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ORIGINAL ARTICLE **OPEN ACCESS**

Identification and Antibacterial Screening of Endophytic Fungi Isolated from *Pongamia pinnata* (Karanj)

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ABSTRACT
Endophytes include a family of microorganisms that grows intra and intercellular tissues of higher plants without causing any symptoms in host plants. The main aspire of this research work was to isolate and screening of antibacterial activity of endophytic fungi against pathogenic bacteria. Because endophytic microorganisms produce a huge amount of secondary bioactive compounds which are similar in chemical composition as secreted by host plant and have tendency to fight against a number of diseases. In present study, a total 6 endophytic fungi were isolated from medicinal plants Pongamia pinnata (Karanj) and observed their antibacterial activity by agar well diffusion method against 4 test bacterial strains such as Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis and Staphylococcus aureus.The present investigation is an effort to search antibacterial bioactive compounds from endophytic fungi. The fungal strain Cladosporium sp. isolated from Pongamia pinnata was showed maximum zone of inhibition against all pathogenic bacteria.
Keywords: *Antibacterial activity, Anti-cancer, Anti-diabetes, Anti-oxidant, Endophytic fungi (Myco-endophyte), Immuno-suppressive.*

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INTRODUCTION

The word "Endophyte" was given by a German scientist Heinrich Anton De Bary, which means the fungi or bacteria found inside the different parts of plant tissues without causing any apparent symptoms in the host. Research on endophytes is rapidly increasing in recent, due to production of plethora of metabolites bearing therapeutic properties. Generally, all types of microorganisms have been found in endophytic association with Plants such as fungi, bacteria or actinomycetes [1, 2].

These endophytes guard their hosts from infectious organisms and unfavorable state by producing secondary metabolites [3]. These are not host specific there are different types of endophytic fungi can be isolated from the various parts of the same plant. Endophytic fungi serve as a possible source of natural products for development of different type of drugs in modern medicine, agriculture and in industries. Niranjana Devi *et al*.,[4] isolated the endophytic fungi *Peniclillium* sp. from *Camellia sinesis.* The fungal culture was extracted with ethyl acetate and detects the antibacterial activity against *Pseudomonas* sp.Similarly, Sandhu *et al.* [5] isolated 7 endophytic fungi from *Saraca indica* collected from Dumna forest regions of Jabalpur M.P. (India) and observed their antibacterial activity against six pathogenic bacteria like *Bacillus subtilis, Klebsiella pneumonia, Escherchia coli,S. aureus* and *Enterococcus* sp. The *Phomopsis* sp. is also affluent source of biologically active secondary metabolites with their antimicrobial activity [6].

In other study Santos et al., [7, 8] isolated the 65 endophytic fungi from the leaves of the healthy plant *Indigofera suffruticosa* Miller, and among them only 18 fungi especially *Nigrosporasphaerica* and *Pestalotiopsis maculans* give the best activity against test organisms. The endophytic fungi *Taxomyces andreanae* isolated from the plant *Taxus brevifolia* producing Taxol which is used in the treatment of cancer give the alternative approach to obtain easily cheaper product via microorganism fermentation. Some other fungi like *Taxodium distichum Wollemia nobilis, Phyllosticta spinarum, Bartaliniarobillardoides, Pestalotiopsis terminaliae* and *Botryodiaplodia theobromae* have a capacity for production of Taxol [9, 10, 11, 12, 13]. Therefore, the aim of the present study was to explore biotechnological potential of fungal endophytes isolated from *Pongamia pinnata* (Karanj) that have been examine against four pathogenic bacteria.

MATERIAL AND METHODS

Plant material

For isolation of endophytic fungi from different parts of the host plant *Pongamia pinnata* (Karanj) were collected from the Dumna Nature Reserve Park in Jabalpur district of Madhya Pradesh (India). Healthy and mature plant was carefully chosen for sampling. The plant parts were bring to the laboratory in sterilized bags and processed within a few hours after sampling.

Surface sterilization

The different parts of the plant (leaves stem and root) were washed with tap water for 1 to 2 hours for removal of microorganisms and dust debris on the surface of the sample. After that the plant sample leaves, root, stem was cut into small pieces with the help of sterilized blade then surface sterilized by 70% ethanol for 1minute immediately immersed in 4%sodium hypochlorite for 30 seconds and then rinsed in distilled water for 1 minutes. The excess of water adhere with plant sample was blotted in sterilized Whatman filter paper.

