BIOACTIVITY OF THE EXTRACT FROM AN ENDOPHYTIC FUNGI

*NIGROSPORA ORYZAE* ISOLATED FROM *PTEROLOBium HEXAPETALUM* (ROTH.)

S. ASHWINI AND KALYANARAMAN RAJAGOPAL*

Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), VELS University, Pallavaram, Chennai, TamilNadu, India.

**ABSTRACT**

*Nigrospora oryzae* an endophytic fungus isolated from the leaf of a climber *Pterolobium hexapetalum* (Roth.) Sant. and Wagh. produced bioactive compound which are active against human pathogens. The organic extract (ethanol) of the endophyte *Nigrospora oryzae* was separated using HPLC and tested for biological activities. The ethanol extract was tested for antibacterial and anti fungal activity using agar well diffusion method. The extract of *Nigrospora oryzae* was active against *Streptococcus pneumoniae*, *Salmonella typhi* and *Candida albicans*. The extract showed maximum zone of inhibition against *Salmonella typhi* (19.2±0.2 mm) and *C. albicans* (15.6± 0.41mm) at the volume 100µg /ml.

**KEYWORDS**: *Pterolobium hexapetalum*, endophytic fungi, *Nigrospora oryzae*, Bioactivity.

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KALYANARAMAN RAJAGOPAL

Department of Biotechnology, School of Life Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), VELS University, Pallavaram, Chennai, TamilNadu, India.
INTRODUCTION

Pterolobium hexapetalum (Roth.) Sant. and Wagh is one of the large scrambling or climbing shrubs that grow commonly in dry deciduous forest, or as pioneer plants in open land. It is a climbing shrub belonging to Caesalpiniaceae family having significant medicinal properties. The leaves and stem bark are used against fever, tooth ache, chest pain, delivery pains, antidote to dog bite, wound healing, constipation, piles, ulcers, skin infections, venereal diseases, jaundice and diarrhoea. Phytochemical screening reveals the presence of main bioconstituents like flavonoids, phenols, and alkaloids, which proved in supports of their bioactivity, as potential antibacterial drug in comparison to the control drugs with leaf and bark Hot water and Methanol extracts. Endophytic microorganisms are to be found in virtually every plant on earth. These organisms reside in the living tissues of the host and do so in a variety of relationships, ranging from symbiotic to slightly pathogenic. Because of what appears to be their contribution to the host plant, the endophytes may produce a plethora of substances of potential use to modern medicine, agriculture, and industry. Novel antibiotics, antimycotics, immunosuppressant’s, and anticancer compounds are only a few examples of what has been found after the isolation, culture, purification, and characterization of some choice endophytes in the recent past. The potential prospects of finding new drugs that may be effective candidates for treating newly developing diseases in humans, plants, and animals are great. Hence, in the present study the dominant endophyte N. oryzae isolated from the leaf of P. hexapetalum crude extract was screened for the antimicrobial activity.

MATERIALS AND METHODS

The endophyte Nigrospora oryzae obtained from the Vels University Culture Collection (No. VUCC 151). The endophyte was isolated from leaf tissue of Pterolobium hexapetalum was selected for this study, because of their high colonization frequency from the leaf tissue among other seven endophytic fungi were isolated.

Extraction of Bioactive Compounds

The selected endophytic fungus was grown in Czapek's liquid media incubated for 21 days at 120 rpm. Before the extraction the media along with mycelium was macerated and separated using Whatman No 1 filter paper. To extract equal volume of ethanol was added with culture filtrate and the compounds were separated using separating funnel and concentrated in rotary vacuum evaporator. The dry semi solid residue was redissolved in ethanol for further analysis.

HPLC analysis of crude extract

The ethanol extract of the fungal endophyte sample was analyzed using High Performance Liquid Chromatography (Schimadzu 9A). C18 ODS Reverse phase Gemini 110A, 5U column (250 X 4.6 mm) was used. The mobile phase Acetonitrile/water (60:40) were used and were read at the wavelength of 215nm, flow rate 0.5ml/min.

