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European Journal of Experimental Biology, 2014, 4(2):35-43

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Antimicrobial activity and phytochemical analysis of crude extracts of endophytic fungi isolated from *Plumeria acuminata* L. and *Plumeria obtusifolia* L.

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ABSTRACT

Endophytes are the chemical synthesizers inside plants and have been extensively investigated for their endophytic microbial complement. Different parts of Plumeria acuminata and Plumeria obtusifolia were subjected to the isolation of endophytic fungi and identified morphologically and also screened for antimicrobial activity against Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhimurium and Candida albicans. The identification of the prospective endophytic fungi Colletotrichum gloeosporioides (PAS-1) and Fusarium oxysporum (POS-3) was confirmed by molecular technique of 18s rDNA analysis and were selected for the production of secondary metabolites which were grown in rice medium and extracted with ethyl acetate and were screened for their antimicrobial activity by agar well diffusion method and Phytochemical analysis. The extract of C. gloeosporioides gave effective inhibition to the tested organisms except C. albicans; whereas extract of F. oxysporum gave effective inhibition against tested organisms except P. aeruginosa. Phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, steroids, phenol and phenolic compounds.

Key words: *Plumeria acuminata, Plumeria obtusifolia,* Endophytic fungi, antimicrobial, Phytochemicals.

INTRODUCTION

There is a steady demand and need for new antimicrobial agents as infectious diseases are still a worldwide problem and the development of resistance by the pathogens is a growing concern [1, 2]. The problem extends beyond the clinical application of antimicrobial drugs and many microorganisms of agricultural concern are also known to have acquired resistance to commonly used antimicrobial chemicals [3], which indicates an increasing want for new bioactive compounds. Historically, a majority of the compounds have been isolated from the natural environment, particularly plants, and have been used in the treatment of many diseases and illnesses. Many of the drugs available commercially are derived from these natural products and have become potential drug sources [4]. While plants have been a major source of new lead compounds for drug discovery, attention has more recently turned to endophytes as these microorganisms are seen as having great potential as sources for new bioactive compounds [5]. Thus, there is a growing need for new, environmentally-friendly antimicrobial agents that may be used safely in agriculture [6].

Endophytic fungi, which colonize plants internally without apparent adverse effects, occur ubiquitously in plants [7] and do not have pathogenic effects on its hosts [5]. They produce a number of compounds which can inhibit pathogens. They are relatively not much explored and offer potential sources of novel natural products for exploitation in medicine, agriculture and the pharmaceutical industry [8].

Plumeria acuminata and *Plumeria obtusifolia* familiarly known as temple tree and jasmine tree belong to the family *Apocynaceae* are widely distributed throughout India. Traditionally, different parts of these plants have been useful in the treatment of a variety of diseases [9]. The plant material is widely used as a purgative, remedy for diarrhea and cure for itch. The milky juice is employed for the treatment of inflammation and rheumatism [9, 10].

Plumeria species contain abundant amounts of phytoconstituents comprising of iridiods, tannins, alkaloids, plumieride, fulvoplumierin plumieric-acid, lupeol-acetate, oxymethyldioxycinnamic-acid, lupeol, acetyl-lupeol, alpha-amyrin, beta-amyrin, caoutchouc, cerotic-acid Plumericine, isoplumericine, beta-dihydroplumericic-acid, Quercetin, quercetin-glycoside, phenyl-ethyl-alcohol, kaempferol, l-(+)-bornesitol, linalol, citronellol, farnesol, geraniol [11].

Many endophytic fungi have been identified from different plants belonging to *Apocynaceae* have shown antimicrobial properties [12, 13]. The endophytic fungus *Phomopsis sp*. isolated from the leaves of *Plumeria acutifolia* gave strong antibacterial activity [14]. The present study is aimed at the isolation and identification of endophytic fungi from *P. acuminata* and *P. obtusifolia*, screening for their antimicrobial activity, production and extraction of antimicrobial metabolites and analysis of different phytochemicals.

MATERIALS AND METHODS

2.1. Sample collection for endophytic fungi:

Different plant parts of *P. acuminata and P. obtusifolia* collected from Dhanvantri vana located in Mariyappanapalya, Department of Forestry, Government of Karnataka, Bangalore, Plant samples were excised using a sterile knife and brought to the laboratory in sterile polythene bags. Herbarium of plant samples were prepared and deposited in Institute of Ayurveda and Integrative Medicine (FRLHT), No. 74/2, Jarakabande kaval, Attur (Post) Via Yelahanka, Bangalore- 560064 with the accession FRLHT Coll. No. 74064 and 74065 for *P. acuminata and P. obtusifolia* respectively.

