Antibiotic potential of *Lunularia cruciata* (L.) Dum ex. Lindb (bryophyta) of Kumaon Himalaya

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**Antibiotic activity of Lunularia cruciata** was tested against five pathogenic bacteria (*Agrobacterium tumefaciens*, *Xanthomonas phaseoli*, *Escherichia coli*, *Bacillus subtilis* and *Erwinia chrysanthemi*) and three pathogenic fungi (*Alternaria alternata*, *Sclerotinia sclerotiorum* and *Pyricularia oryzae*). The plant extract was prepared in various solvents (ethanol, methanol, acetone, chloroform and water). All the crude organic extracts of *L. cruciata* showed substantial antibacterial activity while antifungal activity was not found against any of the fungal strains tested. The aqueous extract of the plant did not exhibit any antimicrobial activity. The ethanol extract showed maximum growth inhibition against the test microorganisms (*A. tumefaciens*, *X. phaseoli*, *E. coli* and *B. subtilis*; ZOI = 10 to 20 mm) followed by methanol, acetone and chloroform extracts (ZOI = 8 to 12 mm). The inhibitory activity of these extracts was found very effective against bacteria compared to standard antibiotic ampicillin (10 mcg) used as positive control.

**Key words:** Antibacterial activity, *Lunularia cruciata*, bryophyte, ampicillin, azithromycin.

**INTRODUCTION**

Bryophyta represents a small and delicate group of terrestrial plants which grow profusely in groups during rainy season exhibiting various growth forms on a wide range of habitats. Owing to their potential to live on a variety of habitats, they are exposed to various biotic and environmental dangers. To cope with these dangers, a variety of secondary metabolites are synthesized in their tissues as defence system (Herout, 1990) and many of these compounds have shown interesting biological activity.

These plants have been used for their medicinal properties for more than 400 years in North America, China and Europe to cure burns, bruises and external wounds. More than 30 to 40 species of bryophytes are used as herbal medicine in China (Ding, 1982). Judith (2007) reported that some of the Chinese medicines that contain mosses like Grimmia, Atrichum, Polytrichum and Thuidium were used as antibacterial and anti-inflammatory agents. Germans used mosses, *Ceratodon purpureus* and *Bryum argenteum* to cure fungal infections (Frahm, 2004). In India, people of Kumaon Himalaya use *Marchantia polymorpha* and *M. palmata* to cure burns, abscesses and to reduce pus formation, while paste of *Riccia* spp. is applied on the ring worm disease of skin (Pant and Tewari, 1989). The Gaddi tribes of Himachal Pradesh, India use *Plagiochasma appendiculatum* for the cure of burns, boils and blisters of skin (Kumar et al., 2000).

Singh et al. (2011) reported the use of *Conocephalum conicum*, *P. appendiculatum*, *B. argenteum* and *Mnium marginatum* by traditional healers for burn infection. In spite of these uses of bryophytes in human health, their medicinal importance is not exploited completely. In many countries, about 80% of therapeutic substances are obtained from medicinal plants which belongs to angiosperms while less work is done on non-vascular...
plants like bryophytes which are known to contain numerous potentially useful compounds including oligosaccharides, polysaccharides, sugar, alcohols, amino acids, aliphatic compounds, phenylquinones, aromatic and phenolic substances (Asakawa, 1979a, 1981; Pant and Tewari, 1990). The universal demand for natural antimicrobiological therapeutics and the rise of antibiotic resistant bacteria have motivated scientists to search for new natural source with potential pharmaceutical capabilities (Cowan, 1999).

The liverwort, Lunularia cruciata Dum. is a thallose mat forming liverwort of family Lunularieae, that grows profusely as a weed in the flower pots within glass house. The species is also reported from exposed but moist and cool retaining walls and brickworks. Generally, it is distributed in eastern Himalaya and south India but also reported from Nainital district of western Himalaya (Kanwal, 1977). The prostrate irregularly branched thallus is light green in colour and can be identified easily due to the presence of half moon shaped gemma cups on the dorsal surface. Wilson and Schwabe (1964) isolated a compound from L. cruciata and M. polymorpha. In this compound, a plant hormone has the property of growth inhibition and promotion of dormancy in liverworts. Valio et al. (1969) identified this compound as lunularic acid. Pryce (1971a) isolated this plant growth-regulator, lunularic acid from this species. This liverwort also expressed antimicrobial and to a less extent antifungal activities against several microorganisms (Basile et al., 1998; Ielpo et al., 1998).

The main objective of the present study was to assess the antibiotic potential of L. cruciata extracts against a wide range of bacteria in order to use it as a source of new antimicrobial agent.

MATERIALS AND METHODS

The plant material was collected from the glass house, Department of Botany, Kumaon University, Nainital in the month of June 2010. The collection was done when the plant had attained maturity.

