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Screening of the Antibacterial Properties of Lichen *Roccella belangeriana* (Awasthi) from Pichavaram Mangrove (*Rhizophora* Sp.)

G. Karthikaidevi, G. Thirumaran, K. Manivannan, P. Anantharaman, K. Kathiresan and T. Balasubaramanian

Centre of Advanced Study in Marine Biology,
Annamalai University, Parangipettai, Tamilnadu, India

Abstract: *Roccella belangeriana* were extracted from different solvents like Acetone, methanol, diethylether, ethanol, ethyl acetate, petroleum ether, chloroform and aqueous extracts against 12 bacterial strains. The present study exhibit maximum antibacterial activity was recorded from chloroform extracts against *Enterococci* sp. and minimum activity was noted from ethyl acetate extract against *Klebsiella pneumoniae, Enterococci* sp., *Salmonella* sp. and *Shewanella* sp.

Key words: Antibacterial activity · *Roccella belangeriana* · Different solvent extracts · Pichavaram mangrove

INTRODUCTION

Medicinal plants are well known natural sources for the treatment of various diseases since ancient times. According to a report issued by the World Health Organization (WHO), plant species that are currently used for medicinal purposes are about 20,000. Lichens have been used for medicinal purposes throughout the ages, such as *Cetraria islandica*, (Iceland moss), *Lobaria pulmonaria* and *Cladonia* species were reported to be effective in the treatment of pulmonary tuberculosis [1]. As lichens are symbiotic associations between a fungus (mycobiont) and an alga (Photobiont), a number of lichens screened for antibacterial activity in the 1940s and 1950s following the discovery of penicillin from a fungus.

Lichens are an important food for many animals, including man [2]. They are used in production of alcohols and paints, as well as in the perfume and pharmaceutical industries. In addition to this, lichen has been used in folk medicine for centuries; their biological properties were long ago recognized by Native Americans, Indians and Europeans, who use in their traditional medicines to treat a variety of animals [3].

Many different bioactive secondary metabolites have been isolated from lichens [4] and some of them are used in pharmaceutical sciences. Several lichen extracts have been used for various remedies in folk medicine and screening the lichens has revealed the frequent occurrence of metabolites with antibiotic, antimycobacterial, antiviral, antitumour, analgesic and antipyretic properties [1, 5-7].

Lichen forming fungi produce antibiotic secondary metabolites that protect many animals from pathogenic microorganisms [6]. A number of investigators have studied the antibacterial and antifungal activity of lichens. The first study of the antibiotic properties of lichens was carried out by Burkholder [8]. Vartia [1] reported antibacterial activity for several lichens and other researchers have since then studied the antibacterial activity several lichens against gram-positive and gram-negative bacteria, as well as the antifungal activity of lichen extract [9-12]

The development and spread of microbial resistence to available antibiotics has prompted investigators to study antimicrobial substances from other sources. Owing to pronounced antimicrobial activity of some of their secondary metabolites, lichens (Together with algae, micro fungi and higher plants) are attracting much attention among researchers as significant new sources of bioactive substance.[7,13]. In this context the purpose of the present study was to conduct invitro evaluation of the antibacterial activity manifested by acetone, methanol, diethyl ether, ethanol, ethyl acetate, petroleum ether, chloroform and aqueous extracts from one species of lichen (*R.belangeriana*) in relation to test microorganisms that included agents of human.

Corresponding Author: P. Anantharaman, CAS in Marine Biology, Annamalai University, Parangipettai, Tamilnadu, India-608502

MATERIALS AND METHODS

Collection and Identification: Lichen was collected from Pichavaram mangrove area (Lat.11°27'N: Long.79°47'E) and dried at room temperature for 48 h. various taxonomy books were used for identification of lichen sample [14-17].

Preparation of Extract: The air dried powdered lichen (10 g) was extracted in 250 ml of (acetone, methanol, diethyl ether, ethanol, ethyl acetate, petroleum ether, chloroform and water) Using a soxhlet extraction for 72 h at room temperature not exceeding boiling point of the solvent [18]. The extracts were filtered using Whatman filter paper (No.1) and then concentrated in vacuo at 40°C using a rotary evaporator. The obtained residues were kept in a freezer at-80°C until testing like antibacterial activity against some disease causing bacteria (*Klebsiella pneumoniae, Escherichia coli, Staphylococcus* sp*, Enterococci* sp *Proteus* sp *Streptococcus* sp, *Pseudomonas aeruginosa, Vibrio parahaemolyticus, Salmonella* sp, *Shewanella* sp *Vibrio flurialis, Vibrio splendidus*).

Antibacterial Assay

Disc-Diffusion Method: The agar-diffusion method was followed for antibacterial susceptibility test [19]. The 6 mm discs were impregnated with 20µl of the extracts and placed in an inoculated Muller Hinton agar. Then the plates were incubated at 35°C for 24 hrs.Control was maintained with solvent alone.

