Evaluation of Antibacterial activity of Endophytic Fungi Isolated from Cordia *Macleodii* Medicinal Plant.

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Abstract- **The present study is based on the isolation and identification of endophytic fungi from common medicinal plants of satpura region of chhindwara of madhya pradesh and the screening of selective isolated endophytic fungi for their antimicrobial activity against pathogenic bacteria (*Escherichia coli* and *Staphylococcus aureus*). Nine species of endophytic fungi were identified from the selected plants. The ethanol extract of three endophytic fungi (*Fusarium sp.,Microsporium., Mucor ., Nigrospora spp., Aspergillus flavus., . Cladosporium spp., Alternaria sp., Curvularia lunata., and Aspergillus niger.,)* were selected for the screening of antimicrobial activity. Both the tested bacterial pathogens were found susceptible to the fungal extracts. Results showed significant increase in the inhibition zone diameter with increase in the concentration of fungal extract (100-400 µg/ml). Against *E. coli*, *Alternaria* sp. and *Fusarium* sp. were found to be more effective than *Aspergillus* sp., whereas against *S. aureus*, *Alternaria* sp. and *Fusarium* sp. showed almost similar activity. The *Aspergillus* sp. was observed with significant activity but lesser than the other eight fungal extracts. The study concludes the potential antimicrobial activity of the endophytic fungi against *E. coli* and *S. aureus* which could be due to bioactive secondary metabolites. Further study required the isolation of novel bioactive compounds for use in medicines.**

Index Terms- **Endophytic fungi, cordia *macleodii*, medicinal plants, antimicrobial activity, pathogenic bacteria, bioactive compound.**

I. INTRODUCTION

The Medicinal plants are known for their medicinal values since the ancient times. The medicinal properties in plants are due to some chemical substances, called secondary metabolites that when enter in the human body, produce a definite function. Researchers have now started to isolate these chemical substances from the entophytic fungi residing in the tissues of medicinal plants because, both medicinal plants and their endophytes are important source of discovery of natural products. Endophytic fungi are basically the group of fungi that colonize living internal tissues of plants without causing any immediate or harm effect except when the host is under stress conditions. Hirsh HV and Braun U *et.al*., (1992).

The medicinal plants Cordia *macleodii* (Boraginaceae) is called to have medicinal properties. The leaf, stem and root extracts of these medicinal plants are used ethanopharmaceutically for their medicinal properties viz., antioxidant, anti-inflammatory, wound heeling and anti microbial activity Martin, P. *et. al.,* (1997). Therefore, present study is based on the isolation and identification of endophytic fungi from some common medicinal plants and the screening of selective endophytic fungi for their antimicrobial activity. The investigations of the antimicrobial activity of natural products have opened new ways for drug development in the control of antibiotic resistant pathogens. Therefore, initial objectives of this work are to isolate and identify the endophytic fungi from medicinal plants Cordia *macleodii* (Boraginaceae).

The benefit of symbiotic relationship for the endophyte is that the host plant is able to supply the necessary nutrients and compounds required for the endophyte to complete its life cycle and unlike the host plant, many endophytes are able to survive under quite extreme and inhospitable condition. They play an essential role to provide protection to their host against attack by other pathogen and environmental condition. They can co-evolve with plant host and possess species-specific interaction.

Approximately, one million species of entophytic fungi residing in plants Dreyfuss MM and Chapela

IH. *et.al.,*(1994). Over the last decade, numerous studies have been done on fungal endophytes of medicinal plant. Tan RX and Zou WX. e*t.al*.,(2001). In addition, endophytes have also been investigated in search for new secondary metabolites. Schulz B and Boyle C. *et.al.*(2005).

II. METHODOLOGY

Collection of Plant Samples

The healthy plant parts (Leaves, stem, root) of the plant Cordia *macleodii*, were collected from labhaghogri and sawari forest area of chhindwara district , Madhya Pradesh. The collected plant parts were kept in sterile bags and brought to the laboratory. and processed immediately to reduce the chances of contamination.

Surface Sterilization of Plant Materials

The healthy plant segments were cleaned completely in running tap water to remove the dust particles. Then the segments were subjected to sequential immersion in surface sterilization agents such as 70% ethanol and 5% sodium hypochlorite solution for 2 minutes to remove the surface contaminants. Again it was washed thrice in sterilized distilled water to remove the sterilization agents Schulz. *et. al.,* (1993). Then all the segments were blotted and dried using sterile blotting paper.

