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Research Article

ANTIMICROBIAL ACTIVITY OF JANIARUBENS AGAINST AN ENTERO PATHOGEN

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ABSTRACT

Objective: The aim of the present study was to evaluate the antimicrobial activity of crude extracts of Janiarubens against Vibrio parahemolyticus. **Methods:** The methanol, ethanol, chloroform and acetone extracts of the red alga Janiarubens were prepared by cold maceration method and their antimicrobial activity was determined by disc diffusion method. Basic phytochemical tests were used to screen the phytochemical constituents present in the extracts. **Results:** The extracts upon phytochemical screening showed the presence of alkaloids, triterpenoids, steroids, tannin, saponin, coumarins, terpenoids, quinine, phytosteroids, phlobatannins, and flavonoids. Among the four solvents tested, methanol and ethanol extracts of seaweeds exhibited the best activity. **Conclusions:** The red alga J. rubens has significant antimicrobial activities. Further studies must be carried out to identify the main active constituent (s) and its safety levels.

Keywords: Janiarubens, antibacterial activity, disc diffusion method, Luria Bertani Agar Millermedium, Vibrio parahemolyticus .

INTRODUCTION

In mariculture, diseases of microbial origin can cause huge economic losses worldwide. Bacteria, mainly of the genus *Vibrio* have been identified as the main causative agents responsible for the most common infections in fish and shellfish, called *Vibriosis*[1-3]. Moreover, these microorganisms can accumulate in the reared animal's flesh and become a serious threat to human health. *Vibrio* parahemolyticus is one such pathogen.

Vibrio parahemolyticus is a curved, rod shaped, non-sporulating gram negative enteric bacterium commonly found in the coastal waters. It is associated with three major syndromes of clinical illness, i.e., gastroenteritis, travelers' diarrhea and septicemia. Humans beings get infected with this pathogen through consumption of raw or undercooked sea food mainly with molluscan shellfish such as clams, mussels, etc. [4].The evolution of microorganism resistance to antibiotics has resulted in a growing need for new antibacterial compounds that are effective in veterinary medicine and characterized by limited undesirable side effects. There is currently great interest in the screening of microalgae for biologically active metabolites such as polyphenolic compounds, halogenated compounds, etc. which have already been proved for antibacterial activities.

The red alga Janiarubens (L.) Lamx. (syn. Corallinarubens L., family Corallinaceae, Rhodophyta) commonly distributed in the Mediterranean, Black Sea, North-Eastern Atlantic (from Norway to Morocco), Indian Ocean and the China Sea, and has been reported to have many biological activities [5]. The aqueous extract of J. rubens showed antibacterial and antifungal activity whereas its dichloromethane extracts showed the presence of antiproliferativeactivity on the KB tumor cell line (human buccal epidermal carcinoma) [6,7].Significant analgesic, antipyretic and antiinflammatory effects were reported in the J. rubens collected from the Egyptian seashore [6]. Around seven brominated diterpenes of the parguerene and isoparguerene series have been isolated[8]. The anthelminthic activity of some isolated compounds has also been reported, but neither aqueous nor alcohol extracts of J. rubens exerted molluscicidal activity [4]. The present study deals with the screening of antimicrobial effects of the various extracts of J. rubensagainst an enteropathogen i.e., Vibrio parahemolyticus.

MATERIALS AND METHODS

Collection of algae

The algae Janiarubens Linn was collected manually from East Coast of Bay of Bengal during May 2017.It was identified and authenticated by Dr. S. Nirmala, Head of the Department of Botany, Osmania University, Telangana, India. A Voucher specimen (PH- 805) was deposited in the herbarium of the college. The biomass samples were cleaned with double distilled water several times to remove epiphytes, extraneous matter, airdried in a hot air oven at 75^{o}C for 24 h and weighed. The dried algae were cut into pieces, powdered and passed through #200 sieve.

Preparation of extracts

20 grams of pulverized samples were extracted with 100 ml of solvents such as methanol, ethanol, chloroform, and acetone. The samples were preserved in the dark away from the light for four days with intermittent shaking. The resultant extracts were passed through filter paper, and the filtrate was concentrated in an oven at 50° ± 1 °C for one hand stored at 4^{0}C until further use. All solvents used were of analytical reagent grade and obtained from Sigma Chemical Co.