Isolation of endophytic fungi

The isolation of endophytic fungi from the host plant was done by using the standard protocol of Petrini [14].Surface sterilized of 4 segment of the plant sample were put on the Potato Dextrose Agar plates having streptomycin for inhibit the growth of bacteria. These plates were incubated in a fungal incubator at 26±1°C for 5 to 7 days. The Petri-dishes were observed every day to make sure that the growth of endophytic fungal colonies. The fungal isolate obtained were transferred on agar slants having PDA media for preservation and stored at 4°C for further studies. The isolated endophytic fungal strains were identified on the basis of their morphological characteristics at species and genus level by using standard procedure [15, 16].

Data analysis

The colonization frequency (CF %) of each endophytic fungi was calculated as described by Suryanarayanan *et al*., [17] and Hata *et al*.,[18].

$$\text{Colonizing frequency \%} = \frac{\text{Number of segments colonized by fungi}}{\text{Total number of segments observed}}$$

Test bacterial strains

The pathogenic bacteria strain such as *Escherichia coli, Klebsiella pneumonia, Bacillus subtilis* and *Streptococcus aureus* were used for detection of antibacterial potential of secondary metabolites secreted by endophytic fungi. The bacterial cultures were received from the laboratory of Bio Design Innovation Centre, Ekatm Bhawan, Rani Durgavati University Jabalpur (M.P.) India.

Extraction of bioactive compounds

The endophytic fungi were grown in Potato Dextrose broth and incubated at 26±1°C in the incubator. After 7, 14 and 21 days of incubation the crude culture broth was filtered by using Whatman filter paper1 and observe the antibacterial activity against the four pathogenic bacteria by using Agar Well Diffusion method [19].

Antibacterial activity

The crude secondary metabolites of the endophytic fungi were tested for their antibacterial activity against *Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis* and *Streptococcus aureus.* Antibacterial activity of the metabolites was confirmed by using Agar well diffusion method. The nutrient agar plates were seeded with 25 µl of bacterial suspension. Wells were made aseptically in the seeded media using sterile cork borer and appropriate amount of the bioactive metabolite were pipette (80µl) into sterile well and incubated at 37°C in bacteriological incubator for 24 hrs. Finally, plates were observed for zones of inhibition and their diameter was measured with the help of Hi-Antibiotic zone scale, Hi Media.

RESULTS AND DISCUSSIONS

Isolation of endophytic fungi

In the present study a total 6 endophytic fungi were isolated from the different parts of the plant *Pongamia pinnata* (Karanj) as depicted in table no.1. A 24 segment (leaves 8, root8 and stem 6) of the plant were processed for isolation of fungi. All isolated endophytic fungi were belonging to Eusacomycetes, Dothideomycetes and Eurotiomycetes. Similarly, Sandhu et al.,[20] isolated 10 endophytic fungi from the different parts of the plant *Ricinus communis* and identified as *Aspergillus fumigatus, Aspergillus japonicas, Aspergillus niger, Fusarium semitectum, Curvularia pallescens, Phomahedericola, Alternaria tenuissima, Fusarium solani, Drechsleraaustralien* and *Aspergillus repens.* In other studyLiang et al., [21] also isolated the 65 fungal species from the *Indigofera suffruticosa* Miller.

Table 1: Endophytic fungi isolated from different parts of *Pongamia pinnata*

S. No.	Plant parts	Name of Endophytic fungi	Class
1.	Leaf	*Curvullaria*sp.	Euacsomycetes
2.	Leaf	*Alternaria alternata*	Dothideomycetes
3.	Leaf	*Cladosporium* sp.	Dothideomycetes
4.	Stem	*Aspergillus fumigatus*	Eurotiomycetes
5.	Stem	*Sterile mycelia*	-----
6.	Root	*Aspergillus niger*	Eurotiomycetes

Table 2. Colonization frequency (%) of the endophytic fungi

S. No.	Plant parts	Name of endophytic fungi	No. of isolates	(%) Frequency of colonization
1.	Leaf	*Curvullaria*sp.	3	37.50 %
2.	Leaf	*Alternaria alternata*	4	50.00 %
3.	Leaf	*Cladosporium* sp.	2	25.00 %
4.	Stem	*Aspergillus fumigatus*	2	25.00 %
5.	Stem	*Sterile mycellia*	4	50.00 %
6.	Root	*Aspergillus niger*	5	83.33 %