2.2. Isolation of endophytic fungi:

Different parts of fresh healthy *P. acuminata and P. obtusifolia* plants were cut into small pieces (5 mm × 2 mm) using sterile blade and washed with sterile distilled water. The samples were surface sterilized by dipping into 2% sodium hypochlorite for 60 seconds and 70% ethanol for 5 seconds, rinsed with sterile water and allowed to surface-dry under sterile conditions [14]. The surface sterilized samples were placed on Potato Dextrose Agar (PDA) plates amended with 50 mg/L tetracycline to suppress the bacterial growth and incubated at 28ºC to 30ºC for 2 to 3 days. The hyphal tip of endophytic fungi growing out from the plant tissue was transferred to fresh PDA plates amended with 50 mg/L tetracycline. After incubation at 30ºC for 7 to 14 days, purity of the culture was determined by colony morphology. Colonization rate (CR) was expressed as percentage of total number of isolates obtained from different tissue segments divided by total number of isolates obtained from overall tissue segments incubated. Isolation rate (IR) was calculated as number of isolates obtained from segments divided by total number of segments [15].

2.3. Identification of endophytic fungi:

2.3.1. Morphological identification:

The endophytic fungi were identified based on the cultural characteristics, morphology of the fruiting bodies and spores, using standard manuals [16, 17, 18].

2.3.2. Molecular identification:

The potential endophytic fungi were grown in 50 ml Potato Dextrose Broth (PDB) for 5-6 days at 28º C. The mycelia was harvested and washed with distilled water and ground with liquid nitrogen. The nucleic acid was extracted using the cetyl Trimethyl Ammonium Bromide (cTAB) method [19]. Polymerase chain reaction was carried out using universal primers ITS1 and ITS4 [20]. The PCR products were visualized in 0.8% agarose gel using ethidium bromide and UV trans illuminator. Prior to sequencing, amplicons were cleaned using GenElute™ PCR clean-up kit according to the manufacturer's instructions and sequencing was carried out in an ABI automated

DNA sequencer. The sequencing PCR was set up using ABI-BigDye® Terminatorv3.1 Cycle Sequencing Kit. BLAST analysis was carried out in the NCBI database and sequences are submitted to NCBI.

2.4. Screening the Antimicrobial activity of endophytic fungi:
Endophytic fungi isolated from medicinal plants of *P. acuminata and P. obtusifolia* were subjected to screening for antimicrobial activity against the human pathogenic bacteria *Staphylococcus aureus* (NCIM No. 2079), *Bacillus cereus*(NCIM No. 2106), *Pseudomonas aeruginosa* (NCIM No. 2200), *Escherichia coli* (NCIM No. 2256), *Salmonella typhimurium* (NCIM No. 2501) and yeast *Candida albicans* (NCIM No. 3471)

2.4.1. Agar Plug method:
Cylindrical pieces were cut out from well grown culture of the endophytic fungi strain on PDA. The blocks were placed on the Petri dishes inoculated with a fixed amount of test-microorganisms grown on Nutrient agar medium (NA) for bacteria and Sabouraud Dextrose Agar medium (SDA) for yeast (10^6 cells/ml). The cultures were kept for 12 hours at 2 - 8 °C for the diffusion of antimicrobial substance and thereafter they were incubated for 24 hours at 37ºC for bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *E. coli*, *Salmonella typhi*); 48 hours at 28ºC for yeast(*Candida albicans*). The antimicrobial activity was measured in millimeter (mm) as clear zone of inhibition [21].

2.5. Production and extraction of secondary metabolites:
Endophytic fungi exhibiting broad spectrum of antimicrobial activity were subjected for the production of secondary metabolites. The fresh mycelia of endophytic fungi were grown on PDA plates at 28±2 ºC for 3-6 days and were inoculated into 1000 ml flasks containing 200gms of unpolished rice, soaked with 200 ml distilled water (autoclaved twice at 121 ºC for 20 min), followed by incubation for 30 days at 28±2 ºC [22]. The incubated flasks were filled with 300ml of ethyl acetate and allowed to stand for one day, shaken thoroughly and filtered. The above procedure was repeated until most of the metabolites were extracted. The ethyl acetate filtrate was extracted with pure distilled water to remove debris and other particles. Finally ethyl acetate extract was treated with anhydrous sodium sulphate to remove the moisture content and dried under rotary evaporator [23].

2.6. Antimicrobial activity of crude extract of the endophytic fungi by well diffusion method:
The extracted secondary metabolite was dissolved in DMSO at different concentrations of 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml; poured into the 5mm diameter well made in Petri dishes containing NA for bacteria, SDA for yeast and inoculated with a fixed amount of test-microorganisms (10^6 cells/ml). The cultures were kept for 12 hours at 2- 8 °C for the antimicrobial metabolite diffusion and thereafter they were incubated at an appropriate temperature for the growth of test-microorganisms. The zone of inhibition was measured in mm. [24].

2.7. Phytochemical analysis:
The ethyl acetate extract of the potent endophytic fungi were subjected to chemical constituent analysis [25, 26]. The test details as follows:

2.7.1. Test for Alkoloids:
A drop of ethyl acetate extract was spotted on the pre coated TLC plate followed by spraying with Dragendorffs reagent. The orange red or brown coloration indicates presence of alkaloids.

2.7.2. Test for Steroids and Terpenoids:
One ml of ethyl acetate extract, 1ml of chloroform, 2-3 ml of acetic anhydride and 1 to 2 drops of concentrated sulfuric acid were added. The dark green coloration of solution indicates the presence of steroids and dark pink or red coloration of solution indicates presence of terpenoids.

