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ANTIBACTERIAL ACTIVITY OF BROWN SEAWEED *PADINA TETRASTROMATICA* HAUCK FROM SOUTH EAST COAST OF TAMIL NADU, INDIA.

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ABSTRACT

The aim of our investigation was to evaluate the antibacterial activities of brown seaweed *Padina tetrastromatica* against Gram positive bacteria (*Staphylococcus aureus, Enterococcus faecalis, Streptococcus pyogenes*) and Gram negative bacteria (*Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus vulgaris, Escherichia coli,*). The antibacterial activity was carried out by disc diffusion method.The petroleum ether extract showed the better result than acetone, chloroform, ethanol,aqueous extracts. The strong antibacterial activity was noted in petroleum ether extracts against *S. aureus* (26.6±0.47 mm) and the minimum inhibition (7.6± 0.47 mm) was recorded against *P. aeruginosa*. *P. vulgaris* was found to be more resistant bacteria to the extracts of *Padina tetrastromatica.* Contrary to this, *P. aeruginosa* was susceptible to the extracts of *Padina tetrastromatica.* The overall antibacterial activity assessed from the above results indicates the presence of active constituents in the extracts and the seaweed can be used in pharmaceutical industry.

KEYWORDS: Antibacterial activity, solvent extracts, disc diffusion method, *Padina tetrastromatica.*

INTRODUCTION

Commercialy available varieties of marine macro algae are commonly refered to as seaweeds. Marine algae are one of the largest producers of biomass in the marine environments.[1] Some bioactive substances isolated from marine algae are chemically classified as brominated, aromatics, nitrogen-heterocyclic, sterols, proteins and sulfated

polysaccharides.[2] Marine algae represent an inexhaustible reservoir of raw materials and cosmetics.[3] Fresh and dry seaweeds are extensively consumed by people expecially living in the coastal areas.[4] Season and habitat of collection and age of marine algae have an influence on their metabolic responses, the nature and levels of proximate constituents.[5] Most of the compounds of marine algae show antibacterial activities,[6] used as direct and indirect human food sources,[7-8] and also used in pharmaceutical industries.[9-11] Marine algae are the rich sources of unsaturated fatty acids and these fatty acids were reported to block growth and systematic spread of humun breast cancer via mechanisms independent of the host immune system, perhaps by peroxidaton of intracellular lipids.[12] Marine algae are being used as food supplements, source of vitamins[13] and also they are used in the treatment of number of noxious effluents containing organophosphorous, pesticides, detergents, antibiotics and other molecule.[14] Recently, there is a growing interest on the discovery of natural phytochemicals which are generally safer than synthetic chemicals.[15] The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases.[16] Approximately, 2500 new metabolites were reported from a variety of marine organisms during the years 1977 to 1987.[17] Marine brown algae have been suggested as an alternative source of blood anticoagulant.[18] Nowadays, the use of antibiotics increased significantly due to heavy infections and the pathogenic bacteria becoming resistant to drugs is common due to indiscriminate use of antibiotics.[19] Bacterial infections causes high rate of mortality in human population and aqua culture organisms.[20] For example *Enterococcus faecalis* is the causative agent of inflammatory bowel disease.[21] *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* cause disease like mastitis, abortion and upper respiratory complaints [22]. *Pseudomonas aeruginosa* is an important and prevalent pathogen among burned patients capable of causing life – threatening illness.[23] Therefore the present study was carried out in order to examine the antibacterial effects of acetone, petroleum ether, chloroform, ethanol, aqueous extracts of marine macroalgal species. The marine alga *Padina tetrastromatica* harvested was from Manavalakurichi coast.

MATERIALS AND METHODS

Collection of samples

Padina tetrastromatica was collected from the Manavalakurichi coastal waters by handpicking. The collected samples were gently rinsed with the seawater and immediately transported to the laboratory, then rinsed with sterile distilled water and shade dried and

grounded into fine powder in mixer grinder.[24] Samples were packed and stored in a refrigerator until the experiments were carried out.

Preparations of solvent extracts

The powdered sample (100g) was extracted in soxhlet apparatus using petroleum ether, chloroform, ethanol and acetone (1000 ml) as solvents for 8hrs at a temperature maintained not more than the boiling point of the solvent. Seaweeds were also extracted with hot-water for the preparation of aqueous solutions. The resultant crude extracts were filtered with Whatman No.1 filter paper. The filtrates obtained were concentrated under vacuum with a rotary evaporator at 40^{o}C to obtain the crude extracts. The crude extracts were collected in an air tight container and stored at 4^{o}C until use.

Test pathogens

The bacterial strains were obtained from Vivek laboratories, Nagercoil, Tamil Nadu, India. Extracts were tested against three Gram positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes* and *Enterococcus faecalis*) and four Gram negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa*).

