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Antimicrobial activities of endophytic fungi isolated from *Catharanthus roseus* (L.) G. Don, *Nothapodytes nimmoniana* (Grah.) Mabb. and *Pongamia pinnata* (L.) Pierre

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The present study is focused on evaluating the antibacterial activity of the endophytic fungi associated with an ethnomedicinally valued plants, *Catharanthus rosues, Nothapodytes nimmoniana* and *Pongamia pinnata* collected from different locations of Maharashtra, India. A total of 81 fungal isolates from the three host plants were investigated for antimicrobial activity against multidrug resistant gram-negative bacterial strains *Escherichia coli*, *Klebsiella* sp., *Enterobacter aerogenes,* gram-positive *Staphylococcus aureus* and a strain of *Candida albicans*. Crude extracts were obtained in Ethyl acetate separately and in a combination of hexane, ether and ethyl acetate. The best result was obtained against *Staphylococcus aureus* in hexane, ether and ethyl acetate combination under *in vitro* plate assay. *Chaetomium globosum* from all the three medicinal hosts, *Colletotrichun* sp. from *N. nimmoniana* and *P. pinnata* and *Phoma* sp. 1 from *N. nimmoniana* inhibited *Staphylococcus aureus* strongly. The minimum inhibitory concentration was determined for the endophytes that showed significant and moderate activities against test pathogens. The highest Minimum inhibitory concentration (25 g/disc) was found in *C. globosum* isolates from all three host plants.

Keywords: Antibacterial, medicinal, endophytic fungi, *Catharathus roseus*, *Nothapodytes nimmoniana, Pongamia pinnata*

INTRODUCTION

The effectiveness of antibiotic medications is being threatened by the emergence of resistant pathogenic bacteria. In recent years, the number of microorganisms that are resistant to multiple drugs (MDR) and antibiotics has rapidly increased. Since this is a global problem, new resistance mechanisms are developing and an increase in MDR is one of the causes of treatment failure and rising death rates (Okla *et al*. 2021). Pathogenic germs that could formerly be treated with antibiotic therapy have now been discovered to be resistant to every type of antibiotic. (Saranraj, 2015). The necessity for novel, cost-effective antimicrobials to treat this expanding range of ailments becomes of paramount significance. (Lahlou, 2013). This prompted a thorough search across new niches and ecosystems for fresh and potent antibacterial compounds. The association between fungi and plants, where they create a variety of bioactive compounds that foster growth, competitiveness, and disease defense, is vital for well-being of people throughout the world (Porras-Alfaro and Bayman, 2011; Teiten *et al.* 2013; Mapperson *et al.* 2014; Bijaya 2015).

In the search for new bioactive strains of endophytic fungi, plants used in traditional medicine have been crucial since it's probable that their therapeutic properties are due to the metabolites produced by their endophytic population (Kaul *et al.* 2012; Kusari *et al.* 2013). All the selected host plants in the present study contain a number of pharmaceutical compounds having anti-microbial, anti-tumor, and wound healing properties (Stessy *et al.* 2017; Durga *et al.* 2020; Akram *et al.* 2021). Despite this potential, the endophytic composition of a variety of

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therapeutic plants has to be explored, including C*atharanthus roseus* (L.) G. Don*, Nothapodytes nimmoniana* (Grah.) Mabb. and *Pongamia pinnata* (L.) Pierre, (Ulukan and Swaan, 2002; Cragg and Newman, 2004; Jalgaonwala *et al.* 2010; Renjimol *et al*. 2018). Though the studies have been conducted over the years on endophytic diversity and distribution from *N. nimmoniana*, *P. pinnata* and *C. roseus* on number of aspects, reports on their antibacterial properties are meager. In the present study we have screened endophytic fungi isolated from these hosts for their antibacterial potential against the multi-drug resistant bacteria.