2.7.3. Test for Phenols and phenolic compounds:
A drop of ethyl acetate extract was spotted initially on a filter paper followed by phosphomolybdic acid spot. The blue coloration indicates presence of phenols and phenolic compounds.

2.7.4. Test for Tannins:
Two to three ml of ethyl acetate extract, 10% alcoholic ferric chloride solution was added. A dark blue or greenish grey coloration of solution indicate the presence of tannins.

2.7.5. Test for Flavonoids:
Two to three ml of ethyl acetate extract, a piece of magnesium strips and 1ml of concentrated hydrochloric acid were added. A pink red or red coloration of solution indicate the presence of flavonoids in the extract.

2.7.6. Test for the presence of Amino acids:
Ninhydrin test: To the extract, 0.25% Ninhydrin reagent was added and boiled for few a minutes. Formation of blue color indicates the presence of amino acid.

2.8. Statistical analysis
Analysis of variance (ANOVA) was used to determine the significance of difference between treatment groups ($p < 0.05$). Means between treatment groups were compared for significance using Duncan's new Multiple Range post test.

RESULTS AND DISCUSSION

3.1. Isolation of endophytic fungi:
Endophytic fungal isolates were grouped as different morpho-types based on morphological characteristics like color, mycelium growth on PDA and the presence of reproductive structures. The total 24 different endophytic fungal isolates were obtained from *P. obtusifolia* whereas in *P. acuminata*, 17 isolates were obtained. The CR of endophytic fungi of *P. obtusifolia*, in leaf (54.17%) was higher than that of stem (25%) and least in flower (20.83%) segments, whereas in *P. acuminata*, CR of stem (41.7%) was higher than that of leaf (35.29%), and least in flower (23.52%) segments (Fig 1). The IR value of endophytic fungi obtained from *P. obtusifolia* was found to be 0.25, 0.3 and 0.65 from flower, stem and leaf segments respectively. Similarly for *P. acuminata*, the IR value found to be 0.2 for flowers, 0.3 and 0.35 for leaf and stem segments respectively (Fig 2). These findings consistent to that of Huang et al [15] reports of endophytic fungi isolated from *P. rubra.*

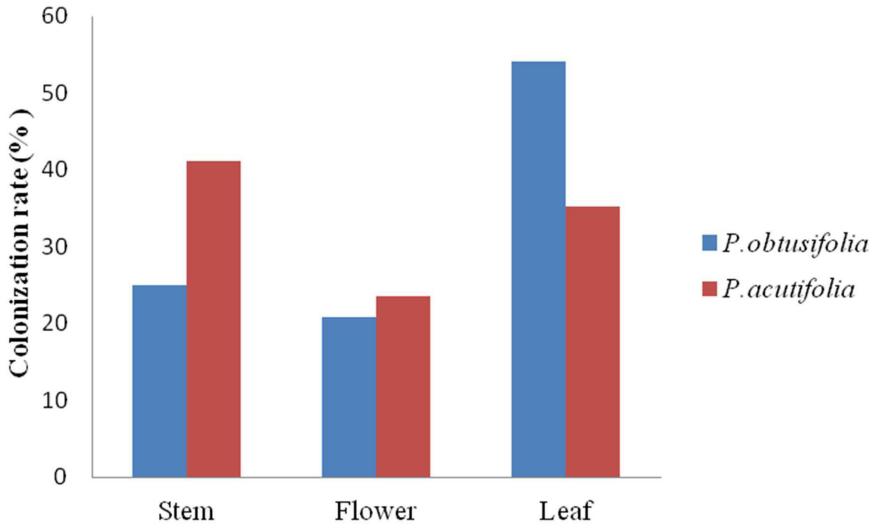

Figure 1: Colonization rate (%) of endophytic fungi

Identification of the endophytes was done based on morphology and conidial characteristics. Among the 24 isolates of *P. obtusifolia*, *Cylindrocephalum* sp., *Chaetomium* sp., *Fusarium* sp., *Pestalotiopsis* sp., *Penicillium* sp. and *Cladosporium* sp., were isolated from stem; *Colletotrichum* sp., *Mycelia sterilia* sp., *Fusarium* sp., *Curvularia* sp. and *Chaetomium* sp., were isolated from the flower; *Colletotrichum* spp., *Fusarium* spp., *Mycelia sterilia* spp., *Pestalotiopsis* sp., *Cochliobolus* spp. and *Chaetomium* spp., were obtained from the leaves. Whereas among 17 isolates of *P. acuminata*, *Colletotrichum gloeosporioides*, *Chaetomium globosum*, *Colletotrichum* sp., *Curvularia* sp., *Mycelia sterilia* spp. and *Aspergillus* sp., were isolated from stem; *Colletotrichum* sp., *Penicillium sp.*, *Aspergillus* sp. and *Uredospore* sp., were isolated from the flower; *Penicillium* sp., *Aspergillus* sp., *Alternaria* sp.,

Mycelia sterilia sp. and *Chaetomium* sp. were obtained from the leaves. These endophytic fungi are previously reported from different plant hosts [27, 28, 29, 30].

