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ANTIBACTERIAL ACTIVITY OF CRUDE METHANOLIC EXTRACT OF MARINE BROWN ALGA *LOBOPHORA VARIEGATA* (J.V.LAMOUROUX)

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ABSTRACT

Seaweeds are the nature most complete and balanced nutrient source; they are potentially prolific source of highly secondary metabolites that leads to development of new pharmaceutical agent. The present study was carried out for the antimicrobial activity at various concentration ranging from 250 to 1000 µg/mL of the marine brown alga *Lobophora variegata* and were tested against *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Salmonella typhi* by disc diffusion method. The zone of inhibition was measured and compared with the standard streptomycin. At the concentration of 1000 µg/mL *Klebsiella pneumoniae* shows the highest activity (4.92 ± 0.002) and on the same concentration *Bacillus subtilis* shows the lowest activity (3.54 ± 0.001). This study established that methanol extracts of brown alga *L. variegata* was highly effective, against gram negative bacteria than gram positive bacterial pathogens at all the concentrations.

KEYWORDS: Antibacterial activity, methanolic extract, *Lobophora variegata* pathogenic bacteria, MIC and MBC, Streptomycin.

1.0 INTRODUCTION

Algae are defined as the photosynthetic, non-vascular plants that contain chlorophyll-a, accessory pigments and with simple reproductive structures.[1] Algae are a large and diverse group of simple plant like organisms ranging from unicellular to multicellular forms. Algae vary in size from microscopic unicellular forms (phytoplankton) to the giant benthic

macrophytes (e.g., *Macrocystis*), which are attached to solid substrata such as rocks or boulders.[1] The largest and most complex marine algae are called seaweeds.[2] Seaweeds are a group of macroscopic marine algae that form the biomass in the intertidal zone and the term seaweeds and sea vegetables are used interchangeably.[3] Seaweeds are also used in pharmaceutical industry for drug development to treat diseases like cancer, acquired immuno-deficiency syndrome (AIDS), inflammation, pain, arthritis, infection from virus, bacteria and fungus.[4]

In recent years, a significant number of novel metabolites with potential pharmacological properties have been discovered from the marine organisms.[5-11] Seaweeds have a unique place in traditional medicine of maritime nations as vermifuges, anesthetics and antibiotics in the treatment of cough, wounds, gout, hypertension, venereal diseases, cancer and a variety of other diseases.[12,13] The chemical substance responsible for the antibacterial activity of marine algae have been identified as organic acids, fatty acids,[14] terpenes, carbonyls, bromophenols,[15] halogenated aliphatic and sulfer containing heterocyclic compounds, isoprenl, brominated hydroquinone and phlorotannins. [14] Some of the compounds produced by seaweeds that have antibacterial action are found to have potential use in mosquito control too.[16] Thus, the screening of marine organism in the presence of novel compounds with therapeutic potential and a variety of biological activities.[17, 11]

Since the finding of antibacterial activities in many species of marine algae from different part of the world and the isolation of some active compounds from them,[18] marine algae have become recognized as potential sources of antibiotic substances.[19] These substances exhibit an appreciable number of distinct biological activities such as antitumoural, antiviral, antifungal, insecticidal, cytotoxic, phytotoxic and antiproliferative actions.[20] So the present study has been designed to evaluate the antibacterial activity of methanolic extract of marine brown alga *Lobophora variegata* on gram positive and gram negative bacteria at various concentrations.

2.0 MATERIALS AND METHODS

2.1 Sample collection and preparation

Lobophora variegata was collected from the intertidal regions of the Mandapam coast (Lat. 09˚ 17.417'N; Long. 079˚ 08.558'E) of Rapid island, the Gulf of Mannar and the collected sample was immediately brought to the laboratory in plastic bags containing water in order to

prevent evaporation. The alga was washed thoroughly with sterilized sea water to remove epiphytes and sand particle, the washed alga was shade dried, ground it as powder.

2.2 Preparation of algal extracts

Seaweed powder was soaked in the 90% methanol with room temperature to make an extract form and kept in hot air oven overnight at 60^{o}C and the extracts were collected and concentrated. The extract was then filtered through a Buchner funnel with Whatman No.1 filter paper. The filtrate was evaporated to dryness under pressure using a rotary vacuum evaporator at 50^{o}C. These crude extracts were then tested for their antibacterial activity against selected human pathogens.