MATERIALS AND METHODS

Plants samples (aerial parts, viz. leaf lamina, leaf mid-rib, petiole, stem and inner bark) of two symptom-less, apparently healthy medicinal plant species, viz. *C. roseus, N. nimmoniana* and *P. pinnata* were collected from different localities of Maharashtra. Samples of *N. nimmoniana* were collected from Lonavala, Mahabaleshwar, Tamhini Ghat and Tilari Ghat, samples of the host *P. Pinnata* were collected from Bhimashankar, Pune, Wai and Yawat and samples of *C. roseus* were collected from Pune, Pimpari, Yawat, Lonawala during monsoon, winter and summer seasons for two consecutive years (2006- 2008). Isolation of the endophytic fungi was carried out as per the protocol (Surynarayanan and Vijaykrishna, 2001; Nimbalkar and Singh, 2022)

Antimicrobial testing

Test microorganisms

For *in vitro* antibacterial testing of endophytic fungi, four clinically significant multi-drug resistant bacterial strains were chosen as test organisms. The gram negative bacterial strains *Klebsiella* sp. (R-2434), *Enterobacter aerogenes* (R-2412), *Escherichia coli* (R-2046) and gram positive *Staphylococcus aureus* (ICU 16) were obtained from Ruby Hall Clinic, Pune. Similarly, strain of *Candida albicans* (NICM 3471) used in the present study was procured from National Collection of Industrial Microorganisms, NCL, Pune.

Fermentation and extraction

Two mycelial agar discs (5 mm diam.) from 5 days old actively growing endophytic culture were inoculated in 100 ml potato dextrose broth in 250 ml Erlenmeyer flasks. Culture broths were filtered through cheese cloth after being incubated for 7 days at 28°C in a rotary shaker (200 rpm). The cell free culture filtrates (CF) were extracted three times using ethyl acetate and a mixture of hexane, ether, and ethyl acetate (100:100:100). The culture filtrates were extracted using the equal amount of solvents in both systems and concentrated until dry. The resulting raw extracts were kept at 4°C till further experiments were carried out. Crude extracts were evaluated for its antibacterial properties by re-dissolving in ethyl acetate (20 mg/ ml). Finally, using the agar well diffusion method, 100 µl aliquots of these solutions were used for the antibacterial plate assay.

Antibacterial Assay

Standardization of bacterial inoculum

The selected bacterial strains were grown in 10 ml of Muller Hilton Broth (MHB) medium for 24 hrs at 37ºC. With MHB medium, the turbidity of the resultant suspensions was diluted to produce approximately 1.5 x 10^8 CFU/ml. This was done by measuring the absorbance at a 625 nm wavelength using spectrophotometer (Shimadzu UV-1601). The acceptable range of turbidity standard is 0.08 – 0.10. The turbidity of bacterial suspension is comparable to 0.5 McFarland turbidity standards [0.05 ml of 1.175% Barium chloride ($BaCl_2.2H_2O$) in 9.95 ml of 1% Sulphuric acid (H_2SO_4)]. This level of turbidity is considered equivalent to approximately 1.5 × 10^8 CFU/ml. The 100 µl of bacterial suspension was inoculated in 90 mm Petri plate containing Muller Hilton Agar (Qualigens) (Anon. 2003).

Agar well diffusion method

The modified agar well diffusion method was employed for preliminary screening of endophytic extracts against selected bacteria and yeast. Plates were prepared with 15 ml of Muller Hilton Agar medium and inoculated with standardized inoculum. About 5 mm diameter wells were cut out using cork borer and filled with 100 µl of endophytic fungal extract. Standard antibiotics like Tobramycin, Vancomycin, Gentamicin sulphate and Nystatin

were used as controls. Equal quantity of ethyl acetate was used as a negative control. The plates were incubated at 37±1ºC for 24 hrs except for *C. albicans* (NCIM 3471) which was incubated at 29±1ºC. All the experiments were carried out in triplicates. Inhibition values were calculated after 24 hrs of incubation and the mean value were calculated (Perez *et al.* 1990; Rojas *et al.* 2006). The zone of inhibition rated as significant (+++) if the inhibition zone was > 20 mm wide, moderate (++) if the zone of inhibition was between 10–20 mm wide, and poor (+) if it was < 7 mm wide.

Determination of minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was evaluated for the fungal extracts exhibiting significant zone of inhibition in the preliminary assay. This test was performed at six different concentrations of each extract like 25, 50, 100, 200, 400, 800 µg/disc employing the agar disc diffusion method (Dilika *et al.* 2000; Leite *et al.* 2006).