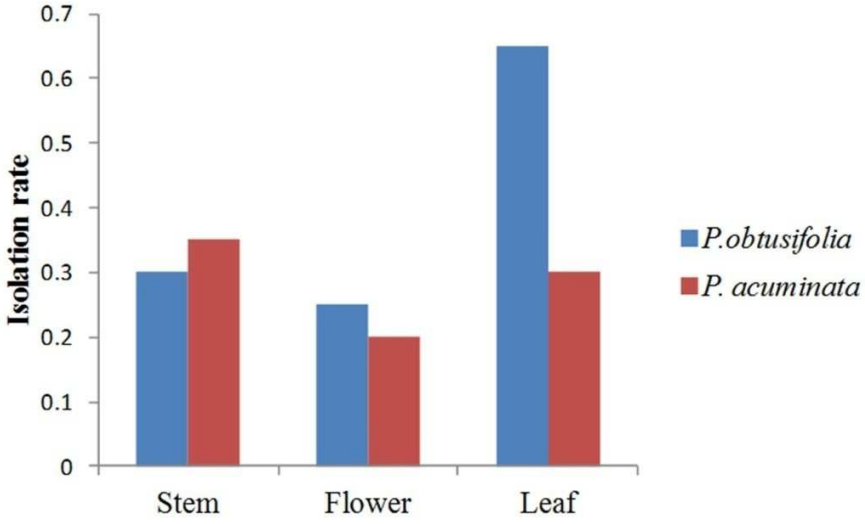

Figure 2: Isolation rate of endophytic fungi

3.2. Preliminary Screening for antimicrobial activity:

Among the 24 endophytic fungi from *P. obtusifolia* screened for antimicrobial activity by agar plug method, 16 endophytic isolates demonstrated activity. Out of 16 isolates, nine isolates showed potential inhibition against *S. aureus* and eight isolates inhibited the growth of *B. cereus*. Only POL-5 isolate suppressed the growth of *P. aeruginosa* and two strains (POS-3 and POS-6) were effective against *C. albicans* (Fig 3). Nine isolates exhibited inhibition zone against *E. coli* and eleven isolates against *S. typhi* (Table 1). Among the tested isolates, endophytic fungal isolate POS-3revealed better antimicrobial activity. The identification was further confirmed by 18s rDNA analysis, as *Fusarium oxysporum* and the sequence was deposited in Genbank with accession no.KF864555.

Figure 3: Inhibition of *C. albicans* by *F. oxysporum* (reverse view)

The antimicrobial potential of endophytic fungi from *P. acuminata* was assessed and it was found that 10 isolates demonstrated activity against the pathogens. Out of them, 4 isolates were inhibitory against *S. aureus* and *B. cereus*; 6 isolates showed potential inhibition against *P. aeruginosa*, *E. coli* and *Salmonella typhi*. Only two isolates (PAS-5 and PAF-1) showed inhibitory potential against *C. albicans* (Table 2). Based on the inferences of the antimicrobial activity of *P. acuminata* isolates, the isolate PAS-1 showed better activity compared to other isolates. The identification of PAS-1 was further confirmed by 18s rDNA analysis, as *Colletotrichum gloeosporioides* and the sequence was deposited in Genbank with accession no. KF864554.

Table 1: Screening for antimicrobial activity by agar plug method of endophytic fungi of *P. obtusifolia*