2.3 Pathogens used for the assay

The bacterial pathogens *Staphylococcus aureus* (ATCC 10832D-5), *Salmonella typhi* (ATCC 202117 CVD 909), *Bacillus cereus* (ATCC 202), *Bacillus subtillus* (ATCC 205), *Klebsiella pneumoniae* (ATCC 31488), *Pseudomonas aeruginosa* (ATCC 207), were purchased from LGC Promochem India Pvt. Ltd, Bangalore, India. The bacterial pathogens were maintained on Nutrient Agar (Hi Media, India).

2.4 Preparation of inoculums

Bacterial inoculums were prepared by transferring a loopful of bacterial culture from fresh culture plates to tubes containing 10 mL of Nutrient Broth (Hi-media) and incubated for 24 hours at 37^{o}C. The tubes were shaken occasionally to aerate and promote growth. These cell suspensions were diluted with sterile Nutrient Broth to provide initial cell counts of about 2 x 10^{3} CFU/mL.

2.5 Antibacterial assay

Antibacterial activity was carried out using the disc diffusion method.[23] The petripates were prepared with 20 mL of sterile Mueller Hinton Agar (MHA) (Hi-media) and the test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The plates were swab inoculated using sterile cotton buds with each of the previously mentioned bacterial pathogens in the concentration of 2 x 10^{3} CFU/mL. Sterile filter paper discs 6 mm in diameters (Whatman No.1) were loaded with extracts (100 µg/mL) and air-dried. Discs containing streptomycin were used as positive controls (100 µg/mL). The discs were placed on Muller Hington Agar (MHA). Plates were incubated for 24 hours at 37^{o}C temperature, the antibacterial assay were done in triplicates. For each plate the zone of inhibition was recorded

in millimeters and it was compared with the control and results were expressed in percentage of inhibition, all the data were statistically analyzed.

3.0 RESULT AND DISCUSSION

The antibacterial activity of the methanolic extract residue of the alga at different concentrations against the tested bacteria were shown in Table.1; Fig.1 & Plate.I. Increasing the concentration of the extract residue resulted in an increase in the activity against all the bacterial pathogens. At a concentration of 750 µg/mg nearly 50% of the activity observed for the standard antibiotic could be noted against all the bacteria. At the concentration of 1000 µg/mL *Klebsiella pneumoniae* shows the highest activity (4.92 ± 0.002) and on the same concentration *Bacillus subtilis* shows the lowest activity (3.54 ± 0.001).

Table.1 Antibacterial activity of various concentrations of methanolic extract residue of *L. variegata*

S.No	Name of the Microorganism	Zone of Inhibition (in mm)				
		250 µg/mL	500 µg/mL	750 µg/mL	1000 µg/mL	Streptomycin
1	*Staphylococcus aureus*	2.42 ± 0.001 (37.46%)	2.83 ± 0.001 (43.80%)	4.12 ± 0.001 (63.77%)	4.83 ± 0.002 (74.76%)	6.46 ± 0.002 (100%)
2	*Bacillus cereus*	1.70 ± 0.001 (17.32%)	2.54 ± 0.002 (25.89%)	3.41 ± 0.002 (34.76%)	4.23 ± 0.001 (43.11%)	9.81 ± 0.002 (100%)
3	*Bacillus subtilis*	1.10 ± 0.001 (15.02%)	1.86 ± 0.001 (25.40%)	2.93 ± 0.002 (40.02%)	3.54 ± 0.001 (48.36%)	7.32 ± 0.002 (100%)
4	*Salmonella typhi*	1.35 ± 0.001 (20.70%)	2.57 ± 0.002 (39.41%)	2.91 ± 0.001 (44.63%)	4.45 ± 0.002 (68.25%)	6.52 ± 0.002 (100%)
5	*Pseudomonas aeruginosa*	1.22 ± 0.001 (19.77%)	2.42 ± 0.001 (39.22%)	3.52 ± 0.001 (57.05%)	4.72 ± 0.001 (76.49%)	6.17 ± 0.002 (100%)
6	*Klebsiella pneumoniae*	2.32 ± 0.001 (27.19%)	2.81 ± 0.001 (32.94%)	3.73 ± 0.002 (43.72%)	4.92 ± 0.002 (57.67%)	8.53 ± 0.002 (100%)