RESULTS AND DISCUSSION

Around 81 endophytic cultures obtained from three different host plants and representing various taxa were evaluated for their antimicrobial activity.

The antibacterial substances produced by endophytic fungi were discovered to be evenly distributed throughout the various groups. As a result, over 87% of ascomycetes, 76% of coelomycetes, 71% of agonomycetes, and 68% of hyphomycetes exhibited antibacterial or anticandidal activity (Fig. 1). Total 61 isolates (75%) of the 81 endophytic cultures evaluated were able to produce compounds that effectively inhibited at least one bacteria or yeast. (Tables 1, 2 & 3). However, *Alternaria alternata*, an endophyte isolated from *P. pinnata*, showed positive action against all examined bacteria and yeast (Table 3). Endophytes, such as *Chaetomium globosum* isolated from all three plants, *Colletotrichum* sp. from *P. pinnata* and *N. nimmoniana*, *Phoma* sp. 1 from *N. nimmoniana,* and *Gliocladium* sp. from *C. roseus*, substantially inhibited (+++) *Staphylococcus aureus* in contrast to other strains of bacteria and yeast.

Endophytic fungi such as *Curvularia lunata, Periconia digitata, Colletotrichum gloeosporioides,* and non-sporulating NS Gr. II (from *C. roseus*), *Cylindrogloeum* sp., *Cladosporium cladosporioides, Fusarium oxysporum, Pestalotiopsis* sp. and non-sporulating NS Gr. II (from *N. nimmoniana*), and *Ascotricha* sp., *Cylindrocarpon* sp., *Fusarium* sp., *F. oxysporum, Myrothecium roridum, Nodulisporium* sp. and non-sporulating NS Gr. II (from *P. pinnata*) showed no activity against any of the selected strains of bacteria and yeast. In comparison to extracts obtained in ethyl acetate, the crude extracts from 18 out of 81 endophytes prepared by combining

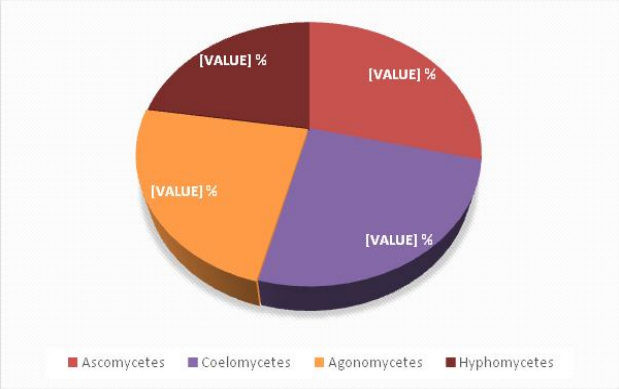

Fig. 1: Group wise distribution of fungal endophytes isolated from all the three plants showing antibacterial or anticandidal activity

solvents (hexane:ether:ethyl acetate) have demonstrated superior activity (Table 1, 2 & 3). Only six endophytic cultures extracted in ethyl acetate, however, demonstrated noteworthy action against a few of the examined bacterial and yeast species. In contrast to the crude extracts in ethyl acetate, which displayed poor activity (+), the extracts of *C. globosum, Phoma* sp. 1, and a non-sporulating isolate NS Gr. IV from *N. nimmoniana* obtained in combination with solvents demonstrated significant (+++) activity against *S. aureus* and moderate (++) activity against *E. coli*. Similarly, crude extracts of *F. verticillioides*, *Xylaria* sp. 1, non-sporulating forms NS Gr. II and NS. Gr. III (against *E. coli*), *Scytalidium lignicola*, *Thielavia icacinacearum* and *Phoma* sp. 2 (against *Enterobacter aerogenes*) obtained in combination of solvents showed moderate (++) activity, which was absent in ethyl acetate extracts. However, when compared to poor (+) or no activity in H-E-E extracts, ethyl acetate extracts of *Xylaria* sp. 2

Table 1: *In vitro* antibacterial assay of crude extracts of fungal endophytes of *Catharanthus roseus*