Preparation of inoculum

The stock cultures were maintained at 4°C on slant slopes of Nutrient Agar medium. Active cultures for the experiments were prepared by transferring a loopful of cells from the stock cultures to conical flasks containing Mueller Hinton Broth (MHB). These conical flasks were incubated at 30°C for 24 hrs and were refered to as seeded broth.

Antibacterial activity

Agar disc diffusion method

Antibacterial activity was evaluated by using agar disc diffusion technique[25] in petri plates. The strains that had been incubated for 24 hours were used for this assay. The cultures of the organisms were seeded on Mueller-Hinton agar plates by using sterilized cotton swabs. The agar surface was allowed to dry for five minutes. Then the discs (6mm) impregnated with the seaweed extracts were placed on the swabbed plate using sterilized forceps. Chloramphenicol (30mcg) disc was used as positive control. The plates were kept in an incubator at 37°C for 24 hrs and the antibacterial activity was determined by measuring the zone of inhibition in millimeter by using graduated scale and recorded. Triplicates were maintained for each test.

Statistical analysis

The values of antibacterial activity of acetone, petroleum ether, chloroform, ethanol, aqueous extracts of *P. tetrastromatica* were expressed in mean ± standard deviation (n=3) for each sample.

RESULTS

The results of primary screening test for antibacterial activities of seaweed *Padina tetrastromatica* using five different solvents (acetone, petroleum ether, chloroform, ethanol, aqueous) were studied against seven human pathogenic bacteria such as *P. aeruginosa, K. pneumoniae, P.vulgaris, E. coli, E. faecalis, S. aureus, S. pyogenes*. The results of antibacterial screening tests are summarized below in Table 1. In acetone extracts, the maximum zone of inhibition was against *S. aureus* (22±0.81 mm), followed *K. pneumoniae* (21.3±0.47 mm), *E.coli* (21.00±0.81 mm), *P. aeruginosa* (20.3±0.47 mm), *E. faecalis* (15.6±0.47 mm) whereas,the extract was failed to inhibit *S. pyogenes* and *P. vulgaris.* Petroleum ether showed highest zone of inhibition against *S. aureus* (26.6±0.47 mm) followed *K. pneumonia* (24.6±0.47 mm), *E. coli* (21.6±0.47 mm) and moderate zone of inhibition was observed against *P. vulgaris* (20.3±0.47 mm), *E. faecalis* (17.00±0.81 mm), *S. pyogenes* (10.3±0.47 mm) and lowest zone of inhibition was observed against *P. aeruginosa* (7.6±0.47 mm).

The zone of inhibition ranged between (10.3±0.47 mm) to (23.3±0.94 mm) in chloroform extract. The ethanol extract showed the maximum activity (20.3±0.47 mm) against *E. coli* and minimum against *P. aeruginosa.* Aqueous extract was unable to inhibit all the pathogens. Among the five extracts petroleum ether exhibited the powerful antibacterial activity against all the seven pathogens.

Table 1: Antibacterial activity of *Padina tetrastromatica* against bacterial pathogens

Solvents	Bacterial pathogens showing zone of inhibition (mm)						
	Escherichia coli	***Klebsiella pneumoniae***	***Proteus vulgaris***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Streptococcus pyogenes***	***Enterococcus faecalis***
Aqueous	-	-	-	-	-	-	-
Petroleum ether	21.66±0.47	24.66±0.47	20.33±0.47	7.66±0.47	26.66±0.47	10.33±0.47	17.00±0.81
Chloroform	21.33±0.47	23.66±0.94	-	10.33±0.47	22.66±0.47	20.00±0.81	14.66±0.47
Ethanol	20.33±0.47	17.33±0.47	-	8.66±0.47	19.33±0.47	10.66±0.47	15.33±0.47
Acetone	21.00±0.81	21.33±0.47	-	20.33±0.47	22.00±0.81	-	15.66±0.47
Positive control Chloramphenicol	38.00±0.00	26.00±0.00	20.00±0.00	-	22.00±0.00	24.00±0.00	28.00±0.00

- = No activity; mm= Millimeter, values are expressed as mean ± standard deviation (n=3)

DISCUSSION

Marine algae are among the major source of various compounds which may be useful for humans. Screening of seaweeds for antimicrobial activity and bioactive constituents is quite imperative. Antibacterial activity of brown seaweeds against both Gram positive and Gram negative bacteria has been established by several scientists. Different solvent such as acetone, ethanol, methanol, [26-28] ethyl acetate [29,30], diethyl ether, petroleum ether [30,31], hexane [19], chloroform [31-33] aqueous [33,34], benzene [35,36] were used earlier to extract bioactive principles from seaweeds. The present investigation was carried out in acetone, petroleum ether, chloroform, ethanol and aqueous extracts.