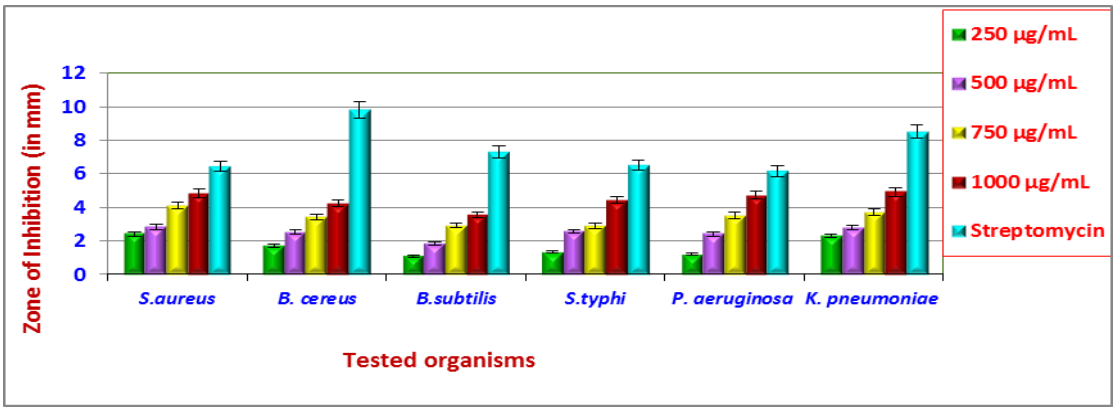

Fig.1 Antibacterial activity of various concentrations of *L. variegata*

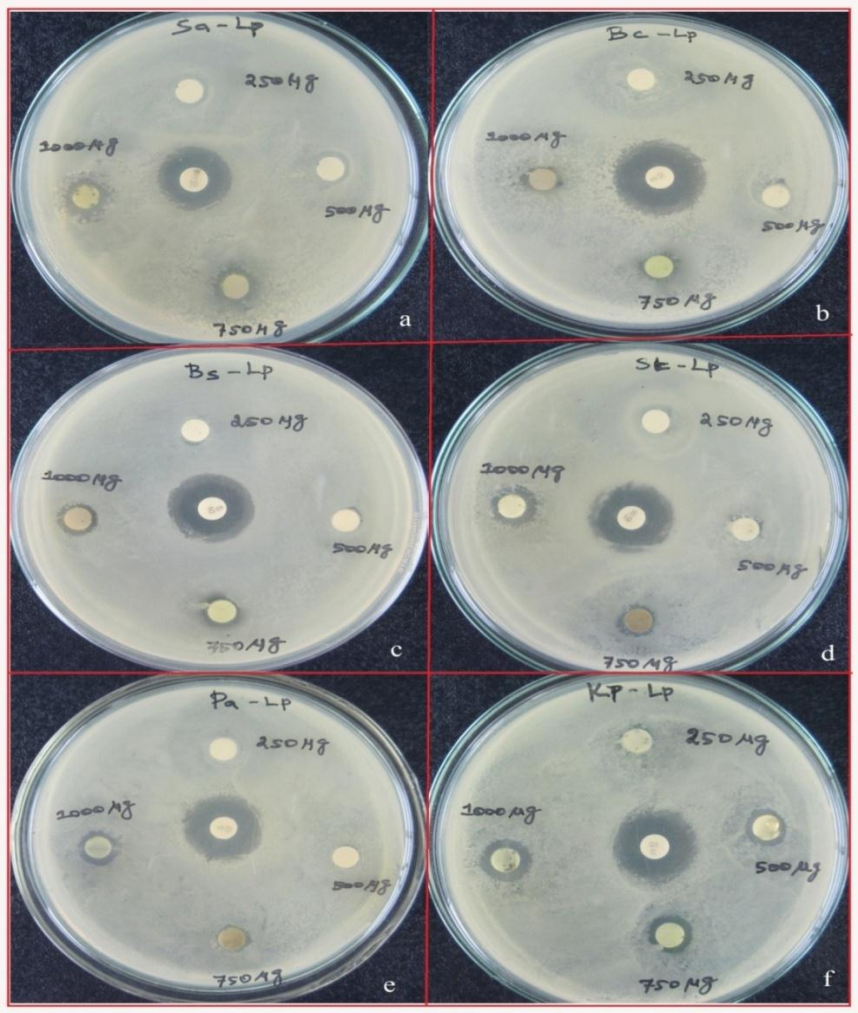

Plate.I Antibacterial activity at various concentrations of *L.variegata*

3.1 Determination of MIC and MBC of algal extracts

Minimum Inhibitory Concentration (MIC) for antibacterial activity was determined by using *K. pneumonia.* The methanolic extract residue of *L. variegata* showed more activity against the tested organism, the MIC was 150 µg/mL, while the Minimum Bactericidal Concentration (MBC) was 225 µg/mL, when compared with standard antibiotic streptomycin for which it shows 180 to 300 µg/mL.

Marine organisms are a rich source of structurally novel and biologically active metabolites. Many chemicals obtained from marine origin showed various biological activities and some of them are under investigation and are being used to develop new pharmaceuticals.[20] The cell extracts and active constituents of various algae are shown to have antibacterial activity *in vitro* against Gram-positive and Gram-negative bacteria.[7] In the present study, the crude methanolic extract residue of brown alga *L.variegata* was evaluated for antimicrobial activity against selective pathogenic bacteria. The findings of the present screening revealed that the

strongest antibacterial activity exhibited by the methanol extract and also showed a broad spectrum of antibacterial activity against human pathogenic bacteria.[8]

Antibacterial activities of seaweeds also varied with the species division. Rao and Parekh.[21] reported that the species of Rhodophyta showed the highest antibacterial activity. Pesando and Caram,[23] found that the highest antibacterial activity was exhibited by the species of Phaeophyta. In the present study, the experimental alga *L. variegata* showed a activity against the tested bacteria, which was in agreement with the findings of seasonal variation of antibacterial and antifungal activities of the extracts of marine algae from Southern coasts of India.[24] It may be probably due to the tested seaweed vertical distribution. The active compounds in the species causing the strong antibacterial activities remain to be identified. Whereas acetone as a solvent for extracting antimicrobial compounds from British marine algae.[26] Similarly, around 20 algae using methanol and ethanol along Idinthakarai coast and they reported that *Bacillus subtilis* and *Staphylococcus sp.*, were highly susceptible to most of the algal extracts.[27]