Identification of endophytic fungi

Identification of these fungal strains was made by using standard protocol of Barnett and Hunter[22] and Aggarwal and Hasija[16] on the basis of their cultural and microscopic properties these fungi show different characteristics. Morphological identification was done according to color, texture, colony diameter and morphology of hyphae and conidia. These fungi were successfully identified as *Curvullaria*sp., *Cladosporium* sp., *Alternaria alternate, Aspergillus fumigatus Aspergillus niger*and one species was not identified as sterile mycelia. Sandhu et al.,[23] also isolated different types of endophytic fungi from *Calotropis procera* (Linn.) R.BR. like *Fusarium solani, Cladosporium herbarum, Curvularia pallescens, Alternaria alternata* and *Drechsleranodulosa.*

Screening of endophytic fungi for antibacterial activity

Antibacterial activity was carried out by Agar Well Diffusion method of crude extract of isolated endophytic fungi against four pathogenic bacteria after 7, 14 and 21 days. Most of the fungi give utmost zone of inhibition after 14 days of but least activity was measured in 7 and 21 days of incubation as shown in figure and table. The endophytic fungi *Cladosporium* sp. showed the highest zone of inhibition against *Klebsiella pneumoniae* (21.90mm), *Streptococcus aureus*(13.83mm), *E. coli* (20.90mm)and *Bacillus subtilis* (21.80mm). Similarly, *Curvularia*sp.give the maximum zone of inhibition against *Klebsiella pneumoniae* (15.76mm), *Streptococcus aureus*(10.76mm), *E. coli* (15.86mm), and*Bacillus subtilis* (16.63mm). The other fungal strain like *Alternaria alternate, Aspergillus fumigatus*and *Aspergillus niger*was not give any satisfactory zone of inhibition against the pathogenic bacteria as shown in Table 3 and Figure 1.

Two metabolites as Altersolanol A and G-hydroxy- 6-methyl produce by *Phoma*sp. isolated from *Taxus wallichinia* show anti-microbial activity[24]. Maria et al.,[25] isolated endophytic fungi from *Acanthus ilicifolius* and *Acrostichum aureum* of Southwest Coast of India and observed their anti-microbial property against *Bacillus subtilis, Enterococcus* sp., *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus*. In further study, Sandhu et al., [26,27]observed the antibacterial activity of crude extract of endophytic fungi isolated from *Bombex ceiba*.

Table 3: Antibacterial activity of endophytic fungal isolates

Fungal isolates	Zone of Inhibition (mm)			
	K. pneuminiae	***E.coli***	***B.subtilis***	**S. aureus**
*Curvularia*sp.	15.76	15.86	16.63	10.76
Cladosporium sp.	21.90	20.90	21.80	13.83
Alternaria alternata	10.06	11.06	13.33	7.76
Aspergillus fumigatus	11.56	15.76	17.7	9.73
Sterile mycellia	00.00	10.33	6.76	5.56
Aspergillus niger	11.10	11.70	15.40	00.00

*Antibacterial activity was expressed in terms of diameter of zone of inhibition (mean ± SD, n=3)