Endophytic fungi			Antimicrobial activity (Zone of inhibition in mm)					
Sl. No	Code	Identification of endophytic fungi	*S. aureus*	*B. cereus*	*P. aeruginosa*	*E. coli*	*S. Typhi*	*C. albicans*
1	POS-1	*Cylindrocephalum* sp.	7 ± 0^{cd}	8.33 ± 0.33^{f}	0^{a}	0^{a}	0^{a}	0^{a}
2	POS -2	*Chaetomium* sp.	7 ± 0^{cd}	6 ± 0^{b}	0^{a}	7.33 ± 0.33^{d}	7.66 ± 0.3^{de}	0^{a}
3	POS -3	*Fusarium oxysporum*	10.33 ± 0.3^{g}	9.33 ± 0.33^{f}	0^{a}	8.66 ± 0.33^{fg}	9.66 ± 0.66^{ij}	10.66 ± 0.33^{e}
4	POS -4	*Penicillium* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
5	POS -5	*Pestalotiopsis* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
6	POS -6	*Cladosporium* sp.	6.66 ± 0.3^{c}	9.33 ± 0.3^{g}	0^{a}	0^{a}	0^{a}	6.33 ± 0.3^{b}
7	POF-1	*Colletotrichum* sp.	0^{a}	0^{a}	0^{a}	7.33 ± 0.33^{d}	8 ± 0^{ef}	7 ± 0^{c}
8	POF-2	*Mycelia sterilia* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
9	POF-3	*Fusarium* sp.	7.33 ± 0.3^{de}	8.33 ± 0.33^{f}	0^{a}	8 ± 0^{e}	8.66 ± 0.3^{fgh}	0^{a}
10	POF-4	*Curvularia* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
11	POF-5	*Chaetomium* sp.	6 ± 0^{b}	6.33 ± 0.3^{bc}	0^{a}	7 ± 0^{cd}	8 ± 0^{ef}	0^{a}
12	POL-1	*Colletotrichum* sp.	0^{a}	0^{a}	0^{a}	0^{a}	6.33 ± 0.3^{bc}	0^{a}
13	POL-2	*Fusarium* sp.	6 ± 0^{b}	6.33 ± 0.3^{bc}	0^{a}	6 ± 0^{b}	6.66 ± 0.3^{bc}	0^{a}
14	POL-3	*Mycelia sterilia* sp.2	6.66 ± 0.3^{c}	7 ± 0^{d}	0^{a}	6 ± 0^{b}	6.66 ± 0.3^{bc}	0^{a}
15	POL-4	*Pestalotiopsis* sp	8.33 ± 0.33^{f}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
16	POL-5	*Cochliobolus* sp.	0^{a}	0^{a}	6.66 ± 0.3^{c}	0^{a}	0^{a}	0^{a}
17	POL-6	*Mycelia sterilia* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
18	POL-7	*Chaetomium* sp.	6.66 ± 0.3^{c}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
19	POL-8	*Colletotrichum* sp.	7.33 ± 0.3^{de}	6.66 ± 0.3^{cd}	0^{a}	0^{a}	7 ± 0^{cd}	0^{a}
20	POL-9	*Fusarium* sp.	0^{a}	0^{a}	0^{a}	6.66 ± 0.33^{c}	6 ± 0^{b}	0^{a}
21	POL-10	*Chaetomium* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
22	POL-11	*Colletotrichum* sp.	0^{a}	7.66 ± 0.3^{e}	0^{a}	8 ± 0^{e}	9.33 ± 0.33^{hi}	0^{a}
23	POL-12	*Colletotrichum* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
24	POL-13	*Cochliobolus* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}

In each column, mean values followed by the same letter are not significantly different according to DMRT at p = 0.05.

Table 2: Screening for antimicrobial activity by agar plug method of endophytic fungi of *P. acuminata*

Endophytic fungi			Antimicrobial activity (zone of inhibition in mm)					
Sl. No	Code	Identification of endophytic fungi	*S. aureus*	*B. Cereus*	*P. aeruginosa*	*E. coli*	*S. Typhi*	*C. albicans*
1	PAS-1	*Colletotrichum gloeosporioides.*	7.66 ± 0.33^{e}	8.33 ± 0.33^{f}	6 ± 0^{b}	8.33 ± 0.33^{ef}	9 ± 0.57^{ghi}	0^{a}
2	PAS -2	*Chaetomium globosum.*	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
3	PAS -3	*Colletotrichum sp.*	8.33 ± 0.33^{de}	9.33 ± 0.33^{f}	0^{a}	0^{a}	0^{a}	0^{a}
4	PAS -4	*Curvularia sp.*	8.33 ± 0.33^{g}	9 ± 0^{b}	0^{a}	0^{a}	0^{a}	0^{a}
5	PAS -5	*Mycelia sterilia sp.*	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	6.33 ± 0.33^{b}
6	PAS -6	*Aspergillus sp.*	0^{a}	0^{a}	8 ± 0^{d}	8 ± 0^{e}	9.66 ± 0.33^{ij}	0^{a}
7	PAS -7	*Mycelia sterilia sp.*	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
8	PAF-1	*Colletotrichum* sp.	0^{a}	0^{a}	7 ± 0^{d}	9 ± 0^{g}	8.33 ± 0.33^{efg}	7.33 ± 0.33^{d}
9	PAF -2	*Penicillium sp.*	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
10	PAF -3	*Aspergillus sp.*	0^{a}	0^{a}	6.66 ± 0.33^{c}	9.66 ± 0.33^{h}	8 ± 0^{ef}	0^{a}
11	PAF -4	*Uredospores sp.*	0^{a}	0^{a}	8.66 ± 0.33^{e}	9 ± 0^{g}	8.66 ± 0.88^{fgh}	0^{a}
12	PAL-1	*Penicillium sp.*	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
13	PAL-2	*Aspergillus sp.*	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
14	PAL-3	*Alternaria* sp.	0^{a}	0^{a}	7 ± 0^{d}	10.33 ± 0.33^{i}	9.33 ± 0.33^{j}	0^{a}
15	PAL-4	*Mycelia sterilia* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
16	PAL-5	*Chaetomium* sp.	7 ± 0^{cd}	7.66 ± 0.3^{e}	0^{a}	0^{a}	0^{a}	0^{a}
17	PAL-6	*Mycelia sterilia sp.*	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}

In each column, mean values followed by the same letter are not significantly different according to DMRT at p = 0.05.

