Antimicrobial activities of the lichen *Roccella belangeriana* (Awasthi) from mangroves of Gulf of Mannar

G Karthikai Devi, P Anantharaman*, K Kathiresan & T Balasubramanian
Centre of Advanced Study in Marine Biology, Faculty of Marine Science, Annamalai University
Parangipettai, Pin – 608 502, Tamilnadu, India.

*Email: paraman_cas@yahoo.co.in*

Received 17 November 2009; revised 26 July 2010

The present study, attempted to test antimicrobial activity of the mangrove lichen *Roccella belangeriana* collected from the Gulf of Mannar Biosphere Reserve area. The lichen was extracted in different solvents: acetone, methanol, diethylether, ethanol, ethyl acetate, petroleum ether, chloroform and water and tested against 14 bacterial strains and 3 fungal strains by well diffusion assay. Regarding antibacterial activity the maximum zone of inhibition was recorded in methanol extracts against *Vibrio cholerae* and the minimum zone of inhibition was in ethyl acetate extract against *Klebsiella pneumoniae*, *Enterococci* sp., *Salmonella* sp. and *Shewanella* sp. Regarding the antifungal activity, the maximum zone of inhibition was recorded against *Aspergillus niger*, and the minimum was noted against *Rhizophus* sp.

**Introduction**

Lichens are symbiotic organisms composed of fungi (mycobionts) and algae (phycobiont), and about 20,000 species of them have been recorded worldwide. The lichens are an important food for many animals, including human. They are used in production of alcohols and paints, as well as in the perfume and pharmaceutical industries. Lichens have also been used in folk medicine to treat a variety of animals for centuries by Native Americans, Indians and Europeans.

Bioactive secondary metabolites have been isolated from lichens 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, antmycobacterial, antiviral, antitumour, analgesic and antipyretic properties. Lichen forming fungi produce antibiotic secondary metabolites that protect many animals from pathogenic microorganisms. The first study on the antibiotic properties of lichens was carried out by Burkholder. The development and spread of microbial resistance to available antibiotics has prompted investigators to study antimicrobial substances from other sources like lichens which attract much attention of researchers as significant new sources of bioactive substance. The present study was conducted to test antimicrobial activity of mangrove derived - lichen (*R. belangeriana*).

**Materials and Methods**

Lichen was collected from mangrove area Kurusadai Island (Latitude 9º15’N; Longitude 79 º12’E), Gulf of Mannar, dried at room temperature for 48 h and identified. The air-dried powdered lichen (10 g) was extracted separately for 72 h at room temperature in 250 mL of acetone, methanol, diethyl ether, ethanol, ethyl acetate, petroleum ether, chloroform and water. The extracts were filtered using Whatman filter paper (No.1) and then concentrated in vacuo at 40°C using a rotary evaporator. The residues obtained were kept in a freezer at - 80°C until they were used.

The extract was tested for inhibition of growth against 14 bacteria and 3 fungi by using well diffusion technique. The air-dried powdered lichen (10 g) was extracted separately for 72 h at room temperature in 250 mL of acetone, methanol, diethyl ether, ethanol, ethyl acetate, petroleum ether, chloroform and water. The extracts were filtered using Whatman filter paper (No.1) and then concentrated in vacuo at 40°C using a rotary evaporator. The residues obtained were kept in a freezer at - 80°C until they were used.

The extract was tested for inhibition of growth against 14 bacteria and 3 fungi by using well diffusion technique. The air-dried powdered lichen (10 g) was extracted separately for 72 h at room temperature in 250 mL of acetone, methanol, diethyl ether, ethanol, ethyl acetate, petroleum ether, chloroform and water. The extracts were filtered using Whatman filter paper (No.1) and then concentrated in vacuo at 40°C using a rotary evaporator. The residues obtained were kept in a freezer at - 80°C until they were used.

The extract was tested for inhibition of growth against 14 bacteria and 3 fungi by using well diffusion technique. The air-dried powdered lichen (10 g) was extracted separately for 72 h at room temperature in 250 mL of acetone, methanol, diethyl ether, ethanol, ethyl acetate, petroleum ether, chloroform and water. The extracts were filtered using Whatman filter paper (No.1) and then concentrated in vacuo at 40°C using a rotary evaporator. The residues obtained were kept in a freezer at - 80°C until they were used.