In reference,[28] screened the antimicrobial activity of *Hydroclathrus clathratus* using methanol extract along the Gulf of Mannar Coast and reported that *Pseudomonas aeruginosa* was more susceptible than the other bacterial pathogens. In the present study, seaweed of *L. variegata* shows promising results against antibacterial pathogens. This finding supports that.[28] who demonstrated that some marine plants showed antibacterial activity against three bacterial strains. In the present study, the extracts of *L. variegata* exhibited a broad spectrum of antibacterial activity and inhibited both Gram-positive and Gram-negative strains. Methanol was the best solvent for extracting the effective antimicrobial compounds used in these experiments. This could be related to the high solubility of bioactive metabolites in the methanol. Antibacterial activity depends on both the species and the efficiency of the extraction method. It is clear that the organic solvents always provide a higher efficiency in extracting compounds for antimicrobial activities compared to water-based method.[29]

The present investigation shows that there are remarkable differences in the activity (%) of methanolic extracts of *L. variegata* against the tested pathogenic species. It is amazing that, mostly, the antimicrobial activity towards Gram-positive species is lower than that detected against Gram-negative bacteria when compared with streptomycin as standard. This may be due to the more complex structure of the cell wall of Gram negative bacteria (Pesando and Caram, 1984). Kandhasamy and Arunachalam (2008) revealed that Gram-positive organisms

were more susceptible to the crude extracts of algae used. Similar kind of work was also carried out by.[30] However, the exact mechanism and the compound responsible for the antimicrobial activities are currently unclear.

4.0 CONCLUSION

The present investigation has proven that marine brown alga *L. variegata* possesses antimicrobial activity by the presence of some inhibitory compounds in the crude methonolic extracts. Therefore, it is suggested that further works should be performed on the isolation, identification, purification and characterization of these bioactive compounds, which are responsible for antibacterial activity.

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