Preliminary phytochemical analysis

The extracts were tested for the presence of phytochemicals as described by Sadasivam and Manickam [9]

Test microorganisms

Marine water samples collected from the coastal areas of Tamil Nadu (India) were diluted up to ten folds and streaked on to the plates containing sterile TCBS medium and were incubated at 37^{0}C for 48hrs. Bluish green colored colonies thus produced were spreaded on to the surface of trypticase soy salt agar plate containing 4% NaCl and were incubated for 24 h at 37 °C. The bacterial colonies were identified using conventional bacterial methods such as Gram staining, motility and a series of biochemical tests [10].

Preparation of medium for antibiotic activity

Readymade dehydrated Luria Bertani Agar Millermedium supplied by Hi-Media was used for testing the antimicrobial activity of algal extracts. 4 grams of the dehydrated medium was dissolved in 100 ml of distilled water and heated to boiling to dissolve the medium completely following the instructions are given by the manufacturer. The medium was distributed into clean glass tubes and plugged with cotton and sterilized by autoclaving at 15 lbs/sq. Inch pressure at 121^{0}C for 20 min.

Inoculum

The culture of test organisms was used as inoculum. A sterile bacterial colony from trypticase soy salt agar plate wastransferred into the flask containing sterilieL.B medium (Luria Bertani Miller)and allowed to grow at 37^{0}C for 18 to 24 hr. The cultures from broth were centrifuged, and a suspension of cells was made with the sterile saline (10%). This culture suspension was used for further studies.

Antimicrobial testing

Disc diffusion method was employed for testing the antimicrobial activity [11,12].Adequate amounts of autoclaved Luria Bertani Agar Millermedium was dispensed into sterile plates, and allowed to solidify under aseptic conditions. Loopful of the test organisms (0.1 mL) were added to the surface of the medium and spreadedin the different directions with the help of sterile spreader. Sterile filter paper discs of around 6mm diameter were dipped into the solution containing 20–30 µL of the respective J. rubens extracts and placed on to the surface of the medium. Care was taken to maintain sufficient distance between the discs to prevent the overlapping of the zone of inhibition.

The bacterial plates were then incubated at 37° ± 0.1 °C for 24 h .After incubation, all plates were observed for zones of growth inhibition, and the diameters of these zones were measured in millimeters. All tests were performed under sterile conditions in duplicate and repeated three times. Tobramycin discs (10 µg/disc) was used as positive controls. Methanol, ethanol, chloroform, and acetone were used as negative controls.

RESULTS & DISCUSSION

Vibrio parahemolyticusisolates produced green to bluish color colonies and showed the faster growth within a day.Suspicious colonies were further tested by sub-culturing onto the chromagar medium.The strain was further identified through series of sub culturing and analyzing enzymatic properties.*Vibrio parahemolyticus* developed mauve colored colonies,clearly visible under normal lighting conditions on chromagar media.Results of characterization of the bacterial isolates are given in table 1.

Preliminary phytochemical analysis of the different extracts showed the presence of phytochemicals, such as alkaloids, triterpenoids, steroids, tannin, saponin, coumarins, terpenoids, quinine, phytosteroids, phlobatannins and flavonoids (table 2).

The results of the antibacterial activity are given in table 3.Among the four solvents tested, methanol and ethanol extracts of seaweeds exhibited the best activity.

Fig. 1:Colonies of *V. parahaemolyticus* on a. TCBS agar *b. Chromagar* media

Table 1: morphological and biochemical characterization of the bacterial isolate.

Name of the test	Result
Growth on TCBS plates	Showed bluish green colored colonies
Indol test	+
VP test	–
Citrate test	–
Production of H, S	–
Casein hydrolysis	–
Starch hydrolysis	–
Cytochrome oxidase	+
Gelatin hydrolysis	+
Fibrionogen hydrolysis	+
Lipase hydrolysis	+
Collagen hydrolysis	–
Elastin hydrolysis	–
Acid from	
Maltose	–
Glucose	+
Mannitol	+
Cellobiose	+
Galactose	+
Lactose	–
Arabinose	+
P-Galactosidase (ONPG test)	+
Arginine dihydrolase	–
Lysine decarboxylase	+
Ornithine decarboxylase	+
Tryptophan deaminase	–
Growth at % NaCl:	
0	–
3	+
6	+
8	+
10	+
Growth at	
4	–
25	+
37	+
40	+

Table 2: Preliminary phytochemical screening of red seaweed extracts.