3.3. Production and extraction of secondary metabolites:

The two isolates *C. gloeosporioides* (PAS-1) and *F. oxysporum* (POS-3) were found to be more promising antimicrobial activity and hence were selected for the production and extraction of secondary metabolites. The ethyl acetate extracts of the *C. gloeosporioides* was yielded around 500mg/200g of red rice media and around 400mg/200g by *F. oxysporum.*

3.4. Antimicrobial activity of crude extract by agar well diffusion method:

The crude extract of the potential endophytic fungi *C. gloeosporioides* and *F. oxysporum* isolates exhibited a broad spectrum of antimicrobial activity against the test pathogens, when compared with that of standard positive control tetracycline for bacteria and fluconazole for yeast. According to Rios and Recio [31] extracts of natural origin showing antimicrobial activity above 100 µg/mL concentration should be avoided; therefore, the crude extract was

maintained at concentrations of 20–100 µg/mL. The zone of inhibition of test pathogens ranged from 6 mm to 23.33 mm at concentrations of 20–100 µg/mL of tested crude extracts.

The two fungal extracts of *C. gloeosporioides* and *F. oxysporum* showed MIC at 20 µg/mL against *S. aureus* and *B. cereus*; however, there was no inhibition at concentration below 20 µg/mL. Both the fungal isolates were found to be inhibitory to *E. coli* and *S. typhi* at concentrations ranging from 20µg/mL-100µg/mL (Table 3). The fungal isolate *C. gloeosporioides* did not show any inhibitory activity against *C. albicans* for tested concentrations. *F. oxysporum* did not have an inhibitory effect against *P. aeruginosa* at the tested concentrations of 20 µg/mL-100 µg/mL. The activity of crude extracts was found to be similar to that of reference antibacterial agent, tetracycline and also exhibited antifungal activity against yeast *C. albicans* to the reference antifungal agent, fluconazole. This suggests the metabolites are broad spectrum in nature and that they have potential application as antimicrobial agents [32, 33]. The activity of *F. oxysporum* extract at 100 µg/mL, can be compared with the standard fluconazole with analogous zone of inhibition against *C. albicans*. Many new and interesting bioactive metabolites such as antibiotics, antiviral, anticancer and antioxidant compounds, which are of pharmaceutical, industrial and agricultural importance have been reported and characterized from fungal endophytes [8].

The endophytes are potentially inhibited against human pathogens bacteria and *C. albicans* and can diminish the growth of the harmful bacteria or fungi by their different mode of action. The present results correlated with the previous findings [1, 30, 34, 35, 36] who have earlier reported antimicrobial activities of different endophytes. *Fusarium* spp. has been reported as endophyte from several plants with diverse biological activity [37, 38, 39] and it provides an opportunity to discover novel bioactive metabolites. The bioactivity of endophytic fungus of *C. gloeosporioides* was previously reported from *Artemisia annua* [40] and *P. acutifolia* [41]. The ethyl acetate extracts of *C. gloeosporioides* and *F. oxysporum* were further screened for the presence of Phytochemicals.

Table 3: Antimicrobial activity of ethyl acetate extracts of endophytes by agar well diffusion method

Endophytic fungi	Crude extract (µg/ml)	Antimicrobial activity (zone of inhibition in mm)					
		S. aureus	*B. cereus*	*P. aeruginosa*	*E.coli*	*S. typhi*	*C. albicans*
C. gloeosporioides	20	7 ± 0.57^{a}	8.66 ± 0.33^{a}	6 ± 0^{b}	8.33 ± 0.33^{a}	9 ± 0.57^{a}	0^{a}
	40	9.33 ± 0.88^{bc}	10.66 ± 0.33^{b}	6.33 ± 0.33^{bc}	8.66 ± 0.66^{a}	9.66 ± 0.33^{ab}	0^{a}
	60	11 ± 0.57^{d}	12 ± 0.57^{c}	6.66 ± 0.33^{cd}	11 ± 0.57^{b}	11 ± 1.15^{b}	0^{a}
	80	13 ± 0.57^{e}	12.66 ± 0.33^{cd}	7 ± 0^{d}	11.66 ± 0.66^{bc}	13.33 ± 0.33^{c}	0^{a}
	100	18.33 ± 1.2^{g}	14 ± 0^{e}	7.66 ± 0.33^{e}	13 ± 0^{c}	14 ± 1.15^{c}	0^{a}
F. oxysporum.	20	8.33 ± 0.33^{ab}	10.66 ± 0.33^{b}	0^{a}	11.66 ± 0.33^{bc}	11.33 ± 0.33^{b}	6.33 ± 0.33^{b}
	40	9 ± 0^{bc}	12 ± 0^{c}	0^{a}	15.33 ± 0.66^{d}	13.33 ± 0.33^{c}	7.66 ± 0.33^{c}
	60	10.33 ± 0.33^{cd}	13 ± 0.33^{d}	0^{a}	18.66 ± 0.66^{e}	16.66 ± 1.2^{d}	9 ± 0.57^{d}
	80	13 ± 0.57^{e}	15.33 ± 0.66^{f}	0^{a}	20 ± 0.57^{e}	20.66 ± 0.66^{e}	11.33 ± 0.33^{e}
	100	14.66 ± 0.66^{f}	18.33 ± 0.33^{g}	0^{a}	21.66 ± 0.33^{f}	23.33 ± 0.33^{e}	12.66 ± 0.33^{f}
Tetracycline	20	27.33 ± 1.2^{h}	15.33 ± 0.66^{f}	8.66 ± 0.33^{f}	16.33 ± 0.33^{d}	11 ± 0.57^{c}	-
Fluconazole	20	-	-	-	-	-	13.66 ± 0.33^{g}

In each column, mean values followed by the same letter are not significantly different according to DMRT at p = 0.05. " –'' Not determined.

