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Bioactivity of Endophytic Fungus *Colletotrichum gloeosporioides* Isolated from *Phlogacanthus thyrsiflorus* Nees

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Abstract

To study antibacterial, antioxidant and total phenolic content of Colletotrichum gloeosporioides an endophytic fungus isolated from Phlogacanthus thyrsiflorus Nees. Fungal endophytes were isolated from Phlogacanthus thyrsiflorus Nees collected from Imphal West, Manipur. Morphological and molecular identification was done for the potential isolate. Extract from fermentation broth of Colletotrichum gloeosporioides were checked for antibacterial activity, antioxidant activity and total phenolic content. Staphylococcus aureus (ATCC25923), Enterococcus faecalis (ATCC29212), Escherichia coli (ATCC25922) and Pseudomonas aeruginosa (ATCC27853) were used for testing antibacterial activity. The crude extract showed an effective antibacterial activity against S. aureus with a zone of inhibition of 13.33±1.52mm. The crude extract of an endophytic fungus Colletotrichum gloeosporioides was studied for total phenolic content and antioxidant activity. Free radical scavenging activityof the extract showed 90%. Total phenolic content is 18.3±0.36mg gallic acid/g of extract. This report of antibacterial and antioxidant activity of endophytic fungus Colletotrichum gloeosporioides residing in Phlogacanthus thyrsiflorus Nees is presented for the first time in this work. The findings indicate that the metabolite of endophytic fungus Colletotrichum gloeosporioides is a potent source of bioactive compounds. Further study is needed for identification of the bioactive compounds.

Keywords: Antibacterial, antioxidant, endophytes, *phlogacanthus thyrsiflorus Nees.*

Introduction

Plant microbiome can be a great field for the welfare of human society. Endophytes exist in close association with plants and can be of valuable resources. *Phlogacanthus thyrsiflorus* Nees is an evergreen shrub which belongs to Acanthaceae family. It is locally known as Nongmankha. Fresh shoot and leaf of this plant is cooked in water and the soup is taken for cough and fever. Inflorescence is eaten raw along with salad; plant part especially leaf are boiled in water and consumed the liquid in stomach ulcer, intestinal disorder and muscular sprain. Researchers have studied the diversity, metabolites and bioactivity of the endophytic fungi associated with various medicinal plants[1]. Endophytes can be defined as microbes residing inside healthy plant tissues without causing any negative impact on the host plants[2]. Endophytes are capable of synthesizing bioactive compounds useful for novel drug discovery [3]. Endophytes have been isolated from various plant that are investigated till now. Endophytic fungi have been described as a repository of varied novel compounds having application in many fields[4,5].

Endophytes have been reported to produce similar compounds to that of the host and it was confirmed when taxol was obtained from an endophytic fungi isolated from *Taxus brevifolia* [6]. The microbial resources of Manipur, particularly the endophytic populations, are mostly unexplored. Thus, an inititive to explore the endophytic fungi was carried out by selecting the medicinal plant *Phlogacanthus thyrsiflorus* Nees. In this work, bioactive endophytic fungus *Colletotrichum gloeosporioides* has been isolated and bioactivities were reported.

Material and Methods

Isolation of endophytic fungi: Samples from healthy living medicinal plant *Phlogacanthus thyrsiflorus* Nees in Imphal West used for isolation of endophytic fungi were collected. The leaf material was thoroughly washed in running tap water and sterile water, it was surface sterilized by immersion in 70% ethanol for 30 sec, 5% NaOCl for 3 min and 70% ethanol for 30 sec, followed by washing in sterile distilled water. The leaves samples were then cut into small fragments and dried on sterile blotting paper and were transferred onto PDA supplemented with antibiotic streptomycin (3mg/100ml) in petri plates and incubated at 28±2˚C until fungal growth was observed. The tips of the fungal hyphae were removed and transfered on PDA.

Cultivation of endophytic fungus: The fresh mycelia of selected endophytic fungus from medicinal host plants were inoculated in 500ml flask containing 200ml potato dextrose broth followed by incubation with a shaking incubator at 140 rpm for 30 days at 28°C. The endophytic fungus culture broth obtained was filtered by Whatmann Filter paper to remove mycelium.

Extraction of metabolites: Metabolite was extracted by using ethyl acetate as organic solvent. Equal volume of the filtrate and ethyl acetate was taken in a separating funnel and shaken vigorously for 10 min. The samples were extracted three times with ethyl acetate. Ethyl acetate collected after extraction was evaporated and the resultant compound was dried in vacuum evaporator using $MgSO_4$ to yield the crude metabolite. The brown coloured crude extract obtained after evaporation was then dissolved in Dimethyl sulphoxide (DMSO) until analysis[7].

Antibacterial assay: Ethyl acetate extracts from endophytic fungus was screened for antibacterial activity against human pathogenic bacteria by agar well diffusion method. Test Bacterial strain include *Staphylococcus aureus* (ATCC25923), *Enterococcus faecalis* (ATCC29212), *Escherichia coli* (ATCC25922) and *Pseudomonas aeruginosa* (ATCC27853). About 1ml of the inoculums of the test pathogen was spread into Muller Hinton Agar plates. A 5mm well was made in each corner of the plate with equal distance using a sterile cork borer. The ethyl acetate extract with different concentrations at 25, 50, 75 and 100µg compared with standard antibiotic were placed in their respective well and the plates were incubated at 37ºC for 48h. DMSO was used as a control. After the incubation, the inhibition zone around the well was recorded and expresses as millimetre.