Phytochemicals	Methanol	Ethanol	Acetone	Chloroform
Alkaloids	+	+	-	-
Triterpenoids	+	+	+	+
Steroids	+	+	+	+
Tannins	+	+	+	-
Saponin	-	-	-	-
Coumarins	+	+	+	+
Terpenoids	+	+	+	+
Qunine	+	+	-	-
Phytoseroids	+	+	+	+
Flavonoids	+	+	+	-
Phlobtannins	+	+	+	-

Table 3: Results of antibacterial activity of the extracts

	Methanol	Ethanol	Acetone	Chloroform	Standard antibiotic
Janiarubens	13mm	14mm	9mm	10mm	12mm

The antibacterial activities of different extracts of Janiarubens are reported in table 3. Methanol and ethanol extracts were shown to have more activity when compared to the remaining extracts. When compared with the standard antibiotic tobramycin, the methanol and ethanol extracts exhibited more potent antimicrobial activity.

It has been reported previously that marine algae can inhibit the growth of microorganisms and the efficacy of the algal extracts depends upon number of factors such as location, seasonality and the solvent used for preparing the extract [13].

Soliman et al. showed that the aqueous extract of J. rubens exerted high antimicrobial activity against Bacillus subtilis and mild activity against Streptococcus aureus [14]. In the studies carried out by Gonzalez del Val et al., the methanol extracts of J. rubens and J. adhaerescens were found inactive against the test microorganisms Pseudomonas aeruginosa, Serratiamarcescens, Enterococcus faecium, Mycobacterium smegmatis, Staphylococcus aureus, Bacillus subtilis and the fungi Candida

albicans, Saccharomyces cerevisiae and Aspergillusfumigatus [15].In the studies carried out by Kumar and Rengasamy ,chloroform extracts and chloroform :methanol (2:1 v/v) extracts of red macro algae exhibited the maximum antibacterial activity, whereas the extracts of benzene, methanol, ethanol showed only traces of activity, which do not comply with our results [16]. In another study it was seen that the methanol and chloroform extracts showed more potent antimicrobial activity than the hexane, dichloromethane, and volatile oil extracts of *J. rubens[17].*

The differences in the results of antimicrobial activities may be due to several factors such as selection of algal species,the solvents used for their extraction,infra-specific variability in the production of secondary metabolites, which may be related to seasonal variations, differences in the extraction protocols to recover the active metabolites and differences in the assay methods [18,19].

Any antimicrobial agent is considered effective, given the size of inhibition zone produced by it measures 2 mm or more. In the present study, the minimum zone of inhibitions obtained was 9 mm and 10mm respectively. Thus the algal extracts were proved to have potent antibacterial activity *against V.* parahemolyticuswhich might be due to the presence of various chemical constituents as described.

The emergence of microbial disease in aquaculture industries implies serious losses. The use of commercial antibiotics for treatment of fish disease produces undesirable side effects. Marine organisms are a rich source of structurally novel biologically active metabolites [20]. Therefore, cell extracts and active constituents of various algae may be potential bioactive compounds of interest in the pharmaceutical industry [21].

CONCLUSION

In conclusion, our results indicate that red alga J. rubens has significant antimicrobial activities, and that the differing activity of the algal extracts on the various organisms may be affected by the extraction protocol. This observation may be of practical importance especially in the treatment of secondary infections where bacteria acts as an opportunistic organism. Further studies are necessary to identify the main active constituent (s) and its safety levels.