3.4. Phytochemical screening:

Chemical analysis was carried out on the isolated endophytic fungal extracts to determine the presence of chemical components as a prospective source for medicinal and industrial use. Their presence is an indicator that they can be exploited as precursors in the development and advancement of synthetic drugs [42, 43]. Phytochemical analysis has been carried out in several plant species but very few reports are available on endophytes [44, 45].

The active metabolites contain chemical groups such as phenols, steroids, flavonoids, quinines, terpenoids, xantones, peptides, cytocatalasins, alkaloids, aliphatic compounds, and phenyl propanoids [6, 46, 47]. In the current study, phytochemical analysis of ethyl acetate extracts of *C. gloeosporioides* extract showed the presence of alkaloids and steroids; whereas *F. oxysporum* extract revealed the presence of flavonoids, phenol and phenolic compounds (Table 4). Our results are in accordance with previous reports [48] wherein the endophytes have shown the presence of different phytochemicals viz alkaloids, steroids [49] phenolic compounds and flavonoids [50] and are known to possess strong antimicrobial activities.

Table 4: Phytochemical analysis of endophytic fungal extracts

Test	Fungal extracts	
	PAS-1	POS-3
Alkaloids	+	-
Steroids	+	-
Terpenoids	-	-
Phenol and phenolic compounds	-	+
Tannins	-	-
Flavonoids	-	+
Proteins and Amino acids	-	-

Note: + Presence, - absence.

CONCLUSION

The present study reveals that the endophytic fungi *C. gloeosporioides* and *F. oxysporum* isolated from *P. acuminata and P. obtusifolia* respectively are effective alternative sources of antimicrobial drugs. Further *Invitro* and *Invivo* experiments are required to establish bioactivity and structural elucidation of these endophytic extracts.

REFERENCES

[1] Suthep Wiyakrutta, N. Sriubolma, W. Panphut, N. Thongan, K.Danwiserkanjana, N. Ruangrungsi, V. Meevootisom, *World J. Microb. Biot.*, **2007**, 20: 265-272.
[2] Devanand Prakash, R. S. Saxena, *Adv. Appl. Sci. Res.*, **2013**, 4(3):98-104
[3] A. Amiri, R. Dugas, Pichot A. L., G. Bompeix, *Int. J. Food Microbiol.*, **2008**, 126:13-19.
[4] A. Daniel, Dias, Sylvia Urban, Ute Roessner, *Metabolites*, **2012**, *2*, 303-336.
[5] A.A.L. Gunatilaka, *J. Nat. Prod.*, **2006**, 69:509-526.
[6] H.B.Q. Tran, M.J. McRae, F. Lynch, C.T. Brett, K. Waldron, Unwin, Hyman, E.A. Palombo, (Ed.) Identification and bioactive properties of endophytic fungi isolated from phyllodes of Acacia species. (Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology **2010**)
[7] B. Schulz, C. Boyle, *Mycol. Res.*, **2005**, 109: 661-686.
[8] G. Strobel, B. Daisy, *Microbiol. Mol. Biol. Rev*., **2003**, 67:491–502.
[9] M. Gupta, U.K. Mazumder, P. Gomathi, V. Thamil Selvan, *BMC Complem. Altern. M.* **2006**, 6:36.
[10] N.L. Owen, N. Hundley, *Sci. Prog*., **2004**, 87: 79-99.
[11] A. Vijayalakshmi, V. Ravichandiran, V. Malarkodi, S. Hemalatha, G. Sudharani, S. Jayakumari, *Asian Pac. J. Trop. Biomed.* **2011**, 1(5): 401-405
[12] V. Gangadevi, J. Muthumary, *World J Microbiol Biotechnol*., **2008**, 24:717-724.
[13] V. Gangadevi, J. Muthumary, *Mycologia Balcanica*., **2008**, 5:1-4.
[14] K. Nithya, J. Muthumary, *Recent Res. In Sci. Tech*., **2010**, 2(4): 99-103.
[15] W. Y. Huang, Y. Z. Cai, K. D. Hyde, H. Corke, M. Sun, *Fungal Diversity*, **2008**, 33:61-75.
[16] H. L. Barnett, B. Barry Hunter, Illustrated genera of imperfect fungi, fourth edition. APS Press: St. Paul, Minnesota. ISBN: 0-89054-192-2, **1998**.
[17] M. B. Ellis, *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England **1971**.
[18] B.C. Sutton, *The Coelomycetes*: *Fungal imperfecti with pycnidia, acervuli and stoma*. Commonwealth Mycological Institute, Surrey, England. **1971**.
[19] K. O'Donnell, E. Cigelnik, N.S. Weber, J.M. Trappe, *Mycologia*, **1997**, 89: 48-65.
[20] S.Shweta, S. Zuehlke, B. T. Ramesha, V. Priti, P. Mohana Kumar, G. Ravikanth, M. Spiteller, R. Vasudeva, R. Uma Shaanker, *Phytochem*., **2010**, 71: 117-122.
[21] N. Denitsa, N. Mariana, *J. Cult. Coll*., **2004-2005,** 4: 29-35.
[22] M. M. Atalla, E. R. Hamed, A. R. El-Shami, *Malaysian J. Microbiol.* **2008**, 4(2): 6-10.
[23] Yuan-Chi Su, Jyh-Jye Wang, Tzu-Tsen LinTzu, Ming Pan, *J. Indian Microbiol. Biotech.*, **2003**, 30: 41–46
[24] S. Visalakchi, J. Muthumary, *Afr. J. Microbiol. Res*., **2009**, 3(9):550-556.
[25] O. Dipali, Somkuwar, Vilas A. Kamble, *Int. J. Pharm. Bio. Sci.* **2013**, 4(2): 383-389.
[26] R. Harisaranraj, S. S. Babu, K. Suresh, *Ethnobotanic. Leaflets.*, **2010**, 14: 84-94.
[27] L. X. Cao, Z. Q. Qiu, J. L. You, H. M. Tan, S. Zhou, *FEMS Microbiol. Letters*, **2004**, 247: 147-152.
[28] J.T. Coombs, *Natl. Sci. forum*, **2002**, 20-22.
[29] V. C. Verma, S. K. Gond, A. Kumar, R. N. Kharwar, G. A. Strobel, *Microbial Ecol*., **2007**, 54: 119- 125.