Endophyte /Pathogens	*Klebsiella* sp.		*E. coli*		*E.aerogenes*		*S. aureus*		*C. albicans*	
	EA *	HEE **	EA	HEE	EA	HEE	EA	HEE	EA	HEE
Alternaria alternata	++	++	++	++	++	+ +	-	-	++	++
Botryosphaeria parva	-	-	-	-	-	-	++	+	++	++
Chaetomium globosum	++	++	++	++	-	-	+++	+++	+	+
Cladosporium oxysporum	-	-	+	+	+	+	-	-	+	+
C. tenuissimum	+	+	-	-	-	-	+	+	+	+
Colletotrichum dematium	-	-	-	-	-	-	+	+	-	-
C. gloeosporioides	-	-	-	-	-	-	-	-	-	-
Curvularia lunata	-	-	-	-	-	-	-	-	-	-
Fusarium oxysporum	-	-	-	-	+ +	++	-	-	-	-
F. sporotrichioides	-	-	-	-	-	-	+	+	+	+
Gliocladium sp.	-	-	++	++	+	+	+ ++	+	+	+
Myrothecium advena	-	-	-	-	-	-	-	-	-	-
M. roridum	-	-	-	-	-	-	+	+	-	-
Nigrospora sphaerica	-	-	-	-	-	-	-	-	+	+
Periconia digitata	-	-	-	-	-	-	-	-	-	-
Phoma sp. 1	-	-	-	-	-	-	-	-	+	+
Phomopsis sp.	-	-	-	-	-	-	+	+	+	+
Phyllosticta conjac	-	-	-	-	-	-	+	+	-	-
Scytalidium lignicola	-	+	-	-	++	++	+	+	-	-
Sordaria fimicola	-	-	-	+ +	+ +	+ +	-	-	+	+
Xylaria sp. 1	-	-	-	-	-	-	-	-	+	+
NS Gr. I	-	-	-	-	-	-	-	-	+	+
NS Gr. II	-	-	-	-	-	-	-	-	-	-
Tobramycin (100 µg)	+++		+		+++		-		-	
Vancomycin (100 µg)	-		-		-		++		-	
Gentamicin sulphate (100 µg)	-		-		-		++		-	
Nystatin (20 µg)	-		-		-		-		+++	

(-) no antimicrobial activity
(+) inhibition zone is less than 7 mm (Poor)
(++) inhibition zone is between 10 mm to 20 mm (Moderate)
(+++) inhibition zone is above 20 mm (Significant)
· (EA) Ethyl acetate
**: (HEE) Hexane:Ether: Ethyl acetate

(against *S. aureus*), non-sporulating NS Gr. IV (against *E. aerogenes*), and *T. icacinacearum* (against *E. coli*) displayed moderate (++) and poor (+) activity.

Chaetomium globosum isolated from all the three plants significantly inhibited (+++) the gram positive *S. aureus,* while it showed moderate (++) inhibition against *Klebsiella* sp. and *E. coli*.

Significant (+++) and moderate (++) suppression against *S. aureus, Klebsiella* sp., and *E. aerogenes* were displayed by *Colletotrichum* sp. isolated from *N. nimmoniana*. However, *Colletotrichum* sp. isolated from *P. pinnata* inhibited *E. coli* (++), *E. aerogenes* (+), *S. aureus* (+++) and *C. albicans* (+). Other strains of the same endophytic species isolated from *P. pinnata* and *N. nimmoniana*, however, showed only moderate activity against *E. aerogenes*, despite *C. gloeosporioides* (*from C. roseus*) exhibiting no activity for all the test pathogens. The extracts of *C. dematium* isolated from *C. roseus* showed no activity against all the test pathogens, except *S. aureus*. When compared to H-E-E extracts, ethyl acetate extract of *Colletotrichum* species from *P. pinnata* strongly (+++) inhibited *S. aureus*. Similarly, when compared to the extracts in H-E-E, the crude extracts of *Gliocladium* sp. obtained in ethyl acetate considerably (+++) inhibited *S. aureus* and ethyl acetate extracts of *S. lignicola* and *Sordaria fimicola* (from *C. roseus*) were found to have no effect on *Klebsiella* sp. and *E. coli,* respectively, when compared with H-E-E extracts. Across the different fungal groups, the percentage of strains having anti-cadida action was more or less consistent (22% ascomycetes, 32% hyphomycetes, 24% coelomycetes, and 22 non-sporulating isolates).