Preparation of extracts

The thoroughly washed plant material was blotted dry to remove the extra moisture. The sample was crushed in mortar and pestle along with a pinch of sterilized sand for the preparation of the extract in water and organic solvents.

To prepare stock solution, 50 g of the crushed material was added to 200 ml of solvents (w/v 50 g/200 ml). Solvents used for extraction were ethanol, methanol, acetone, chloroform and water. Each extract was shaken for at least 6 h and then filtered with Whatman filter paper no. 1.

Microorganisms used

Five (Gram +ve and Gram -ve) bacteria: A. tumefaciens (Gram-ve) MTCC No. 609, E. coli (Gram -ve) MTCC No. 40, B. subtilis (Gram +ve) MTCC No. 121, were procured from the Institute of Microbial technology, Chandigarh, India and X. phaseoli (Gram-ve) and E. chrysanthemi (Gram-ve) obtained from Plant Pathology Department, G. B. Pant University, Pantnagar, India were used in this investigation.

Screening of antibacterial activity

Antibacterial tests of selected microorganisms were carried out by using disc-diffusion method (Bauer et al., 1966). Nutrient agar (Hi Media, Laboratories, Mumbai, India) was poured in sterilized Petri plates (90 mm size) and cooled at room temperature (20 ± 2°C). A small sterile cotton swab was dipped into the 24 h old culture of bacteria and was inoculated by streaking the swab over the entire agar surface. This process was repeated twice or more by rotating the plates approximately 60° to ensure even distribution of inoculum. After inoculation, the plates were allowed to dry at room temperature (20 ± 2°C) for 20 min in laminar flow for settling down of inoculums. The whatman no. 1 filter paper discs (5 mm) loaded with 40 µl of extract were placed on to the surface of the bacteria seeded agar plates and allowed to diffuse for 5 min. The Petri plates inoculated with bacterial strains were incubated at 37 ± 1°C for 24 h.

The three antibiotics, Gentamycin (10 mcg), Azithromycin (15 mcg) and Ampicillin (10 mcg) used as positive control and were also placed onto the agar plates while filter paper discs (5 mm) loaded with respective solvents were used as negative control. After 24 h of incubation, the diameter of inhibition zone was measured in mm (including the disc size). Each extract was tested in triplicates and was performed twice. The values of zone of inhibition (Z0I) were expressed as near value with standard error of mean (SEM).

RESULTS AND DISCUSSION

The results indicated that all the bacterial strains were sensitive to the tested extracts (ethanol, methanol, acetone and chloroform) of L. cruciata except aqueous extract. The order of inhibitory activity of different extracts of L. cruciata was ethanol > methanol > acetone > chloroform. In ethanol extract, the maximum inhibition zone was 20±0.33 mm for A. tumefaciens; 19±0.57 mm for B. subtilis; 18±0.33 mm for E. coli; 15±0.33 mm for E. chrysanthemi and 10±0.66 mm for X. phaseoli (Table 1). The bacterial strains were found more sensitive to the tested extracts in comparison to commercially available susceptibility disc of ampicillin (10 mcg); positive control (Figures 1 and 2).

Asakawa (1998) reported that the crude extracts of bryophytes possessed inhibitory activity against germination, root elongation and coleoptile growth of rice, wheat, lettuce and radish. Several plant growth inhibiting substances were already known in bryophytes among which the most widely studied compound is lunularic acid obtained from the thallus of L. cruciata.

Gorham (1978) observed that lunularic acid inhibited the germination of Marchantia gemmae at lower concentration and 20% cress root growth at higher concentration. In the present investigation, it is obvious that the extract of L. cruciata were effective against the pathogenic bacteria perhaps due to the presence of
Table 1. Antibacterial activity of *Lunularia cruciata* extracts on pathogenic bacteria diameter of inhibition zone (mm)*.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>C</th>
<th>A</th>
<th>E</th>
<th>M</th>
<th>W</th>
<th>G</th>
<th>Az</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. tumefaciens</em></td>
<td>9±0.57</td>
<td>9±1.00</td>
<td>20±0.33</td>
<td>10±0.57</td>
<td>na</td>
<td>25±0.45</td>
<td>31±0.9</td>
<td>na</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>8±0.66</td>
<td>9±0.00</td>
<td>18±0.33</td>
<td>11±1.45</td>
<td>na</td>
<td>27±0.37</td>
<td>32±0.93</td>
<td>na</td>
</tr>
<tr>
<td><em>X. phaseoli</em></td>
<td>8±0.33</td>
<td>8±0.33</td>
<td>10±0.66</td>
<td>11±0.57</td>
<td>na</td>
<td>25±0.34</td>
<td>29±1.21</td>
<td>na</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>10±0.33</td>
<td>10±0.33</td>
<td>19±0.57</td>
<td>12±0.33</td>
<td>na</td>
<td>25±0.48</td>
<td>25±1.74</td>
<td>na</td>
</tr>
<tr>
<td><em>E. chrysanthemi</em></td>
<td>8±0.57</td>
<td>9±0.00</td>
<td>15±0.33</td>
<td>8±0.00</td>
<td>na</td>
<td>24±0.34</td>
<td>24±2.16</td>
<td>na</td>
</tr>
</tbody>
</table>

