Antimicrobial Activity of Endophytic Fungi Isolated from Medicinal Plant *Hugonia mystax* L.

G. Abirami" and M. Boominathan

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Abstract

Endophytic fungal isolates obtained from the leaf samples of medicinal plant *Hugonia mystax* L. was evaluated for its antimicrobial potential. The endophytic fungal isolates namely *Aspergillus* sp., *Chaetomium* sp., *Curvularia* sp., *Dreschelara* sp., *Fusarium* sp., *Penicillium* sp., *Colletotrichum* sp., *Nigrospora* sp., *Pestalotiopsis* sp. and *Phyllosticta* sp. were tested for antimicrobial activity against various bacterial and fungal pathogens. It was noted that the isolated endophytes showed prominent antimicrobial activity against the tested pathogens. Significant inhibition zone was observed among the different ethanol extracts showed the antimicrobial potential of the endophytes. Two endophytic isolates namely *Penicillium* sp. and *Colletotrichum* sp. showed highest zone of inhibition against the tested pathogens compared to all other endophytic isolates. The findings of the present study may be directed towards the identification of potential antimicrobials from the isolated endophytes in near future.

Keywords: Endophytes, *Hugonia mystax* L., ethanolic extracts, antimicrobial activity, zone of inhibition.

Introduction

The phenomenal increase in the world population leads to the emergence of new diseases, drug-resistant bacteria, parasitic protozoa and fungi. This has created an alarm in the scientists to go for the research on different natural resources which can be safe and acts as pharmaceutical agents to face challenges against the emerging diseases. In these consequences, many medicinal plants are used in a traditional manner for treatment of various ailments (Ong and Nordiana, 1999). The interrogation raised is whether the potential activity is due to the extracts of the medicinal plants or the mutualistic beneficial endophytes existing in their internal tissues. Many reports have been identified that the bioactivity like antibacterial and antifungal were contributed by the substances produced by the endophytes which resides in the host plant. Endophytes are the symbiotic microbes that inhabit into the internal plant tissues without causing any apparent harm to their host (Hirsch and Braun, 1992). These endosymbiotic fungi found to synthesis bioactive secondary metabolites (Schulz et al., 2002; Strobel, 2003) like flavonoids, phenols, saponins, tannins and terpenoids which are used to protect the host from infectious diseases, insect attack, parasitic infections and herbivore tissue invading pathogens. Extremely ubiquitous endophytic fungi exist universally in various plants (Rodríguez et al., 2009), performing significant physiological (Malinowski et al., 2004) and ecological (Tintjer and Rutgers, 2006) role in their host life. *Hugonia mystax* Linn. is an important medicinal plant which is highly beneficial to cure many diseases, other species namely *H. ferruginea* Wight and Arn., were also reported in India (Santapau and Hendry, 1983; Pullaiah and Chellaiah, 1997).

The tribal people of Kalakad Mundanthurai used the roots of *H. mystax* to treat Rheumatism (Sutha et al., 2009). In Tiruvannamalai hills in Tamil Nadu, the leaves of the plant are used to treat skin diseases. Ethnobotanical study revealed that, the roots of the plant were used as astringent, antihelmintic (Padel et al., 2010) and also to treat dysentery, snake bite and fever (Balasubramaniam et al., 1997). Petroleum ether, chloroform and ethanolic extracts of stem, leaf and fruit of *H. mystax* are reported to possess antimicrobial activity against human pathogens like *Streptococcus pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Candida albicans*. Several natural products produced by endophytic fungi have unique structures and large bioactivities which applied in agricultural, industrial and medicinal fields. Approaches for development of new antibiotics have been pursued, such as combinatorial chemistry tools but only a few broad spectrum antibiotics are reported to be produced by the pharmaceutical industry at the present time (Coates and Hu, 2007). Keeping the above facts in view, this study was aimed to determine the antimicrobial activity of various endophytic fungi isolated from medicinal plant *Hugonia mystax* L.

Materials and methods

Collection of plant material: Healthy *Hugonia mystax* Linn. plants were collected from Puthupattu sacred grove region, South India, Pondicherry during the month of February 2014 (Fig. 1). The plant was authenticated by Dr. Kumaresan, Asst. Prof., Dept. of Botany, Bharathidasan Womens College, Pondicherry, Tamil Nadu, India.
Fully matured healthy leaves from its various parts were excised with an aseptic scalpel and placed in a sterile plastic bag for storage at 4°C until use.