The extract was tested for inhibition of growth against 14 bacteria and 3 fungi by using well diffusion technique. The air-dried powdered lichen (10 g) was extracted separately for 72 h at room temperature in 250 mL of acetone, methanol, diethyl ether, ethanol, ethyl acetate, petroleum ether, chloroform and water. The extracts were filtered using Whatman filter paper (No.1) and then concentrated in vacuo at 40°C using a rotary evaporator. The residues obtained were kept in a freezer at - 80°C until they were used.

The extract was tested for inhibition of growth against 14 bacteria and 3 fungi by using well diffusion technique. The air-dried powdered lichen (10 g) was extracted separately for 72 h at room temperature in 250 mL of acetone, methanol, diethyl ether, ethanol, ethyl acetate, petroleum ether, chloroform and water. The extracts were filtered using Whatman filter paper (No.1) and then concentrated in vacuo at 40°C using a rotary evaporator. The residues obtained were kept in a freezer at - 80°C until they were used.
incubated for 24 h at 37°C. The areas of inhibited bacterial growth were observed as clear halos (zones) around the wells. Antibacterial activity was measured as diameter of zone of inhibition, excluding the well diameter. Similarly antifungal activity was tested on Sabouraud’s Dextrose Agar (SDA) medium against Aspergillus sp., Penicillium sp., and Rhizopus species.

Results and Discussion

Antimicrobial activity of the lichen Roccella belangeriana against the test organisms change were observed on the basis of the presence or absence of inhibitory zones (Tables 1 and 2). Antibacterial assay by using aqueous extract showed the minimum activity recorded against Vibrio sp. (5 mm), V. splendidus (6 mm) and Enterococci sp. (7 mm). The maximum activity was found against Klebsiella pneumoniae (14 mm). The acetone extract showed the minimum activity recorded against Vibrio sp. (5 mm), Streptococcus sp. (5 mm), Enterococci sp. (8 mm). The maximum activity was observed against Klebsiella pneumoniae (16 mm).

The methanol extract showed the minimum activity against Vibrio flurialis (5 mm), Vibrio parahaemolyticus (7 mm), Pseudomonas aeruginosa (8 mm). The maximum activity was observed against V. cholerae (26 mm). Ethyl acetate showed the minimum activity against shewanella sp. (5 mm), and Escherichia coli (8 mm). The maximum activity was observed against Staphylococcus aureus (18 mm), and Vibrio cholerae (18 mm).

The chloroform extract showed the minimum activity against Vibrio sp. (6 mm), Streptococcus sp. (6 mm), Vibrio flurialis (8 mm) and Enterococci sp. (8 mm). The maximum activity was noted against Enterococci sp. (24 mm). The ethanol extract showed the minimum activity against Vibrio parahaemolyticus (8 mm), Salmonella sp. (9 mm) and Vibrio cholerae (9 mm). The maximum activity was noted against Enterococci sp. (18 mm).

The diethyl ether extract showed the minimum activity against Vibrio flurialis (5 mm), Proteus sp. (5 mm), Enterococci sp. (8 mm). The maximum activity was noted against Streptococcus sp., (9 mm), and shewanella sp. (9 mm). The petroleum ether extract showed minimum activity observed against Vibrio cholerae (4 mm), Streptococcus sp., (3 mm), Pseudomonas aeruginosa (4 mm), and Klebsiella pneumoniae (4 mm). The maximum activity was noted against Vibrio flurialis (11 mm).

Antifungal assay by using the aqueous extract showed the minimum activity against Aspergillus niger (5 mm) and the maximum activity against Penicillium sp. (8 mm) and Rhizopus sp. (8 mm).