REFERENCES

1. Toranzo A.E., Magariños B., Romalde J.L., A review of the main bacterial fish diseases in mariculture systems, Aquaculture, 2005, 246:37-61.
2. Ponprateep S., Somboonwiwat K., Tassanakajon A., Recombinant anti-lipopolysaccharide factor isoform 3 and the prevention of Vibriosis in the black tiger shrimp, Penaeusmonodon, Aquaculture, 2009, 289:219-224.
3. Radjasa O.K.S., Sabdono A., Prayitno S.B., Hutabarat S., Phylogenetic diversity of the causative agents of vibriosis associated with groupers fish from Karimunjawa Islands, Indonesia, Curr. Res. Bacteriol, 2009, 2:14-21.
4. Rippey SR. Infectious diseases associated with molluscan shellfish consumption. ClinMicrobiol Rev. 1994 Oct; 7(4):419-25.
5. Awad NE. 2004. Bioactive brominated diterpenes from the marine red alga Janiarubens (L.) Lamx. Phytother Res 18: 275–279.
6. Soliman FM, El-Tohamy SF, Fathy MM, Ramdan A, Afify NA, Sanad OA. Phytochemical and biological investigation of Janiarubens (L.) Lamx. Amino acids, proteins, nitrogenous bases and biological screening. J Drug Res Egypt. 1994b. 21: 155–164.
7. El-Masry MH, Mostafa MH, Ibrahim AM, El-Naggar MM. Marine algae that display anti-tumorigenic activity against Agrobacterium tumefaciens. FEMS Microbiol Lett.1995, 128: 151–155.
8. Aknin M, Miralles J, Kornprobs JM. Sterol and fatty acid distribution in red algae from the Senegalese coast. Comp Biochem Physiol.1990; 96B: 59–63.
9. S. Sadasivam, A. ManickamBiochemical Methods for Agricultural Sciences.New Age International Ltd., New Delhi, India.1996,1-97
10. Colakoglu FA, Sarmasik A, KoseogluB.Occurrence of Vibrio spp. and Aeromonas spp. in shellfish harvested off Dardanelles cost of Turkey. Food Control.2006,17:648–652
11. Collins CM, Lyne PM. 1989. Microbiological Methods. Butterworths: London.
12. Bradshaw LJ. 1992. Laboratory Microbiology, 4th edn. Emeritus California State University, Fullerton, Saunders College Publishing: New York, USA
13. Febles CI, Arias A, Gil-Rodriguez MC, Hardisson A, Sierra Lopez A.In vitro study of antimicrobial activity in algae (Chlorophyta, Phaeophyta and Rhodophyta) collected from the coast of Tenerife (in Spanish). AnuariodelInstituto de Estudios Canarios.1995, 34: 181–192.
14. Soliman FM, El-Tohamy SF, Fathy MM, Ramdan A, Afify NA, Sanad OA. Phytochemical and biological investigation of Janiarubens (L.) Lamx. Amino acids, proteins, nitrogenous bases and biological screening. J Drug Res Egypt. 1994b,.21: 155–164
15. Gonzalez del Val A, Platas G, Basilio A et al. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). Int Microbiol.2001, 4: 35–40
16. Kumar KA, Rengasamy R. Evaluation of antibacterial potential of seaweeds occurring along the coast of Tamil Nadu, India against the plant pathogenic bacterium Xanthomonasoryzaepv. oryzae (Ishiyama) Dye. Bot Marina.2000,43: 409–415
17. N.U. Karabay-Yavasoglu, A. Sukatar, G. Ozdemir, Z. Horzum.Antimicrobial activity of volatile components and various extracts of the red alga Janiarubens.Phytother. Res.2007,21:153-156.
18. Tariq VN. 1991. Antifungal activity in crude extracts of marine red algae. Mycol Res. 1991,95: 1433–1440.
19. Itoh N, Shinya M. Seasonal evolution of bromomethanes from coralline algae (Corallinaceae) and its effect on atmospheric ozone. Mar Chem 1994,45: 95–103
20. RahamatUnissa, Komati BhanuRekha, Daggupati Amani, SowmyaKonakanchi, SaranyaArun Kumar. Production of l-arginase under SSF and its optimization. Mintage Journal of Pharmaceutical & Medical Sciences.2018;7(1):24-29.
21. E. Rodrigues, S. Tilvi, C.G. Naik. Antimicrobial activity of marine organisms collected off the coast of East India. J. Exp. Biol. Ecol.2004, 309:121-127.

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