

Antibacterial Activities of Brown Marine Algae (*Sargassum wightii* and *Turbinaria ornata*) from the Gulf of Mannar Biosphere Reserve

P. Vijayabaskar and V. Shiyamala

Post Graduate Research Department of Microbiology, Ayya Nadar Janaki Ammal College
(Autonomous), Sivakasi-626124, Tamilnadu, India

Abstract: Southeast coast of India is a unique marine habitat infested with diverse seaweeds. Therefore, the present study was initiated to explore bioactive potential of major seaweeds. The brown algae, *Sargassum wightii* and *Turbinaria ornata* were collected from Gulf of Mannar, South-east coast. The methanolic extracts of both seaweeds (*S. wightii*, *T. ornata*) were tested against various Gram positive and Gram negative human pathogenic microbes. The finding envisages that methanol extracts of *T. ornata* could be utilized as a good source of antimicrobial agent in pharmaceutical industry.

Key words: *Sargassum wightii* · *Turbinaria ornata* · Polyphenol · Human pathogens · TLC

INTRODUCTION

Commercially available varieties of marine macro algae are commonly referred to as seaweeds. Macro algae can be classified as red algae (rhodophyta), brown algae (phaeophyta) or green algae (chlorophyta) depending on their nutrient and chemical composition. Seaweeds serve as an important source of bioactive natural substances. They have some of the valuable medicinal value components such as antibiotics, antioxidant [1,2], anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas. From the literature, it is observed that the edible seaweeds contain a significant amount of the protein, vitamins and minerals essential for the human nutrition [3].

Most of the compounds of marine algae show antibacterial activities [4,5]. Many metabolites isolated from marine algae have been shown to possess bioactive efforts [6-8]. Among the different compounds with functional properties, antioxidants are the most widely studied. Moreover the important role of antioxidants in human health has been demonstrated thus increasing the interest in such products and their demand by consumers. Marine algae serve as important resources for bioactive natural products [9,10]. Oxidative stress is an important

factor in the genesis of pathology, from cancer to cardiovascular and degenerative disease [11,12]. Fayaz *et al.* [3] suggested the utility of *Kappaphycus alvarezzi* for various nutritional products including antioxidant for use as health food or nutraceutical supplement. Different parts of the thalli are also known to differ in their antimicrobial potential. The present study was undertaken to evaluate the phenol content of the brown seaweeds, *Sargassum wightii* and *Turbinaria ornata* and their antibacterial activity against human pathogens.

MATERIALS AND METHODS

Collection and Processing of Marine Algae: The brown algae, *Sargassum wightii* (Fuciales/phaeophyta) and *Turbinaria ornata* (Fuciales/phaeophyta) were collected from the intertidal region of Mandapam coast (Lat. 9°17'N; Long 79° 19'E) of Gulf of Mannar, South-east coast of India. Algal samples were cleaned of epiphytes and extraneous matter and necrotic parts were removed.

Polyphenol Extraction from Seaweeds: In the laboratory, samples were rinsed with sterile distilled water, shade dried, cut into small pieces and powdered in a mixer grinder. They were stored in polyethylene bags or airtight container at room temperature. 100 g of *S. wightii* and *T. ornata* (powder) were extracted with 500 ml of

methanol (2:1) in a Soxhlet extractor for 6 hours. The extraction was repeated twice. The total extracts were filtered and the obtained filtrates (crude extracts) were concentrated under reduced pressure to dryness. The concentrated extracts served as the crude seaweed polyphenol for further analysis.

Estimation of Total Phenolic Content: Phenolic contents of crude methanolic extracts were estimated by the method of Taga *et al.* [13]. 100µl crude sample was mixed with 2ml of 2% sodium carbonate and allowed to stand for 2 minutes at room temperature in the dark. Absorbance of all the sample solutions was measured at 720nm using spectrophotometer. Gallic acid was used as a standard and a calibration curve was prepared with a range of concentration from 10 to 200mg/l. Phenolic content was expressed as gallic acid equivalent per gram (GAE/g) of extract.

Identification of Phenolic Compounds by TLC: Thin-layer chromatography (TLC) was performed on a silica gel plate. An aliquot of each sample was spotted on the silica gel plate with a developing solvent system of chloroform/methanol (10:1, v/v). The spots were checked under a UV detector and then visualized by spraying the plates with spraying solution (1% potassium ferric cyanide in water and 1% ferric chloride in water) and then visualized under UV [14].

Antibacterial Activity

Bacterial Strains Used for Assay: Test organisms used were MTCC cultures. The pathogenic bacteria were cultured on Nutrient broth at 37°C for 18 hours before inoculation for assay. *Bacillus subtilis*, *E. coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Shigella flexneri* and *Staphylococcus aureus* were used as test organisms and the bioassay was done using agar plate diffusion test. The bacterial stock cultures were maintained at 4°C.

Evaluation of Antimicrobial Activity: Antibacterial activity was measured using a well diffusion method according to the Berghe and Vlietinck, [15]. Standard antibiotic, Ampicillin was used as control. The plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the area in which bacterial growth was inhibited around the well. The average of three replicates for each extract, antibiotic was calculated.