Isolation of endophytic fungi

The endophytic fungi were isolated by imprint technique Petrini, *et. al.,* (1986). The sterilized plant segments were cut into small sections and Then dried leaf, stem and root pieces were placed on agar media supplemented with chloramphenicol 150 mg/l. to suppress the bacterial growth. All plates were placed in BOD at 27º incubated until the fungal growth appeared. The unsterilized plant segments and the finally rinsed distilled water were placed on the agar plates as a control to check for surface contaminated fungi. The plates were observed once a day for the growth of endophytic fungi. The fungi that grown out from the tissues was isolated and stocked. The cultures were maintained on PDA slants at 4°C for further screening Rajeswari, *et. al.,* (2016).

Pure culturing:

The plant discs were observed within few days for the growth of endophytic fungi. Hyphal tips growing out the plated discs were immediately transferred into potato dextrose agar for making pure culturing. The fungal isolates were identified based on their morphological characters. Purification was done by transferring the hyphal tip to the fresh PDA plates. Continuous sub culturing was done to maintain the culture. Ibrahim, *et. al.* (2017).

Identification of endophytic fungi:

The Endophytic fungal isolates were identified up to genus level based on the morphological features such as colony morphology, pigmentation, growth pattern, spore structures, and other hyphal characteristics with the help of the standard mycological manuals. For the identification of endophytic fungi, small thin hyphae of unknown endophytic fungi were isolated through forceps from fungal petriplate (pure culture) and kept on glass slide. The thin hyphae of fungus treated with cotton blue stain. Then fungal hyphae or mycelium structure observed under the microscope in 10X and then 40X. Morphological features of fungus were recorded and photograph were taken using camera Lucida. Further identification of fungi was done on the basis of morphological structure using standard identification manuals and previously reported literature. Subramanian CV.*et.al.*(1971).

Mass cultivation of endophytic fungi:

Mass cultivation of selected endophytic fungi was done by following the method of previous study on fungal endophytes. Karunai Selvi B.*et.al.,* (2014). Fungal endophytes were mass cultivated on PDA broth by placing agar blocks of actively growing pure culture (3mm in diameter) in 250 ml Erlenmeyer flask containing 100 ml of the medium. The flasks were incubated at room temperature for 3 weeks with periodical shaking at 150 rpm. After the incubation period, the cultures were taken out and filtered through sterile cheesecloth to remove the mycelial mats. Mycelia mats were dried and used for antimicrobial activity.

Evaluation of antimicrobial activity of endophytic fungi:

Extraction of metabolites from endophytic fungi:

Ethanol solvent systems was used for metabolites extraction. Three species of fungi isolated from *Cordia macleodii* were extracted with ethanol. The solvent was taken in a separating funnel and shaken vigorously with dried mycelia mats. The solution was then allowed to stand, the cell mass got separated and the solvent so obtained, was collected.

Solvent was evaporated on hot water bath to yield the crude extract. Raviraja NS. *et.al.*(2006).

Selection of test organisms:
The bacterial culture of most common human pathogens (*Escherichia coli*, *Staphylococcus aureus*) were used to evaluate the antimicrobial activity of endophytic crude extracts. Both test pathogens were isolated from wound of patient. The selected microbial cultures were maintained using sub culturing techniques. Nutrient agar slants were used for bacterial culture maintenance. Bacterial cultures after 24 hours' incubation period at 37º c were kept in refrigerator.

Evaluation of antimicrobial activity of endophytic fungi:
Antimicrobial activity against selected gram negative and positive bacteria was evaluated by following the method of Wang. Wang FW. *et.al.*(2007). The crude extract was dissolved in dimethyl sulphoxide (DMSO) for antimicrobial bioassay. 1 ml of DMSO was taken and 20 mg weight of crude was dissolved for stock preparation. 5-20 µl of fungal extract containing 100-400 µg/ml was taken from stock solution on sterilized whatman filter paper discs. Total five discs were placed on nutrient broth media on a Petri plate. One disc has Chloramphenicol of 20 µl (5mg/ml) was used as positive control.

The magnitude of antimicrobial activity was assessed by the diameter of inhibition zones relative to those of positive and negative controls. The zone of inhibition was measured and compared with the control. Three replicates were maintained in each case. The plates were incubated at 37° for 24 h. After incubation the diameter of the clear zone was measured, the averages were calculated.

III. RESULTS

Isolation and identification of endophytic fungi:
Endophytic fungi was isolated from the medicinal plant leaves (sterilized). It took 7-15 days of time period for the growth of fungi from the discs of sterilized leaves. For checking whether it is endophytic or epiphytic fungi the procedure was repeated 3 times. For the identification of endophytic fungi the first attempt involved comparing their features as exhibited in culture with those of known species of fungi. By measuring morphological character nine fungi were identified (Table 1). *Alternaria* sp. , *Aspergillus* sp*., Fusarium* sp., *Mucor*, *Nigrospora spp. Cladosporium spp. Curvularia lunata, Microsporium spp.* The characteristics features of all the isolated fungi are shown in Table 1.