Brown seaweeds posses a variety of bioactive secondary metabolites and other natural products in their thalli,[37-41] which are responsible for their strong antibacterial activity. In the present study, petroleum ether extract of seaweed found to posses strong antibacterial activity. This result finds similarity with those obtained by Omer *et al.,* [30] who reported that the petroleum ether extract showed strong antibacterial activity against bacterial pathogens. These strong activities related to brown algae may be due to the phenolic compounds such as phlorotannins, eckol and eckol- related compounds that have strong bactericidal activity.[42] Of the five solvents tested, petroleum ether was found to be the best solvent for isolation of antibacterial compounds from the tested marine algae followed by chloroform, ethanol, acetone and aqueous extracts. In our study, chloroform extract of *P. tetrastromatica* showed maximum antibacterial activity especially against *K. pneumoniae* and also inhibited all the tested pathogens except *P. vulgaris.* This result could be related to Subba *et al.*[33] Rhimou *et al.,*[43] who reported that chloroform extract had moderate level of antibacterial activity. The present study also reported that *P. vulgaris* (Gram negative bacteria) was resistant to chloroform, ethanol, acetone and aqueous extracts of *P.tetrastromatica.* In another study conducted by Muhamad *et al.,*[44] ethanol showed significant activity against both Gram positive and Gram negative bacteria.

Kolanjinathan and Stella[2] indicated that acetone was the best solution for extracting the effective antimicrobial meterials. But in our study actone extract inhibited in *P. aeruginosa, K. pneumoniae, E. coli, E. faecalis, S. aureus* and was unable to inhibit in *P. vulgaris* and *S. pyogenes.* The variation in antibacterial activity may be due to the method of extaction, solvents used in extraction and season at which samples were collected.[20]

Among Gram positive organisms, the petroleum ether extracts of *P. tetrastromatica* showed maximum level of inhibition zone (26.6±0.47 mm) especially against *S. aureus.* The large diameter zones of inhibition represent the high sensitivity of the microorganisms to the seaweed extracts and vice versa.[45] The zone of inhibition of the different extracts of the tested algae was compared with the positive control Chloramphenicol impregnated discs.

In the present study *P. vulgaris*, the Gram negative bacteria was resistant to aqueous, chloroform, ethanol and acetone extracts of *P. tetrastromatica. P. aeruginosa* appeared to be the most sensitive bacterial species, as it was affected by all the five extracts while *P.vulgaris* was influenced by only one extract. In Gram negative bacteria the outer membrane acts as a barrier to many environmental substances including antibiotics.[46] The presence of thick murine layer in the cell wall also presents the entry of the inhibitors.[20] The clinical pathogen *P. aeruginosa* responsible for causing the nosocomial infections was inhibited effectively by all the five extracts of *P.tetrastromatica.* In the present study the aqueous extract was unable to inhibit in *P. aeruginosa, S. pyogenes, P.vulgaris, K. pneumoniae, E.coli, E. faecalis,* and *S. aureus.* This result contradicts with the result of Khandhasamy and Arunachalam[20] which reported that aqueous extract of *P.tetrastromatica* inhibited most of the tested human pathogens and fish pathogens Johnsi *et al.,*[34] reported that aqueous extract of *P. tetrastromatica* inhibited *P. aeruginosa.*

The earlier investigations Reichelt and Borowitzka,[47] Burkholder *et al.,* [48] Caccamese *et al.,*[49] and Takaki *et al.,*[50] found higher antibacterial activity in the extracts of brown algae than the red algae extracts.While Majin and Tan wel,[51] obtained positive results from red and brown algae. Similarly Selvi and Selvaraj,[52] Viachosi *et al.,*[53] Veeragurunathan and Geetha[54] and Vallinayagam *et al.,*[32] noted higher activity in brown algae. Johnson *et al.,*[55] suggest that *P. tetrastromatica* can be used as antiviral, antibacterial, antiparasitic agents and to treat the diseases like ulcer, gonnorrhoea, leucorrhoea etc.

CONCLUSION

In the present study *Padina tetrastromatica* collected from Manavalakurichi coast of India was checked for their antibacterial activity. The seaweed extracts of *P. tetrastromatica* possessed noticeable activity against Gram positive and negative bacteria when compared with standard chloramphenicol. The highest zone of inhibition (26.66±0.47 mm) was observed in petroleum ether extract of *P. tetrastromatica* against *S. aureus*. *P. tetrastromatica* evoked strong antibacterial activities against the tested pathogens except *P. vulgaris* and

therefore *P. tetrastromatica* may have potential bioactive compounds. 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 antibacterial components in *P. tetrastromatica* for its pharmaceutical application.

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