RESULT

Total Phenol Content: The total phenolic content of the seaweed extracts were measured by the Folin-Ciocalteu method. The results were expressed as gallic acid equivalents (GAE). *Turbinaria ornata* found to have the highest phenolic content (43.72 ± 1.63 mg GAE/g extract). This is followed by *Sargassum wightii* (35.98 ± 1.75 mg GAE/g extract).

Identification and Determination of Phenolic Constituents by TLC: The phenolic compounds present in *Turbinaria ornata* and *Sargassum wightii* were tentatively detected by TLC. After spraying with the solution composed of 1% potassium ferric cyanide and 1% ferric chloride, the appearance of blue colour spot in the TLC chromatogram indicated the presence of phenolic compounds (Fig. 1).

Antibacterial Activity of Seaweed Polyphenols:

Turbinaria ornata showed the highest activity against the growth of *Bacillus subtilis* (20 ± 0.62 mm) and *E. coli* (16 ± 0.58 mm). Besides this, the extract showed moderate activity against the growth of *Shigella flexnerii* (14 ± 0.49 mm), *Staphylococcus aureus* (15 ± 0.53 mm) and moderate activity towards all other pathogens. *Sargassum wightii* strongly inhibited the growth of *E. coli* (18 ± 0.55 mm) and *Aeromonas hydrophila* (15 ± 0.78 mm) and moderate activity against *Bacillus subtilis* (12 ± 0.53 mm) and *Pseudomonas aeruginosa* (12 ± 0.53 mm) (Table. 1).

Table 1: Antibacterial activity of seaweed polyphenols against human pathogens

Test Organisms	Zone of Inhibition (mm)		
	Standard	<i>T. ornata</i>	<i>S. wightii</i>
<i>Aeromonas hydrophila</i>	22 ± 0.45	15 ± 0.78	15 ± 0.78
<i>Bacillus subtilis</i>	28 ± 0.56	20 ± 0.62	12 ± 0.53
<i>Escherichia coli</i>	24 ± 0.49	16 ± 0.58	18 ± 0.55
<i>Enterococcus faecalis</i>	14 ± 0.37	10 ± 0.52	6 ± 0.59
<i>Klebsiella pneumoniae</i>	22 ± 0.43	14 ± 0.56	10 ± 0.49
<i>Proteus vulgaris</i>	18 ± 0.32	12 ± 0.64	8 ± 0.55
<i>Pseudomonas aeruginosa</i>	16 ± 0.45	10 ± 0.65	12 ± 0.53
<i>Shigella flexneri</i>	20 ± 0.36	14 ± 0.49	10 ± 0.50
<i>Staphylococcus aureus</i>	28 ± 0.41	15 ± 0.53	9 ± 0.47



Fig. 1: TLC analysis of polyphenols of *Sargassum wightii* (SW) and *Turbinaria ornata* (TO) Compared to the Standard Gallic acid (GA)

DISCUSSION

Marine organisms are a rich source of structurally novel and biologically active metabolites. Secondary or primary metabolites produced by these organisms may be potential bioactive compounds of interest in the pharmaceutical industry. To date, many chemically unique compounds of marine origin with various biological activities have been isolated and some of them are under investigation and are being used to develop new pharmaceuticals [16]. The cell extracts and active constituents of various algae have been shown to have antibacterial activity *in vitro* against Gram-positive and Gram-negative bacteria [17].

Phenolic compounds may affect growth and metabolism of bacteria. They could have an activating or inhibiting effect on microbial growth according to their constitution and concentration [18, 19]. The brown seaweeds, *Turbinaria ornata* and *Sargassum wightii* extracts were active against nine pathogens such as *Aeromonas hydrophila*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Shigella flexneri* and *Staphylococcus aureus*. This may be due to active components which are present in the seaweed extracts. These results indicate that the extracts contained different antibacterial substances and reflect the variety of secondary metabolites [20].

The result shows that the methanol extract possesses a strong antimicrobial activity against both positive as well as negative when compared with ampicillin as standard. However, the exact mechanism and the compound responsible for the antimicrobial activities are currently unclear. Therefore, it is suggested that further works should be performed on the isolation and characterization of the compound.

The present investigation has also proved that seaweed polyphenols (*T. ornata* and *S. wightii*) possess antimicrobial activity to achieve excellent results by inhibiting the growth of maximum human pathogenic bacteria. This may be due to the masking of antibacterial activity by the presence of some inhibitory compounds in the crude extracts [21].

CONCLUSION

The seaweed extracts of *Turbinaria ornata* possessed noticeable activity against positive and negative bacteria when compared with standard ampicillin. It is evident from the present study that the methanol extracts of *T. ornata* could be utilized as a good source of antimicrobial agent in pharmaceutical industry. However, the active components responsible for the antibacterial activities need to be evaluated. Therefore it is suggested that further works may be performed on the isolation and identification of the antimicrobial components in *T. ornata* for its pharmaceutical application.

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