Table 2: *In vitro* antibacterial assay of crude extracts of fungal endophytes of *Nothapodytes nimmoniana*

Endophytes /Pathogens	*Klebsiella* sp.		*E. coli*		*E. aerogenes*		*S. aureus*		*C. albicans*	
	EA*	HEE**	EA	HEE	EA	HEE	EA	HEE	EA	HEE
Alternaria alternata	++	+ +	+ +	+ +	++	+ +	-	-	+	+
Chaetomium globosum	+ +	++	++	++	-	-	+	+ ++	-	-
Chaetomium sp. 1	-	-	-	-	-	-	-	-	+	+
Cladosporium cladosporioides	-	-	-	-	-	-	-	-	-	-
C. oxysporum	-	-	-	-	-	-	-	-	+	+
Colletotrichum gloeosporioides	-	-	-	-	+ +	++	-	-	-	-
Colletotrichum sp.	++	+ +	-	-	+ +	+ +	+ ++	+ ++	-	-
Cylindrogloeum sp.	-	-	-	-	-	-	-	-	-	-
F. oxysporum	-	-	-	-	-	-	-	-	-	-
Fusarium verticillioides	-	-	-	+ +	-	-	-	-	+	+
Mycosphaerella sp.	-	-	+	+	+	+	+	+	++	++
Myrothecium sp.	-	-	-	-	+	+	-	-	-	-
Nigrospora sphaerica	-	-	-	-	-	-	-	-	-	-
Periconia digitata	-	-	-	-	-	-	+ +	++	+	+
Pestalotiopsis sp.	-	-	-	-	-	-	-	-	-	-
Phoma sp. 1	-	-	-	-	-	-	+	+ ++	+	+
Phoma sp. 2	-	-	-	-	-	+ +	-	-	-	-
Phomopsis sp.	-	-	-	-	-	-	-	-	+	+
Scytalidium lignicola	+	+	+	+	-	+ +	+	+	-	-
Thielavia icacinacearum	-	-	+	-	-	+ +	+	+	-	-
Xyalaria sp. 1	-	-	-	-	-	-	+ +	+	+	+
Xylaria sp. 2	-	-	-	+ +	+ +	++	-	-	+	+
NS Gr. I	-	-	-	-	-	-	-	-	-	-
NS Gr. II	-	-	-	+ +	-	-	-	+	-	-
NS Gr. III	-	-	-	+ +	+ +	+ +	-	-	+	+
NS Gr. IV	-	+	+	+ +	++	+	-	-	-	-
NS Gr. V	-	-	-	-	-	+	-	+	+	+
NS Gr. VI	-	-	-	-	-	-	-	-	+	+
NS Gr. VII	-	-	-	-	-	-	+	+	+	+
Tobramycin (100 µg)	+++		+		+++		-		-	
Vancomycin (100 µg)	-		-		-		++		-	
Gentamicin sulphate (100 µg)	-		-		-		++		-	
Nystatin (20 µg)	-		-		-		-		+++	

(-) no antimicrobial activity
(+) inhibition zone is less than 7 mm (Poor)
(++) inhibition zone is from 10 mm to 20 mm (Moderate)
(+++) inhibition zone is above 20 mm (Significant)
· (EA) Ethyl acetate
** (HEE) Hexane:Ether: Ethyl acetate

The crude extracts from all three plants that demonstrated moderate and significant activity (++ or +++) against test pathogens were chosen to determine the minimal inhibitory concentration (MIC). By using the agar disc diffusion method, the MIC was estimated to be between 25 and 800 g/ disc (Table 4). The MIC values below 200µg/disc are given in table 4. With MICs of 50, 50, and 25 g/disc, the extracts of *C. globosum* from all three host plants were the most effective against *Klebsiella* sp., *E. coli*, and *S. aureus.*

Although there are numerous medications available to treat bacterial infections, which are frequently fatal, many of these organisms have developed multi-drug resistance. In order to occasionally overcome the resistance, it is therefore inevitable to look for novel chemicals. Although the biosynthesis of antimicrobial substances by endophytes has been studied by different scholars (Huang *et al.* 2001; Li *et al.* 2001; Strobel *et al.* 2001, 2002; Harper *et al.* 2003; Du *et al*. 2020), only a few reports are available from India (Raviraja *et al.* 2006; Mohanta *et al.* 2008; Kharwar *et al.* 2010; Mahadevamurth *et al.* 2016). The experimental investigation also shown the enormous antibacterial potential of endophytes.