*All the values are mean of three determinations. C, A, E, M and W denote chloroform, acetone, ethanol, methanol, aqueous extracts, respectively. G, Az, A - Gentamycin, Azithromycin, Ampicillin (+ve control), respectively. na, not active.

Figure 1. Antibacterial activity of *Lunularia cruciata* extracts against some pathogenic bacteria. (A), chloroform extract; (B), acetone extract; Ag, *Agrobacterium tumefaciens*; Bs, *Bacillus subtilis*; Eco, *Escherichia coli*; Ec, *Erwinia chrysanthemi*; Xp, *Xanthomonas phaseoli*; 1, extract; 2, 3, 4, positive controls (gentamycin, ampicillin, azithromycin), 5- negative control (solvent).

Figure 2. Antibacterial activity of *Lunularia cruciata* extracts against some pathogenic bacteria. (A), ethanol extract; (B), methanol extract; Ag, *Agrobacterium tumefaciens*; Bs, *Bacillus subtilis*; Eco, *Escherichia coli*; Ec, *Erwinia chrysanthemi*; Xp, *Xanthomonas phaseoli*; 1, extract; 2, 3, 4, positive controls (gentamycin, ampicillin, azithromycin), 5- negative control (solvent).
lunularic acid.

The effectiveness of different extracts of *L. cruciata* differed largely due to the relative solubility of various secondary metabolites in different solvents and it appears that these metabolites were more soluble in organic solvents compared to water thus resulting into the absence of antibacterial activity in aqueous extract of *L. cruciata*.

Banerjee and Sen (1979) examined 52 species of bryophyte for antimicrobial activity. Out of these species, 29 were active against at least one of the test bacteria. They reported that the crude ethanolic and methanolic extracts of *L. cruciata* exhibited antibacterial activity against *Staphylococcus aureus*, *E. coli*, and *Salmonella typhi*. Similarly, in our study these extracts of *L. cruciata* exhibited substantial antibacterial activity against *E. coli*, *B. subtilis* and other bacteria.

Joshi (1993) found that the water and DMSO extracts of *Dumortiera hirsuta* and *L. cruciata* possessed antibacterial activity against *S. aureus*, *E. coli*, *Klebsiella pneumoniae* and *B. subtilis*. In all the aforesaid studies, the water extract of *L. cruciata* was as active as those of alcoholic extracts but in the current study only the water extract of *L. cruciata* was found inactive against all the selected bacterial strains.

This may perhaps be due to the insolubility of secondary metabolites in water at low temperatures, compared to the other similar studies carried out at higher temperatures. Also, there is possibility that the plant collected from different geographical areas and ecological setup showed variable antimicrobial spectra. Further, the synthesis of chemical constituents in the tissues of plants may be an adaptation to their ecological setup (Anderson et al., 1974; Karunen, 1978). Besides, the variation in ageing (Banerjee and Sen, 2001) and solvents may be responsible for the variation in results. Basile et al. (1998) evaluated the action of acetone extract of *L. cruciata* against 13 bacteria (Gram +ve and Gram -ve) and 2 fungal pathogens and found substantial antibacterial activity. Pryce (1972) reported the presence of lunularin (dihydrostilbene) and lunarlic acid (3, 4′ dihydroxybibenzyl-2′ carboxylic acid) from *Conocephalum conicum*, *M. polymorpha* and *L. cruciata*. The lunularic acid is identical to the known compound dihydrogenic acid derived from hydrogenol glucoside isolated from the higher plant *Hydrangea macrophylla*. Perhaps, majority of the liverworts owe their antimicrobial potential by the content of lunarlic acid. *L. cruciata* is also known to possess a flavonoid heteroside luteolin 7-O- glucoside and the flavonoid aglycone quercetin compound (Jovkovic et al., 2008). This yellow pigment quercetin was found to be the most biologically active of the flavonoids and many medicinal plants owe much of their activity due to their high quercetin content. Perhaps the antibacterial activity of *L. cruciata* observed in our study may be due to the presence of lunarlic acid and another quercetin like secondary metabolites.

In general, the bioactivity data indicated that the tested extracts have potential to inhibit the growth of bacteria. However, there is still need for more experimental work to explore and isolate the specific chemical compounds from the plant which exhibit promising antimicrobial property.

REFERENCES