Free radical scavenging activity: The free radical scavenging activity was assayed by DPPH method[8]. Free radical scavenging assay were performed to study the antioxidant potency of ethyl acetate extract of endophytic fungus. Sample (3mL at 0.025g/mL) was mixed with a DPPH solution (45µg/mL, Sigma) in HPLC grade methanol (Merck), vortexed well at room temperature and left standing exactly for 10 min. The UV/VIS absorbance was measured at 517 nm serving the methanol without DPPH solution as blank solution. A reference solution (125µg/mL) of Butylated hydroxyl toluene (BHT, Sigma) in methanol was used taking 100% radical scavenging activity. The scavenging percentage was calculated by the following equation:

$$\% \text{ Radical Scavenging activity} = (A_0 - A_5) \times 100 / (A_0 - A_5)$$

where, A_0, A_5 are the absorbance values of DPPH + Sample solution at 0.0 min and after 0.5 min, respectively; A_0, A_5 are the absorbance values of DPPH + BHT at 0.0 min and after 0.5 min.

Total phenolic content: Dilutions of Sample (0.5ml) were oxidised with 2.5mL Folin-Ciocateau for 5 minutes at room temperature. Then it was neutralized with 2mL Sodium Carbonate resulting in blue colour. The mixture was incubated for 2 hrs at room temperature and measured at 650nm with the spectrophotometer. Quantification was done on the basis of the standard curve of gallic acid. The entire test was carried out in triplicate. The results obtained were expressed as gallic acid equivalent (GAE), i.e., mg gallic acid/g of extract.

Statistical Analysis: In this study all the test were conducted in triplicates. The results obtained were calculated as mean ± standard deviation (SD). Coefficients of determination (R^2) were calculated using Microsoft Excel 2007.

Results and Discussion

Isolation and identification: *Colletotrichum gloeosporioides* : Identification of the endophytic fungus was done by observing conidia and mycelia , and was confirmed by rDNA sequencing. The colonization frequency was found to be 62%. Molecular characterization was carried out and it revealed that the fungus sequence showed 100% homology with *Colletotrichum gloeosporioides* from nucleotide database of NCBI. This sequence is submitted in genebank as *Colletotrichum gloeosporioides* strain NP13 and can be accessed with the Accession number KC662170.

Antimicrobial activity: The crude metabolites of the endophytic fungus isolated from the medicinal plant *Phlogacanthus thyrsiflorus* Nees displayed considerable antibacterial activity against the test pathogenic bacteria. The crude extract of *Colletotrichum gloeosporioides*, showed effective inhibition against the test bacterial strain. Antimicrobial activity against *Staphylococcus aureus* was highest with zone of inhibition 13.33±1.52mm, *Pseudomonas aeruginosa* (11.3±1.5mm). The result was not satisfactory against *Escherichia coli* and *Enterococcus faecalis.*

Free radical Scavenging activity: Free radical scavenging activity was measured by DPPH method. Using this method antioxidant capacity of the endophytic fungal isolate was assessed. The sample was found to possess scavenging property. The ethyl acetate extract of *Colletotrichum gloeosporioides* showed the scavenging property of 90%.

Total Phenolic content: Total Phenolic content was estimated by using Folin- Ciocalteu colorimetric method. Total content of phenolic in metabolites from *Colletotrichum gloeosporioides* is 18.3±0.36 mg gallic acid/g of extract.

Discussions: Endophytic fungi are reported as sources of bioactive compounds and secondary metabolites that has application in biological control[9]. Endophytes are thought to use chemical compounds to mediate interactions with other antagonists[10]. In this study *Colletotrichum* have been reported as endophyte and has been isolated from a wide host range[11-13]. *Colletotrichum gloeosporioides* is present as an endophytic fungus in *Artemisia Mongolic* and has been reported to produce a new antimicrobial metabolite, colletotric acid[14]. *Colletotrichum musae* and *C.gloeosporioides* are found as an endophytes in banana plant[15]. There is an report of metabolites extracted from the endophytic fungus *Colletotrichum* sp. that showed strong antimicrobial activity against various strain[16]. Recent reports have found that hundreds of natural products including alkaloids, flavonoids, and steroids, have been obtained from endophytes. The bioactive compounds isolated from

endophytes are known to have antibiotics, immunosuppressants, anticancer agents and biological control agents.

Colletotrichum gloeosporioides was isolated from *T. Mairei* as an endophyte[17]. Thirty-nine endophytic fungi identified as *Colletotrichum* spp. associated with Brazilian pepper tree or aroeira (*Schinus terebinthifolius* Raddi. Anacardiaceae) in Paraná state, Brazil[18]. Various novel compounds having the property of antibiotics, antimycotics, immunosuppressants, and anticancer compounds are isolated from endophytes [19,20] . *Colletotrichum gloeosporioides* from *Phlogacanthus thyrsiflorus* Nees can be a lead source for pharmaceutical industry.

Conclusion

In conclusion we can report that a fungal endophyte *Colletotrichum gloeosporioides* has been isolated from *Phlogacanthus thyrsiflorus* Nees which shows a good bioactivity. A further study can be performed to identifiy the bioactive compounds present in the extract. This study indicates that this plant supports endophytes with significant bioactive potential.

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