In this investigation, samples of leaf lamina, leaf midrib, stem, petiole, and inner bark from *Cathranthus roseus*, *Nothapodytes nimmoniana*, and *Pongamia pinnata* collected from various locations were used to evaluate 81 endophytic fungus for their antibacterial activity. The *in vitro*

Table 3 : *In vitro* antibacterial assay of crude extracts of fungal endophytes of *Pongamia pinnata*

Endophytes /Pathogens	*Klebsiella* sp.		*E. coli*		*E. aerogenes*		*S. aureus*		*C. albicans*	
	EA*	HEE**	EA	HEE	EA	HEE	EA	HEE	EA	HEE
Alternaria alternata	+ +	++	++	++	++	++	+	+	+	+
Ascotricha sp.	-	-	-	-	-	-	-	-	-	-
Chaetomium globosum	++	++	++	++	-	-	++	+++	-	-
Chaetomium sp. 1	-	-	++	++	++	++	-	-	+	+
Chaetomium elatum	+	+	-	-	-	-	+	+	+	+
Cladosporium cladosporioides	-	-	-	-	-	-	-	-	-	-
C. oxysporum	-	-	-	-	-	-	+	+	+	+
Colletotrichum gloeosporioids	-	-	-	-	+ +	++	-	-	-	-
Colletotrichum sp.	-	-	++	++	+	+	++	+ +	+	+
Cylindrocarpon sp.	-	-	-	-	-	-	-	-	-	-
F. oxysporum	-	-	-	-	-	-	-	-	-	-
F. solani	-	+	-	-	++	++	+	+	-	-
Fusarium verticillioides	-	-	-	-	-	-	-	-	+	+
Fusarium sp.	-	-	-	-	-	-	-	-	-	-
Gnomoniella pongamiae	-	-	-	-	-	-	+	+	-	-
Myrothecium roridum	-	-	-	-	-	-	-	-	-	-
Nigrospora sphaerica	-	-	-	-	-	-	+	+	-	-
Nodulisporium sp.	-	-	-	-	-	-	-	-	-	-
Periconia digitata	-	-	-	-	++	++	+	++	+	+
Phoma sp.1	-	-	-	-	-	-	+	+ +	+	+
Phomopsis sp.	-	-	-	-	-	-	-	-	+	+
Phyllosticta conjac	-	-	-	-	-	-	+	+	+	+
Scytalidium lignicola	-	-	-	-	-	-	+	+	-	-
Xylaria sp. 1	-	-	-	-	-	-	-	-	+	+
NS Gr. I	-	-	-	-	-	-	-	-	-	-
NS Gr. II	-	-	-	+ +	+ +	+ +	-	-	+	+
NS Gr. III	-	-	-	-	-	-	-	-	+	+
NS Gr. IV	-	-	-	-	-	-	-	-	+	+
NS Gr. V	-	-	-	-	-	-	-	-	-	-
Tobramycin (100 µg)	+++		+		+++		-		-	
Vancomycin (100 µg)	-		-		-		++		-	
Gentamicin sulphate (100 µg)	-		-		-		++		-	
Nystatin (20 µg)	-		-		-		-		+++	

(-) no antimicrobial activity
(+) inhibition zone is less than 7 mm (Poor)
(++) inhibition zone is from 10 mm to 20 mm (Moderate)
(+++) inhibition zone is above 20 mm (Significant)
(EA) Ethyl acetate ** (HEE) Hexane:Ether: Ethyl acetate

Table. 4: Determination of minimum inhibitory concentration (MIC) of selected fungal crude extracts

