this study the author has recorded the antibacterial activity of the Seaweed against specific isolates (*Staphylococcus aureus, Bacillus subtilis, Lactobacillus acidophilus, Pseudomonas aeruginosa, E.coli., and Proteus mirabilis*). The antibacterial activities were carried out by the agar well diffusion method.

These results showed that the antibacterial activity was recorded in the 100µg/mL concentration. In low concentration (40µg/mL), the highest activities were seen in ethanol extract . This study is also similar to our study which also shows antibacterial properties against selected isolates.[18]

The limitations of this study are the crude extract taken from the seaweed species was not screened and in this study only three clinical isolates were used and for the future prospect in this study the antibacterial activity can be done on various species and future studies can be done on anti cancer activity of seaweed (*Ulva* sp.).

CONCLUSION

The study concluded that the seaweed (*Ulva* sp.) has very good antibacterial activity against the selected isolates (*Klebsiella* sp., *Streptococcus* sp., *Salmonella* sp.*)* and *Streptococcus* sp. had more MIC when compared to other isolates.

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