[30] V. C. Verma, S. K. Gond, A. Kumar, A. Mishra, R. N. Kharwar, A. C. Gange, *Microb. Ecol*., **2009**, 57: 749-756.
[31] J. L. Rios, M. C. Recio, *J. Ethnopharmacol*., **2005**,100:80-84.
[32] K. J. Sanmati, Pradeep Mishra, *Eur. J. Exp. Biol*., **2011**, 1 (2):1-6.
[33] G. Sibi, Kalpana Kaushik, K. Dhananjaya, K. R. Ravikumar and H. Mallesha, *Adv. Appl. Sci. Res*., **2013**, 4(2):259-261
[34] M. Corrado, F. Katia, K. F. Rodrigues, *J. Basic. Microbiol*., **2004**, 44(2): 157-160.
[35] H. Q. Li, X. J. Li, Y. L. Wang, Q. Zhang, A.L. Zhang, J. M. Gao, X. C. Zhang, *Biochem. Syst. Ecol*., **2011**, 39: 876-879.
[36] K. Ramasamy, S. M. Lim, A. B. Bakar, N. Ismail, M. S. Ismail, M. F. Ali, J. F. F. Weber, A. L. J. Cole, *Phytothear. Res*., **2010**, 24(5): 640-643.
[37] Y. Shiono, M. Tsuchinari, K. Shimanuki, T. Miyajima, T. Murayama, T. Koseki, H. Laatsch, K. Takanami, K. Suzuki, *J. Antibiot*., **2007**, 60: 309.
[38] A. Kour, A. S. Shawl, S. Rehman, P. Sultan, P. H. Qazi, P. Suden, R. K. Khajuria, V. Verma, *World J. Microbiol. Biotechnol*., **2008**, 24:1115-1121.
[39] B.W. Deng, K.H. Liu, W.Q. Chen, X.W. Ding, X.C. Xie *World J. Microbiol. Biotechnol*., **2009**, 25,139-143.
[40] Lu Hong, Wen Xin Zou, Jun Cai Meng, Jun Hu, Ren Xiang Tan, *Plant Sci*., **2000**, 151: 67-73.
[41] K. Nithya, J. Muthumary, *Indian J. Sci. Technol*., **2009**, 2:11
[42] I. R. Jack, K. Okorosaye-Orubite, *J. Appl. Sci. Environ. Manage*., **2008**, 12(4): 63-65.
[43] A. B. Segismundo, P. E. Florendo, P. A. Roman, *UNP Res. J*., **2008**, 17: 1-10.
[44] R.X. Tan, W.X. Zou, *Nat. Prod. Rep*., **2001**, 18: 448-459.
[45] W.Y. Huang, Y. Z. Cai, K. D. Hyde, H. Corke, M. Sun, *World J. Microbiol. Biotechnol*., **2007**, 23:1253-1263.
[46] A. H. Aly, A. Debbab, J. Kjer, P. Proksch, *Fungal Diver*., **2010**, 41:1-16.
[47] K. P. Muthu, P. Ramalingam, B. J. N. N. Sai, *Eur. J. Exp. Biol*., **2011**, 1 (2):172-177.
[48] H. Y. Lai, Y. Y. Yau, K. H. Kim, *BMC Complem. Altern. M*., **2010**, 10: 15
[49] Rekha Bisht, S. Bhattacharaya, *Der Pharmacia Sinica*, **2013**, 4(3):75-79
[50] Poonam C. Patil, Varsha D. Jadhav (Rathod) and Shivprasad D. Mahadkar, *Der Pharmacia Sinica*, **2013**, 4(3):136-142