_isolation and identification of endophytic fungi:_ The collected plant materials were washed thoroughly in running water to remove dirt and debris. The lamina was cut on both the sides of the leaves leaving 0.05 cm of midrib. The midribs were cut into 100 segments approximately of 0.5 cm in size and transferred into a sterile beaker. The segments were surface sterilized by dipping it into 70% ethanol for 1 min, immersed in 4% sodium hypochlorite solution for 90 sec, finally rinsed in sterile distilled water for 20 sec and placed in a sterile plate to remove excess moisture. Using forceps, the surface sterilized 100 segments were inoculated into petri dishes containing sterile PDA medium supplemented with 250 mg/L of chloramphenicol. Each plate contained 10 segments of leaf samples on PDA medium (Fig. 2). Then the plates were sealed with parafilm and kept for incubation at 26-28°C for 3 d in partial dark and light. After 3 d, the plates were observed every day for the growth of endophytic fungi.

The grown endophytes were subcultured in a fresh PDA medium to get a pure culture of it. Endophytic fungi were identified by standard mycology manuals by Ellis (1971), Sutton (1980), Barnett and Hunter (1987) and Domsh et al. (2007).

Antimicrobial activity of endophytic fungi: The isolated endophytic fungi were tested for its antimicrobial activity against various pathogens. Endophytic culture was inoculated in PDA broth and incubated for 7 d at 37°C until the mat formation. Then, 50 mL of ethanol was added and the filtrate was collected using a masculine cloth, dried for overnight at 60°C until semisolid powder was obtained. The dried semisolid powder of each endophytic fungus was tested for antimicrobial activity. Antimicrobial activity was carried out by Agar well diffusion method (Smania, 1999). The bacterial inoculum of Staphylococcus aureus, Escherichia faecalis, Bacillus subtilis and Pseudomonas aeruginosa and other isolates were uniformly spread using sterile cotton swab on a sterile petri dish containing Muller-Hinton agar. About 1 mg/mL of endophytic ethanolic fungal extracts (0.5-12.5 μL) were added to respective wells (6 mm dia), 20 mm apart from one another. Ciprofloxacin (20 μL) was used as the positive control. The plates were incubated for 24 h at 36±1°C under aerobic conditions. After incubation, confluent growth was observed. Inhibition of the growth was measured in mm. All the tests were performed in triplicates and the mean values were recorded.

Results
Totally 10 endophytic fungal isolates were obtained from the leaf samples of _Hugonia mystax_ L. The ten fungal isolates included Aspergillus sp., Chaetomium sp., Curvularia sp., Dreschelara sp., Fusarium sp., Penicillum sp., Colletotrichum sp., Nigrospora sp., Pestalotiopsis sp. and Phyllosticta sp (Fig. 3).

Fig. 3. Endophytic fungal isolates emerging from the leaf segments of _Hugonia mystax_.

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Table 1. Antimicrobial activity of endophytic Aspergillus sp.

<table>
<thead>
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<th>100 μg</th>
<th>150 μg</th>
<th>200 μg</th>
<th>Control* (20 μg)</th>
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Ciprofloxacin was used as positive control at 20 μg concentration.

Table 2. Antimicrobial activity of endophytic Chaetomium sp.

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Table 1 shows the antimicrobial activity of Aspergillus sp. It was noted that highest inhibition against Staphylococcus epidermidis was seen in 200 μg concentration of Aspergillus sp. ethanol extract whereas lowest inhibition was found against Vibrio cholerae at 150 μg concentration. No inhibition was found against Escherichia coli and Candida albicans. From Table 2, it was noted that Chaetomium fungal extract inhibited S. aureus at 200 μg concentration recording an inhibition dia of 18 mm whereas; lowest inhibition was recorded against L. monocytogenes at 50 μg concentration. It was noted from Table 3 that no inhibition was recorded against S. aureus, A. niger and some other species. Curvularia fungal extract potentially inhibited the growth of S. epidermidis whereas lowest inhibition was found against B. subtilis. Table 4 shows the antimicrobial activity of Dreschelara sp. It was noted that highest inhibition was seen against S. aureus in 200 μg concentration whereas lowest inhibition was found against C. albicans. Ethanolic fungal extract of Fusarium sp. effectively inhibited B. subtilis at all concentrations tested. No significant inhibition was found against other isolates tested (Table 5).