RESULTS

The antibacterial activity of acetone, methanol, diethyl ether, ethanol, ethyl acetate, petroleum ether, chloroform and aqueous extracts of the lichen *Roccella belangeriana* against the tested microorganisms was estimated and the basis of the presence or absence of inhibitory zones, in the results were exhibited in (Table 1).

The aqueous extract showed no activity against *Pseudomonas aeruginosa, Staphylococcus* sp*., Salmonella* sp. and *Shewanella* sp. The trace activity was recorded against *Escherichia coli, Vibrio flurialis* and *Proteus* sp. The minimum activity was noted against *Streptococcus* sp. (5 mm) followed by *Vibrio splendidus* (6 mm), *Enterococci* sp. (7 mm) and *Vibrio parahaemolyticus* (7 mm). The maximum activity was found against *Klebsiella pneumoniae* (12 mm).

The acetone extract showed no activity against *Pseudomonas aeruginosa* and *Shewanella* sp. The trace activity was observed against *Salmonella* sp. (2 mm), *Vibrio splendidus* (2 mm) and *Streptococcus* sp. (3 mm), *Vibrio flurialis* (3 mm). The minimum activity was scrutinized against *E.coli*, (7 mm), *Enterococci* sp. (9mm), *Vibrio parahaemolyticus* (9mm) and *Proteus* sp. (11 mm). The maximum activity was observed against *Staphylococcus* sp. (15 mm).

The extracts by way of methanol observed no activity *E.coli, Streptococcus* sp. and *Vibrio parahaemolyticus*. The trace activity was noted against *Klebsiella pneumoniae* (2 mm), *Vibrio flurialis* (2 mm) and *Enterococci* sp. (3 mm), *Shewanella* sp. (3 mm).

Table 1: Dry extracts of lichen *Roccella belangeriana* obtained from Pichavaram mangroves against bacterial pathogens

		Solvent used (Inhibition zone diameter in mm)								
Sl. No	Bacterial pathogens	Aqueous	Acetone	Methanol	Ethyl acetate	Chloroform	Ethanol	Diethyl ether	Petroleum ether	Control
1.	*Klebsiella pneumoniae*	12	14	2	2	3	NS	NS	2	NS
2.	*Escherichia coli*	2	7	NS	8	11	4	4	13	1
3.	*Staphylococcus* sp.	NS	15	23	16	4	2	12	NS	NS
4.	*Enterococci* sp.	7	9	3	2	29	3	2	NS	NS
5.	*Proteus* sp.	3	11	22	4	6	18	NS	NS	NS
6.	*Streptococcus* sp.	5	3	NS	NS	4	NS	NS	3	1
7.	*Pseudomonas aeruginosa*	NS	NS	4	NS	3	NS	NS	NS	NS
8.	*Vibrio parahaemolyticus*	7	9	NS	NS	3	NS	NS	NS	NS
9.	*Salmonella* sp.	NS	2	4	2	3	NS	10	9	1
10.	*Shewanella* sp.	NS	NS	3	2	6	3	4	2	NS
11.	*Vibrio flurialis*	2	3	2	5	4	3	NS	NS	NS
12.	*Vibrio splendidus*	6	2	4	NS	NS	NS	2	NS	NS

NS-No Sensitivity, mm-Millimeter)

The minimum activity was recorded against *Pseudomonas aeruginosa* (4 mm), *Salmonella* sp. (4 mm), *Vibrio splendidus* (4 mm). The maximum activity was observed against *Staphylococcus* sp. (23 mm) and *Proteus* sp. (22 mm).

Ethyl acetate extract showed no activity against *Streptococcus* sp., *Pseudomonas aeruginosa, Vibrio parahaemolyticus* and *Vibrio splendidus.* The trace activity was noted against *Klebsiella pneumoniae* (2 mm), *Enterococci* sp. (2 mm), *Salmonella* sp. (2 mm), *Shewanella* sp. (2 mm) and *Proteus* sp. (4 mm). The minimum activity was recorded against *Vibrio flurialis* (5mm) and *E.coli* (8 mm). The maximum activity was observed against *Staphylococcus* sp. (16 mm).

The chloroform extract showed no activity against *Vibrio splendidus.* The trace activity was observed against *E.coli*, *Pseudomonas aeruginosa, Vibrio parahaemolyticus* (3mm) and *Staphylococcus* sp., *Streptococcus* sp., *Vibrio Splendidus* (4 mm). The minimum activity was recorded against *Proteus* sp., *Shewanella* sp. (6 mm). The maximum activity was observed against *Enterococci* sp. (29 mm).

The ethanol extract showed no activity against *Klebsiella pneumoniae, Streptococcus* sp. and *Pseudomonas aeruginosa, Vibrio parahaemolyticus* and *Salmonella* sp. The trace activity was observed against *Staphylococcus* sp., (2 mm) and *Enterococci* sp., *Shewanella* sp. and *Vibrio flurialis* (3 mm). The minimum activity was recorded against *E.coli* (4 mm). The maximum activity was observed against *Proteus* sp. (18 mm).