Antimicrobial Activity

Agar well diffusion method

The antimicrobial activity of Nigrospora oryzae crude extract was tested against human bacterial and fungal pathogens viz Streptococcus pneumoniae, Salmonella typhi and Candida albicans. It was evaluated by using agar well diffusion method. PDA plates were inoculated with 100µl of standardized inoculum (1.5x10^5 CFU/ml) of each bacterium (in triplicates) and spread with sterile swabs. Wells with a size of 5mm size were made with sterile cork borner in to agar plates containing the bacterial inoculum and the base of the well sealed will a drop molten agar medium. The endophytic fungal extract was added in to the well in two different volumes 50µl and 100µl for both anti fungal and anti bacterial studies. Chloramphenicol dissolved in the DMSO was used control. The plates were incubated at room temperature for ten minutes allowing the diffusion of extract into the agar. After incubation for 24 hrs at 37°C, the plates were observed. Similarly antifungal activity of C. albicans was carried out using GPP medium and incubated at 37°C for 18-24 hours. Fluconazole used as control for anti fungal studies. The activity was detected by inhibition zone around the well. The zone of inhibition was measured and expressed in millimeters. The antimicrobial activity was recorded if the zone of inhibition was greater than 8mm. The mean and standard deviation of the diameter of inhibition zones were calculated.

RESULTS AND DISCUSSION

The HPLC profile of the N. oryzae showed the presence of four different compounds and showed peak value ranges from 4.39, 5.77, 6.47 and 9.78 (Fig 1). The organic extract of the endophytic fungi N. oryzae was tested for antibacterial and antifungal activity the results are presented in Table 1. The ethanol extract of N. oryzae showed varied activity against the test pathogens. The crude extract inhibited the Salmonella typhi growth significantly (19.2±0.2) than the Streptococcus pneumoniae (Table 1) at the highest volume 100µl. The crude extract also inhibited the Candida albicans significantly (15.6± 0.41mm) at the volume 100µg /ml (Table 2). At low volume 50µl none of the organisms were inhibited (Table 1 & 2). Natural products are naturally derived metabolites and or by products from microorganisms, plants, animals. The products have been exploited for human use for thousands of years and plants have been the chief source of compounds used for medicine.

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also Streptococcus pneumoniae and Salmonella typhi were inhibited by the extract of *N.* oryzae (Table 1). *Phomopsis* sp another common endophyte found in various plants produce many active metabolites have been reported as anti-candida compounds \(^\text{15, 16, 17 & 18}\) found that there were wide differences among endophytic fungi in their ability to produce metabolites with antimicrobial activity. In the current investigation also it was established that endophytic fungi isolated from same host exhibit varying degrees of in vitro antimicrobial activities. Uses of diverse solvents also play a very significant role in compounds extractions. The antimicrobial screening results of this study as well as earlier study suggests that the metabolites of endophytic fungi commonly and particularly from climbing shrubs could be a excellent potential source for bio active compounds. Further research is in progress to resolve which fractions of the extracts are accountable for the biological activities and work is ongoing to reveal the chemical structure of the metabolites.

### Table 1

**In vitro antibacterial activity of crude extract of Nigrospora oryzae using ethanol by Agar well diffusion method against human bacterial pathogens**

<table>
<thead>
<tr>
<th>Endophytic fungi</th>
<th>Control</th>
<th>Volume</th>
<th>S. typhi</th>
<th>S. pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nigrospora oryzae</em></td>
<td>18.4±0.3</td>
<td>50µl</td>
<td>16.0±0.40</td>
<td>17.8±0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100µl</td>
<td>19.2±0.20</td>
<td>18.1±0.20</td>
</tr>
</tbody>
</table>

### Table 2

**In vitro antifungal activity of crude extract of Nigrospora oryzae using ethanol by Agar well diffusion method against human fungal pathogen *C.* albicans**

<table>
<thead>
<tr>
<th>Endophytic fungi</th>
<th>Control</th>
<th>Volume</th>
<th><em>Candida albicans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nigrospora oryzae</em></td>
<td>14.2 ± 0.43</td>
<td>50µl</td>
<td>14.0±0.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100µl</td>
<td>15.6± 0.41</td>
</tr>
</tbody>
</table>

### Figure 1

**HPLC analysis of crude extracts of Nigrospora oryzae**

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**CONCLUSION**

In the present study for the time the distribution of fungal endophyte of *Pterolobium hexapetalum* host was studied. *N.* oryzae was the dominant endophyte and produced different compounds in culture. The organic extract of the endophyte actively inhibited human pathogens in vitro. Hence, endophyte fungi from other climbers should be screened for more endophyte diversity and screening of endophytes for bioactive compounds is the need of the hour for novel bioactive compounds. It will be of use to the pharmaceutical industry for the mass production of bioactive compounds using modern technology.

**CONFLICT OF INTEREST**

Conflict of interest declared none.
REFERENCES