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Bacterial Strains</th>
<th>Aqueous</th>
<th>Acetone</th>
<th>Methanol</th>
<th>Ethyl acetate</th>
<th>Chloroform</th>
<th>Ethanol</th>
<th>Diethyl ether</th>
<th>Petroleum ether</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Klebsiella pneumoniae</td>
<td>14</td>
<td>16</td>
<td>13</td>
<td>3</td>
<td>5</td>
<td>NS</td>
<td>NS</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>2</td>
<td>Escherichia coli sp.</td>
<td>3</td>
<td>9</td>
<td>9</td>
<td>8</td>
<td>9</td>
<td>NS</td>
<td>NS</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Staphylococcus sp.</td>
<td>2</td>
<td>13</td>
<td>20</td>
<td>18</td>
<td>8</td>
<td>4</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>4</td>
<td>Enterococci sp.</td>
<td>7</td>
<td>8</td>
<td>18</td>
<td>NS</td>
<td>24</td>
<td>18</td>
<td>8</td>
<td>NS</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>Proteus sp.</td>
<td>NS</td>
<td>11</td>
<td>21</td>
<td>3</td>
<td>6</td>
<td>12</td>
<td>5</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>6</td>
<td>Streptococcus sp.</td>
<td>6</td>
<td>5</td>
<td>NS</td>
<td>4</td>
<td>NS</td>
<td>9</td>
<td>3</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>7</td>
<td>Pseudomonas aeruginosa sp.</td>
<td>NS</td>
<td>2</td>
<td>8</td>
<td>NS</td>
<td>5</td>
<td>NS</td>
<td>NS</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>Vibrio parahaemolyticus sp.</td>
<td>NS</td>
<td>NS</td>
<td>7</td>
<td>4</td>
<td>5</td>
<td>8</td>
<td>NS</td>
<td>NS</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>Salmonella sp.</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>3</td>
<td>NS</td>
<td>9</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>10</td>
<td>Shewanella sp.</td>
<td>3</td>
<td>3</td>
<td>NS</td>
<td>5</td>
<td>NS</td>
<td>11</td>
<td>9</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>11</td>
<td>Vibrio flurialis</td>
<td>2</td>
<td>8</td>
<td>5</td>
<td>NS</td>
<td>8</td>
<td>6</td>
<td>5</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>12</td>
<td>Vibrio splendidus</td>
<td>6</td>
<td>4</td>
<td>8</td>
<td>NS</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>13</td>
<td>Vibrio sp.</td>
<td>5</td>
<td>5</td>
<td>15</td>
<td>NS</td>
<td>6</td>
<td>NS</td>
<td>3</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>14</td>
<td>Vibrio cholerae</td>
<td>8</td>
<td>4</td>
<td>26</td>
<td>18</td>
<td>12</td>
<td>9</td>
<td>8</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

(NS-No Sensitivity)
The acetone extract showed minimum activity against *Aspergillus niger* (7 mm) and the maximum activity against *Rhizophus* sp. (11 mm). The methanol extract showed minimum activity against *Rhizophus* sp. (9 mm) and the maximum activity against *Aspergillus niger* (18 mm). The diethyl ether extract showed minimum activity against *Aspergillus niger* (5 mm) and the maximum activity was found against *Aspergillus niger* (18 mm). The methanol extract showed minimum activity against *Rhizophus* sp. (9 mm) and the maximum activity against *Aspergillus niger* (19 mm). The ethanol acetate extract showed no activity against *Aspergillus niger* and the minimum activity was observed against *Penicillium* sp. (8 mm), and the maximum activity against *Rhizophus* sp. (12 mm). The diethyl ether extract showed minimum activity against *Rhizophus* sp. (4 mm) and the maximum activity was found against *Penicillium* sp. (9 mm). The petroleum ether extract showed no activity against *Rhizophus* sp. The maximum activity was found against *Penicillium* sp. (13 mm) and the minimum against *Aspergillus niger* (11 mm).

The antibacterial activity of lichen extracts are dependent upon the solvent used for extraction. Earlier studies did not find any antibacterial activity of lichens extract in water for reasons that majority of active substances present in the thalli of lichens are either insoluble or poorly soluble in water. But in the present study chloroform extract showed high inhibition compared to other extracts.¹¹,¹²

Rowe et al.¹³ reported that the Turkey lichens, *Evernia prunastri*, *Pseudoevernia furfuracea* and *Alectoria capillaris* are active against Gram-positive bacteria and the *Candida albicans*. About 52% of American lichens are active only against gram positive bacteria⁸. Finnish lichen species inhibit the growth of gram positive and gram negative bacteria¹⁴,¹⁵. Most of the Brazilian lichens are active against gram positive bacteria¹⁶. 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 the lichen *Roccella belangeriana* of the present study inhibited the growth of both gram positive and gram negative bacteria.