The diethyl ether extract showed no activity against *Klebsiella pneumoniae, Proteus* sp., *Streptococcus* sp., *Pseudomonas aeruginosa, Vibrio parahaemolyticus* and *Vibrio flurialis.* The trace activity was observed against *Enterococci* sp., *Vibrio Splendidus,* (3mm) and *Staphylococcus* sp., (2mm). The minimum activity was recorded against *Salmonella* sp. (10mm). The maximum activity was observed against *Staphylococcus* sp., (12mm).

The petroleum ether extract showed no activity against *Staphylococcus* sp., *Enterococci* sp., *Proteus* sp., *Pseudomonas aeruginosa, Vibrio parahaemolyticus, Vibrio flurialis* and *Vibrio splendidus.* The trace activity was observed against *Klebsiella pneumoniae, Shewanella* sp. (2 mm) and *Streptococcus* sp.,(3 mm). The minimum activity was recorded against *Salmonella* sp. (9mm). The maximum activity was observed against *E.coli* (13mm).

DISCUSSION

The present study confirmed the presence and absence of antibacterial substance in the extracts of *R. belangeriana* against 12 pathogens Viz., *K.pneumoniae, E. coli, Staphylococus* sp., *Enterococci* sp., *Proteus* sp., *Streptococcus* sp., *P. aeroginosa*, *V. parahaemolyticus, Salmonella* sp., *Shewanella* sp., *V. flurialis* and *V. splendidus*. The differences of antibacterial activity between lichen extracts were dependent upon the solvent used for extraction. Earlier studies did not find any antibacterial activity of lichens extract in water [20, 21,]. The probable reason for this is that majority of active substances present in the thalli of lichens are either insoluble are poorly soluble in water. But in the present study chloroform extraction for great zone of inhibition compared to other extracts.

Rowe *et al.,* [22] reported that the Turkey lichens, *Evernia prunastri, Pseudovernia furfuracea a*nd *Alectoria capillaris* were active against Gram-positive bacteria and the *Candida albicans*. All these studies indicate that the lichens inhibit mostly Gram-positive bacteria. Even though most of the lichens have been reported to be active against Gram-positive bacteria, the actual factors that affect the selective antibiotic activity have not been identified. However, this may be attributed to the biochemical and physiological variations between Gram-positive and Gram-negative bacteria. If so, it is of great interest to note that *Roccella belangeriana* inhibited the growth of both Gram positive and Gram negative bacteria.

Studies of Burkholder *et al.,* [8] on 100 species of American lichens showed that 52% of lichens were active only against Gram positive bacteria. Vartia [23, 24, 25 and 26] reported that 75 of 149 Finnish lichen species inhibited the growth of gram positive and gram negative bacteria. Silva *et al.,* [27] also observed that most of the Brazilian lichens were active against Gram positive bacteria.

Rankovic *et al.,* [28] screened the antimicrobial properties of acetone, methanol and aqueous extracts of the lichens *Lasallia pustulata, Parmelia sulcata, Umbilicaria crustulosa* and *Umbilicaria cylindrica.* Antimicrobial activities of the extracts of different lichens were estimated by the disc diffusion test for Gram-positive bacteria, Gram-negative bacteria and fungal organisms, as well as by determining the MIC (Minimal Inhibitory Concentration). The obtained results showed that the acetone and methanol extracts of *Lasallia pustulata,*

Parmelia sulcata and *Umbilicaria crustulosa* manifested antibacterial activity against the majority of bacterial strains tested, in addition to selective antifungal activity. The MIC of lichen extracts was lowest (0.78 mg/ml) for the acetone extract of *Lasallia pustulata* against *Bacillus mycoides*. Aqueous extracts of all of the tested lichens were inactive. Extracts of the lichen *Umbilicaria cylindrica* manifested the weakest activity, inhibiting only three of the tested organisms.

Meral Yılmaz *et al.,* [29] screened the antimicrobial activity of the chloroform, diethyl ether, acetone, petroleum ether and ethanol extracts of *Cladonia foliacea*. They were found active against 9 bacteria and fungi. Bacteria *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis, Proteus vulgaris, Aeromonas hydrophila, Streptococcus faecalis* and *Listeria monocytogenes* and the yeasts *Candida albicans* and *Candida glabrata* were the microorganisms whose growth were inhibited by the extracts. From these results, it could be concluded that Gram-positive bacteria we inhibited in general. There was no antimicrobial activity of the extracts against the filamentous fungi tested and bacteria *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia*, *Salmonella typhimirium, Yersinia enterocolitica* and *Pseudomonas syringae.*

Esimone and Adikwu, [30] evaluated the phytochemical constituents, antibacterial, antifungal and cytotoxic properties of the extracts of *Ramalina farinacea.* The ethyl alcohol, chloroform and *n*-hexane extracts (4 mg per disk) showed antibacterial and antifungal activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*, *Trichophyton rubrum* and *Trichophyton mentagrophytes.*

Thus, further study is necessary to characterize the chemical constituents of the extracts from lichen samples. In addition, the data may also suggest that the extracts of lichen species tested possess compounds with antibacterial properties, which require further studies to determine antimicrobial agents for therapy of infectious diseases in human and plant diseases.

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