Host plant	Endophytic fungal species	Minimum inhibitory concentration (µg/disc)			
		Klebsiella sp.	*E. coli*	*E. aerogenes*	*S. aureus*
C. roseus	*Alternaria alternata*	100 (100)*	100 (100)	100 (100)	-
	Chaetomium globosum	50 (50)	50 (50)	-	25 (25)
	Gliocladium sp.	-	-	200 (200)	-
N. nimmoniana	*Alternaria alternata*	200 (200)	200 (200)	100 (100)	-
	Chaetomium globosum	50 (50)	50 (50)	-	25 (25)
	Colletotrichum sp.	-	-	-	50 (50)
	Fusarium verticillioides	-	100 (100)	-	-
	Phoma sp. 1	-	-	-	50 (50)
P. pinnata	*Alternaria alternata*	200 (200)	200 (200)	100 (100)	-
	Chaetomium globosum	50 (50)	50 (50)	-	25 (25)
	Chaetomium sp. 1	-	100 (100)	-	-
	Colletotrichum sp.	-	-	-	100 (100)
	Phoma sp.1	-	-	-	100 (100)

*Values in the parenthesis () refer to the value of duplicate reading

plate assay revealed that 79% of *N. nimmoniana* endophytes, 69% of *P. pinnata* endophytes, and 77% of *C. roseus* endophytes exhibited antibacterial and anticandidal activity. Three-fourths of the endophytes in the current study displayed a wide range of positive action against test yeast and/or bacteria, while approximately 61 % of the endophytes displayed strong inhibition exclusively against test bacteria. These findings collectively provide compelling evidence for the idea that endophytic fungi isolated from various host species inhabiting different ecosystems represent prospective sources of organic antibacterial compounds (Wiyakrutta *et al.* 2004; Guimaraes *et al*. 2008; Phongpaichit *et al.* 2007).

Antimicrobial activities were shown to be evenly distributed across the isolates of endophytes belonging to various taxonomic groups. According to the findings, 30% of all the isolates representing Agonomycetes from various hosts had antibacterial activity. Nearly 87.5% of endophytic Ascomycetes, 76% of Coelomycetes, and 68% of Hyphomycetes shown antibacterial or anti-candidal action in the current study. The endophytes *Chaetomium globosum, Gliocladium* sp., and a few Coelomycetes species, such as *Colletotrichum* sp., *Chaetomium elatum*, and *Phoma* sp. 1, were discovered to be efficient and showed significant inhibition to *Staphylococcus aureus* and other test pathogens. Moreover, species belonging to these genera are known to produce a variety of economically important bioactive compounds. (Zou *et al.* 2000; Stinson *et al.* 2003; Inacio *et al.* 2006; Ding *et al.* 2006; Qin *et al.* 2009). As a result, the endophytic species discovered in this study may be of great interest for use in a variety of fields.

All test bacteria and yeast were successfully inhibited by *Alternaria alternata*, isolated from *P. pinnata*. The outcomes were in line with previous studies of *Alternaria* species isolated from *Quercus variabilis* Blume, which demonstrated high action against test bacteria, including *E. coli, Bacillus subtilis*, and *Pseudomonas fluorescens* (Wang *et al.* 2007). Other studies also suggest that *A. alternata* is a potential source of bioactive compounds exhibiting antimicrobial activities against several tested pathogens (Zhao *et al.* 2005; Qiao *et al.* 2007).

The current investigation also highlighted the potential of *C. globosum*, which exhibited promising and wide-ranging antagonistic action against clinical strains of *C. albicans*, *E. coli*, *S. aureus* and *Klebsiella* sp. The results were somewhat consistent with earlier reports where *C. globosum* isolated from various medicinal plants, including *Vitex negundo*, demonstrated strong inhibition against *S. aureus* while exhibiting negative activity towards *Pseudomonas aeruginosa*, *Salmonella typhi*, *Enterococcus* sp. and *C. albicans* (Raviraja *et al.* 2006). It is remarkable to note that only the *C. globosum* isolate from *C. roseus* out of the three host plants used in the current study demonstrated positive inhibition against *Candida albicans*. Several researchers have also claimed that endophytic *C. globosum* contains a number of additional secondary metabolites. (Bashyal *et al.* 2005; Ding *et al.* 2006; Wang *et al.* 2006; Ge *et al.* 2008).