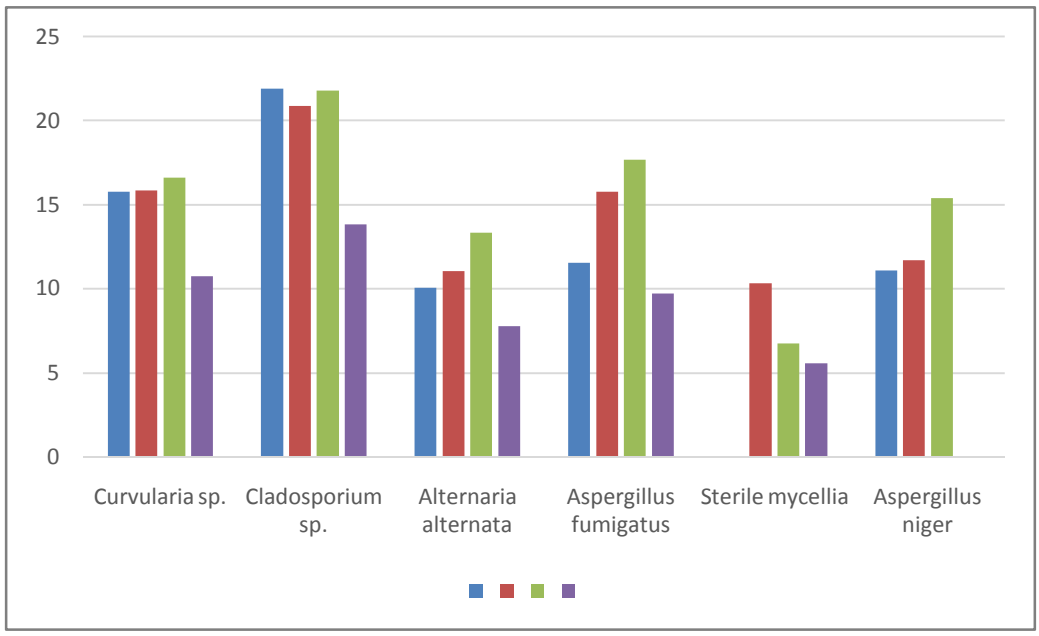
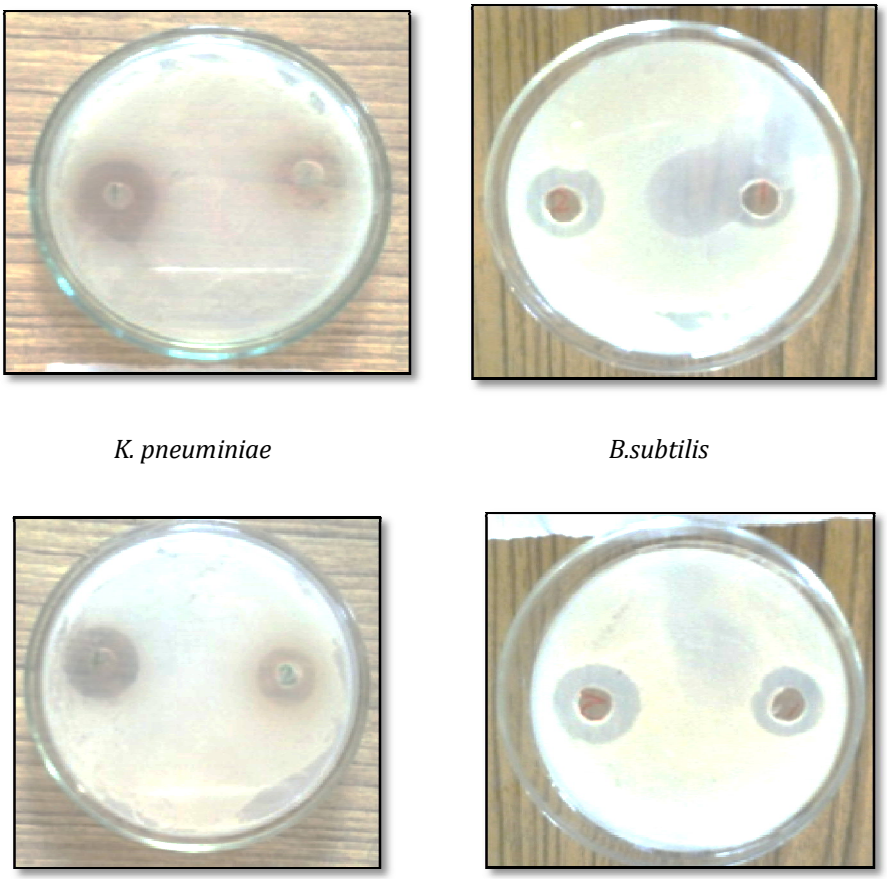


Figure 1. Antibacterial activity of fungal extract isolated from *Pongamia pinnata* against fourPathogenic Bacteria



K. pneuminiae *B.subtilis*

Figure 2. Zone of inhibition of fungal bioactive compounds against pathogenic bacteria strain

CONCLUSION

Endophytic fungi are potential producers of novel bioactive compounds which can be used in pharmaceutical, agricultural and in industries for human well-being. In recent, research precedence has

increased towards endophytes due to their effortless availability and vast production of novel bioactive compounds which fight against a number of pathogenic organisms. In the present study, a total 6 endophytic fungi were isolatedfrom *Pongamia pinnata* and detect their antibacterial activity against five pathogenic bacteria. The endophytic fungi *Curvularia*sp. showed the good zone of inhibition against *Klebsiella pneumoniae* (21.90mm), *Streptococcus aureus*(13.83mm), *E. coli* (20.90mm), and *Bacillus subtilis* (21.80mm). But there is a much more work is needed to recognize the physiology, secondary metabolite production, defensive role and very essential to study these microorganisms at molecular level for better understanding the metabolic pathway that are responsible for the synthesis of various metabolites

CONFLICT OF INTEREST

There is no potential conflict of interest with reference to the current manuscript. All the authors have read the manuscript and agreed to submit the same for publication.

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