Antimicrobial activity of endophytic fungi:
Different concentration of fungal extracts were used for antimicrobial activity assay. Both the tested bacterial pathogens were found susceptible to the fungal extracts (Table 2 and Fig. 3). There was significant increase in the inhibition zone diameter with increase in the concentration of fungal extract (100-400 µg/ml). Against *E. coli*, *Alternaria* sp. and *Fusarium* sp. were found to be more effective than *Aspergillus* sp., whereas against *S. aureus*, *Alternaria* sp. and *Fusarium* sp. showed almost similar activity. The *Aspergillus* sp. was observed with significant activity but lesser than the other two fungal extracts.

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IV. DISCUSSION

Endophytic fungi has ubiquitous nature and found to be associated with the inner tissues of the plants. These fungi positively interact with its environment/plant and benefits its host in various ways. As endophytic fungi have many important properties and one of them is to protect the plant species against harmful pathogens by producing potential metabolites like alkaloids, terpenoids, quinones, lignins, phenols etc. Tan RX and Zou WX.*et.al.,*(2001). Hence, to fulfill the needs of agriculture and pharmaceutical industries a large

scale production of these bioactive compounds must be necessary.From the last few decades, endophytic fungi have been investigated in search of potential antibacterial activity. For example, endophytic fungi (*Phomopsis,Alternaria*, *Colletotrichum* and *Nigrospo ra*) isolated from the leaf tissues of *Tectona grandis* and *Samanea saman* have shown antimicrobial potential against pathogenic bacteria. Sukanyanee C, *et.al.*(2006). Similarly, endophytic fungi isolated from five *Garcinia* plants have also verified the antimicrobial potential of metabolites of 70 fungal isolates against *Staphylococcus aureus, Candida albicans, Cryptococcus neoformans* and *Microsporum gypseu* bacteria. Marcellano JP. *et.al.,*(2017). According to authors some endophytic fungal genera like *Aspergillus, Botryosphaeria, Eutypella, Fusarium, Guignardia, Penicillium , Phomopsis* and *Xylaria* have greater antibacterial activity than others.

In the present study, Eight fungal species (*Alternaria* sp., *Aspergillus* sp., *Fusarium* sp.*,Mucor., Nigrospora* sp*., Cladosporium* sp*., Curvularia lunata,., and Microsporium*) were isolated from the studied medicinal plant species (Table 1). Among all extracted species, Eight fungal species (*Alternaria* sp., *Aspergillus* sp., *Fusarium* sp.*,Mucor., Nigrospora* sp*., Cladosporium* sp*., Curvularia lunata,., and Microsporium*) were selected for identification of antimicrobial activity against *E. coli* (gram negative bacteria) and *S. aureus* (gram positive bacteria). The ethanolic extract of selected fungal species have shown successful activity against the pathogenic bacterial species (Fig. 3 & Table 2). The inhibition diameter increased with increasing concentration of fungal extract (100-400µg/ml) and results showed that *Aspergillus* sp. , and *Alternaria* sp. were more effective than other against *S. aureus* whereas against *E. coli* similar antimicrobial activity was observed by *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp. were more effective than other .

Present study observed a wide range of host adaptability of *Alternaria,, Aspergillus* sp. *Fusarium*, in medicinal plants with potential antimicrobial activity against human pathogenic bacteria. They also observed the role of *Aspergillus* and *Alternaria* fungi in antimicrobial activities against *E. coli* and *S. aureus* and *Fusarium* against *S. aureus, B. cereus* and *E. aerogenes.* Zhang et al. also observed the antimicrobial activity of *Fusarium* sp. against gram positive (*S. aureus*) and gram negative (*E. coli*) bacteria and suggested its role in antimicrobial potential. Zhang H.*et.al,* (2016).

Eight endophytic fungi (*Aspergillus fumigatus*, *A. niger*, *A. repens*, *A. alternata*, *Alternaria* sp., *Phoma hedericola, Fusarium solani* and *F. oxysporum*) were isolated from *Mentha viridis* and shown their antibacterial potential against *E. coli* bacteria . Kumar S, *et.al.,*.(2016). Most of research on endophytic fungi belong to *Aspergillus, Fusarium* and *Alternaria* genus and showed good antibacterial activity. Rani R, e*t.al,* (2017).

The study concluded that endophytic fungi (*Fuserium sp.*, *Aspergillus sp.* and *Alterneria sp.*) isolated from the studied medicinal plants have potential antibacterial activity against *E. coli* and *S. aureus* which may be due to bioactive secondary metabolites. The present study serve as preliminary work and further study is required for the isolation and identification of responsible chemical compounds having potent antimicrobial activity from endophytic fungi.

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