Several previous workers are in support of the present finding. Acetone and methanol extracts of *Lasallia pustulata*, *Parmelia sulcata* and *Umbilicaria crustulosa* manifest antibacterial activity against the majority of bacterial strains tested, in addition to selective antifungal activity. The MIC of lichen extracts is lowest (0.78 mg/ml) for the acetone extract of *Lasallia pustulata* against *Bacillus mycoides*. Aqueous extracts of all of the tested lichens are inactive. Extracts of the lichen *Umbilicaria cylindrica* manifest the weakest activity, inhibiting only three of the tested organisms¹⁹.

Meral Yılmaz et al.²⁰ screened the antimicrobial activity of the chloroform, diethyl ether, acetone, petroleum ether and ethanol extracts of *Cladonia foliacea*. They are 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* are the microorganisms whose growth are inhibited by the extracts. From these results, it could be concluded that Gram-positive bacteria we inhibited in general. There is no

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Fungal Pathogens</th>
<th>Aqueous</th>
<th>Acetone</th>
<th>Methanol</th>
<th>Ethyl acetate</th>
<th>Chloroform</th>
<th>Ethanol</th>
<th>Diethyl ether</th>
<th>Petroleum ether</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Aspergillus</em> sp.</td>
<td>5</td>
<td>7</td>
<td>18</td>
<td>5</td>
<td>19</td>
<td>NS</td>
<td>8</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>2.</td>
<td><em>Penicillium</em> sp.</td>
<td>8</td>
<td>9</td>
<td>11</td>
<td>12</td>
<td>11</td>
<td>8</td>
<td>9</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>3.</td>
<td><em>Rhizophus</em> sp.</td>
<td>8</td>
<td>11</td>
<td>9</td>
<td>13</td>
<td>9</td>
<td>12</td>
<td>4</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

(NS-No Sensitivity)
antimicrobial activity of the extracts against the filamentous fungi tested and bacteria Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhimurium, Yersinia enterocolitica, and Pseudomonas syringae.

Esimone and Adikwu\(^{21}\), have 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) show antibacterial and antifungal activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger, Trichophyton rubrum, and Trichophyton mentagrophytes.

Several compounds, whose structures represent the common chemical classes of lichen metabolites, were screened for in vitro activity against Mycobacterium aurum, a non pathogenic organism with a similar sensitivity profile to M. tuberculosis of the compounds tested, Usnic acid from Cladonia arbuscula exhibits the highest activity with an MIC value of 32 µg/mL.\(^{22}\) Atronorin and lobaric acid, both isolated from Stereocaulon alpinum, Salazinic acid from Parmelia saxatilis and Protolichesterinic acid from Cetraria islandica all show MIC values ≥ 125 µg/mL.\(^{22}\) The antimicrobial activities of crude extracts obtained from some lichen species against numerous microorganisms have been previously screened.\(^{23-25}\) They exhibit a varying antimicrobial activities depending on microorganisms tested. On the other hand, many of crude extracts of various lichen samples usually show an inhibition effects on the growth of Bacillus species.\(^{23,24,26}\)

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 antimicrobial properties, which require further studies to determine antimicrobial agents for therapy of infectious diseases in human and plant diseases.

Acknowledgement

Authors are grateful to authorities of Annamalai University, Gulf of Mannar Biosphere Reserve Trust and University Grants Commission for providing the facilities and financial support to convene the present study.

References


18. Silva, Jose Oliveira D A, Joaquim Efiegnio Maileite, Marcal de Queiroz., Paulo & Lauroz Xavier Filho, Antimicrobial


22 Kristin Ingolfsdottir, Gavin A C Chung, Vilhjalmur G Skulason, Stefan R Gissurarson, Margret Vilhelmsdottir.