Species of *Colletotrichum* has been reported as a ubiquitous endophyte and obtained from different hosts, source, and variety and with potential activities. The bacterial strains *B. subtilis*, *S. aureus*, and *Sarcina lutea* were all inhibited by *C. gloeosporioides* from *Artemisia mongolica* Fisch (Zou *et al.* 2000),. Three of the four species of *Colletotrichum* in the current investigation significantly inhibited the test pathogens. Strong inhibition was demonstrated by *Colletotrichum* sp. from *N. nimmoniana* against *S. aureus, Klebsiella* sp., and *Enterobacter aerogenes*. Significant inhibitory action against *E. aerogenes* was shown by *Colletotrichum gloeosporioides* that was isolated from *P. pinnata* and *N. nimmoniana.* Similarly, *Colletotrichum* sp. isolated from *P. pinnata* strongly inhibited *E. coli, E. aerogenes*, *S. aureus* and *C. albicans.* The antibacterial activity of various *Fusarium* species that were isolated from the three host plants were examined in the present study. When tested against the target bacteria, the antibacterial activity of crude extracts derived from these species differed significantly. The crude extract of *F. oxysporum* isolated from *N. nimmoniana* and *P. pinnata* exhibited no activity against test pathogens.

Contrarily, a crude extract of *F. oxysporum* that was obtained from *C. roseus* strongly inhibited the growth of *E. aerogenes*. The strain-DC-2-30 of *F. proliferatum* (Matsush.) Nirenberg, isolated from *Aquilaria sinensis* (Lour.) Spreng., was reported to have strong antimicrobial activity against five test pathogens, in contrast to the strain-DC-1-42 of the same species, which was only active against *Bacillus subtilis*, *C. albicans*, and *Cryptococcus neoformans* (Gong and Guo 2009). It is therefore necessary to make concerted efforts to explore the potential of endophytes at both the species and strain levels because of the large heterogeneity in the spectrum of antibacterial activity of endophytic fungus that was discovered to be ubiquitous.

The potential of *Gliocladium* species as biocontrol agents is widely known. They produce a variety of antibiotic compounds, which are ecologically important for protecting plants against a wide range of diseases. The endophytic counter parts of *Gliocladium* are also known to express a variety of activities *in vitro* (Stinson *et al.* 2003). The *Gliocladium* species isolated in the present study from *C. roseus* showed significant activity against *S. aureus*. These results imply that *Gliocladium* species are suitable for an endophytic lifestyle and may be a good source of antibiotic compounds.

The literature suggests that there are few reports on anti-candidal activity of endophytic fungi. Only 10.8% of 1510 endophytic fungal strain extracts evaluated by Weber *et al.* (2007) against *C. albicans* were found to be active. Although there were fewer endophytic strains in the current investigation, a higher percentage of strains (55%) were shown to be effective against *Candida albicans*. Several categories of endophytic fungus, including Hyphomycetes (55.55%), Coelomycetes (47.82%), and non-sporulating isolates (57.14%), showed varying degrees of anti-candidal activity. Several plant hosts have occasionally been found to harbour the endophyte *Nigrospora oryzae* (Berk. & Broome) Petch. *N. oryzae* isolated from *Callicarpa tomentosa* was found to have significant inhibition against *Candida albicans* (Raviraja *et al.* 2006). It was an intriguing finding that *N. sphaerica* isolated in the current investigation from *C. roseus* showed positive activity against *C. albicans*, but that the same species from different hosts, *P. pinnata* and *N. nimmoniana*, did not. The endophyte *Chaetomium globosum* from *C. roseus* was also discovered to be active against *C. albicans*, in contrast to the same species recovered by Raviraja *et al.* (2006) from *Vitex negundo* collected from a different location of India, which showed no activity.

The endophytes in the present study were found to have considerable significant antibacterial activity against gram positive bacteria only, in contrast to gram negative bacteria that were highly resistant. Moreover, a significant degree of diversity in the antibacterial activity was seen between endophytic strains from the same host or from other hosts. This strain-to-strain heterogeneity in the production of antimicrobial activity suggests that several strains of the same species should be thoroughly monitored to get probable bioactive natural compounds. It is recommended that the precise chemical components responsible for activity against bacteria be isolated for the development and production of medications.

CONCLUSION

The assessment of the antimicrobial activities of endophytic fungi against pathogens showed that these endophytic fungi have potential as producers of natural antimicrobial substances and might be exploited as an agent for antibacterial activity. The results suggested that either the extract had a good antibacterial efficacy or contained a high concentration of an active principle with favorable biological action.

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