Table 6 shows the antimicrobial activity of endophytic Penicillium sp. Highest inhibition dia of 23 mm was found against Klebsiella pneumoniae whereas inhibition dia of 21 mm was recorded against Escherichia coli and Proteus mirabilis. The ethanolic fungal extract of Penicillium sp. was found active against most of the isolates tested for antimicrobial activity. It was noted from Table 7 that Nigrospora fungal extract potentially inhibited the growth of Staphylococcus aureus and Candida albicans whereas no significant inhibition was found against other isolates tested. Table 8 shows the antimicrobial activity of endophytic Pestaloptiopsis sp. Highest inhibition dia of 20 mm was found against E. coli, S. epidermidis, P. mirabilis and S. aureus whereas no inhibition was found against E. aerogenes. Ethanolic fungal extract of Colletotrichum sp. effectively inhibited Escherichia coli, Staphylococcus aureus, Proteus mirabilis and Klebsiella pneumoniae at 200 μg (Table 9). Table 10 shows the antimicrobial activity of endophytic Phyllosticta sp. Highest inhibition dia of 15 mm was found against Pseudomonas aeruginosa, whereas no significant inhibition was found against other isolates tested.
### Table 3. Antimicrobial activity of endophytic *Curvularia* sp.

<table>
<thead>
<tr>
<th>Tested strains</th>
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<th>100 μg</th>
<th>150 μg</th>
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<th>Control* (20 μg)</th>
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### Table 4. Antimicrobial activity of endophytic *Dreschelara* sp.

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### Table 5. Antimicrobial activity of endophytic *Fusarium* sp.

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### Table 6. Antimicrobial activity of endophytic *Penicillium* sp.

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### Table 7. Antimicrobial activity of endophytic Nigrospora sp.

<table>
<thead>
<tr>
<th>Tested strains</th>
<th>50 μg</th>
<th>100 μg</th>
<th>150 μg</th>
<th>200 μg</th>
<th>Control* (20 μg)</th>
</tr>
</thead>
<tbody>
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<td><em>Staphylococcus aureus</em></td>
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<td>18</td>
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<td>-</td>
</tr>
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<td>14</td>
<td>16</td>
<td>36</td>
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### Table 8. Antimicrobial activity of endophytic Pestalotiopsis sp.

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<th>Control* (20 μg)</th>
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<td>20</td>
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<td>-</td>
<td>-</td>
</tr>
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<td>13</td>
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### Table 9. Antimicrobial activity of endophytic Colletotrichum sp.

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### Table 10. Antimicrobial activity of endophytic Phyllosticta sp.

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<th>Tested strains</th>
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Discussion
In view of the increased global health concern over the failure of currently used antibiotics to many super resistant strains, indiscriminate exploitation of medicinal plants for extraction of antimicrobial agents of plant origin and limitations of plant resources due to various factors like requirement of land for cultivation, environmental competence of plants, seasonal specificity etc. the search for new and effective antimicrobial agents is becoming a necessity (Desale and Bodhankar, 2013). Endophytic fungi have been recognized as useful sources of bioactive secondary metabolites (Schulz et al., 2002; Strobel, 2003). A recent comprehensive study has indicated that 51% of biologically active substances isolated from endophytic fungi were previously unknown (Schulz et al., 2002). Many endophytic fungi have the ability to produce antimicrobial substances (Stinson et al., 2003). *Hugonia mystax* L. is an important medicinal plant which is highly beneficial to cure many diseases (Santapau and Hendry, 1983; Pullaiyah and Chellaiah, 1997). In the present study, totally 10 endophytic fungal isolates were obtained from the leaf samples of *Hugonia mystax* L. The fungal isolates included *Aspergillus* sp., *Chaetomium* sp., *Curvularia* sp., *Dreschelara* sp., *Fusarium* sp., *Penicillum* sp., *Colletotrichum* sp., *Nigrospora* sp., *Pestalotiopsis* sp. and *Phylosticta* sp. It was noted that the isolated endophytes showed prominent antimicrobial activity against the tested pathogens. Significant inhibition zone was observed among the different ethanol extracts showed the antimicrobial potential of the endophytes. The tested endophytes potentially inhibited bacterial isolates and *C. albicans*. The present findings falls in line with the previous reports of Ramesha and Srinivas (2014) who have earlier reported antimicrobial activities of different endophytes.

Conclusion
The present study showed promising antimicrobial activity of 10 endophytic fungal isolates obtained from the leaf samples of *Hugonia mystax* L. The findings of the present study may be directed towards the identification of potential antimiicrobials from the isolated endophytes in near future.

References