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Full Length Research Paper

Antimicrobial and antioxidant activities of endophytes from *Tabebuia argentea* and identification of anticancer agent (lapachol)

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Thirteen different endophytic fungi were isolated from different parts of *Tabebuia argentea*. These endophytic fungal extracts were prepared, using ethyl acetate and evaluated for their phytochemical constituents. *Aspergillus niger* and *Alternaria alternata* yielded saponins, phenolic compounds, anthraquinones, steroids, cardiac glycosides and tannins. Other endophytes yielded less phytochemical compounds compared to plant extracts. Naphthoquinone (natural lapachol) was identified in *A. niger* and *A. alternata*. These two endophytes also exhibited significant antimicrobial activity against an array of pathogenic fungi and bacteria. Endophytic isolates of *A. niger* and *A. alternata* are of particular interest because they showed significant antagonistic activity against all tested bacteria and fungi at different range. The total antioxidant capacity and phenolic content of the fungal cultures ranged from 4299 to 5276 µmol/L and from 2.5 to 2.6 mg gallic acid/100 mL culture respectively. The fungal culture, endophytes, *A. niger* and *A. alternata* showed strongest antioxidant capacity, having the highest levels of phenolics. This is the first report of lapachol (naphthoquinone) producing endophytes and their antimicrobial and antioxidant activities. This investigation reveals that the metabolites produced by a variety of endophytic fungi can be a potential source of novel natural antimicrobials, antioxidants and anticancer agents.

Key words: *Tabebuia argentea*, endophytes, antimicrobial activity, antioxidant activity, lapachol.

INTRODUCTION

Tabebuia argentea (Bignoniaceae) is a large and yellow flowering tree. *Tabebuia* sp. have proven to be a rich source of many organic compounds, especially, of phenolic and poliophenolic nature. Such substances have been classified as cytotoxic, antimicrobial and antifungal (Shen et al., 2002; Hills, 1987) by the presence of anthraquininones and naphtoquinone compounds, such as lapachol. Many natural and synthetic naphthoquinone derivatives and lapachol have extensively been studied due to their ability to interfere with the bioactivities of enzymes known as, topoisomerases, a group of enzymes that are critical for DNA replication in cells (Wuerzberger et al., 1998). The antitumor activity of lapachol may be due to its interaction with nucleic acids and the interaction of the naphthoquinone moiety between base pairs of the DNA helix occurs with subsequent inhibition of DNA replication and RNA synthesis (Murray and Pizzorno, 1998). Other biological activities of lapachol are anti-metastatic activity (Balassiano et al., 2005), anti-microbial and antifungal activity (Da Silva et al., 2003), antiviral activity (Breger et al., 2007), anti-inflammatory (Almeida

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et al., 1990), antiparasitic activity (Murray and Pizzorno, 1998), leishmanicidal activity (Teixeira et al., 2001) and molluscicidal activity (Silva et al., 2005). Endophytic fungi are relatively unexplored producers of metabolites useful to pharmaceutical and agricultural industries (Petrini et al., 1992). Endophytes are the microorganisms that grow inside the plants; both (plant and endophytes) will be beneficial. Fungal endophytes residing within these plants could also produce metabolites similar to or with more activity than that of their respective hosts (Strobel, 2002). Microorganisms are a rich source of biologically active metabolites that find wide-ranging exploitation in medicine, agriculture, and industry (Strobel and Daisy, 2003). Many of the anticancer agents are explored from endophytes rather than host (taxol from *Pestalotiopsis microspora*) (Strobel et al., 1996). Various research groups have identified more than hundreds of endophytic isolates from South Indian medicinal plants that showed promising activity against antitumor and antimicrobial agents (Gangadevi and Muthumary, 2007, 2008). The development of drug resistance in human and pathogenic bacteria and fungi has prompted a search for more and better antibiotics, especially as diseases, caused by pathogenic microorganisms, now represent a clear and growing threat to world health (Raviglione et al., 1995; Pablosmendez et al., 1997). Many of the endophytic fungal strains have attracted special attention because they have the capability of producing different colored pigments with high chemical stability. Globally, there are at least one million species of neophytic fungi in all plants (Ganley et al., 2004), which can potentially provide a wide variety of structurally unique, bioactive, natural products (Tan and Zou, 2001; Huang et al., 2007).

Increasing evidence indicate that reactive oxygen species (ROS), (for example, O2- and OH-) and free radical mediated reactions can cause oxidative damage to biomolecules (for example, lipids, proteins and DNA), eventually contributing to; aging, cancer, atherosclerosis, coronary heart ailment, diabetes, Alzheimer’s disease and other neurodegenerative disorders (Finkel and Holbrook, 2000; Halliwell, 1994). Antioxidants are thought to be highly effective in the management of ROS-mediated tissue impairments. Many antioxidants compound possess anti-inflammatory, anti-atherosclerosis, antitumor, anti-mutagenic, anti-carcinogenic, antibacterial or antiviral activities to a greater or lesser extent (Halliwell, 1994; Cozma, 2004; Mitscher et al., 1996; Owen et al., 2000; Sala et al., 2002). Many endophytic fungi have shown antimicrobial activity and antioxidant properties. However, the endophytes of this plant, their anti-microbial values and antioxidant properties have not been investigated. The present study was aimed at the isolation and identification of different endophytic fungi from *T. argentea,* and was investigated for lapachol presence, antimicrobial and antioxidant potential.

MATERIALS AND METHODS

Plant material

Plant material was collected from the campus of Shridevi Institute of Engineering and Technology, Tumkur, Karnataka, India during 2009 to 2010. The collected plant was authenticated from the Department of Botany, Manasa Gangotri, University of Mysore, Mysore, Karnataka, India and Government Ayurvedic College, Mysore, and herbarium was prepared. Fungi (*Aspergillus flavus, A. niger, Aspergillus nidulans, Aspergillus flaviceps, Alternaria carthami, Alternaria helianthi, Cercospora carthami, Fusarium solani, Fusarium oxysporum, Fusarium verticilloides* and *Nigrospora oryzae*) were collected from authentic stock cultures from the Department of Studies in Applied Botany and Biotechnology, University of Mysore, Mysore, India and were mass multiplied on potato dextrose agar (PDA) medium and incubated under 12/12 h light and darkness for 7 days at 26 ± 2°C, then spore suspension was prepared to adjust the spore load (1×10^5 spores/ml). Bacteria (*Bacillus subtilis, Pseudomona fluorescens, Clavibacter michiganensis* sub sp. *michiganensis, Xanthomonas oryzae* pv. *oryzae, Xanthomonas axanopodis* pv. *malvacearum, Escherichia coli, Pseudomonas aeruginosa, Staphyllococcus aureus* and *Klebsiella pneumonia*) were collected from authentic stock cultures from the Department of Studies in Applied Botany and Biotechnology, University of Mysore, Mysore, India and Department of Studies in Microbiology and Biotechnology, Bangalore University, Bangalore, India respectively and were multiplied on nutrient agar (NA) at 36 ± 2°C. After 2 days, culture was harvested and prepared at the final concentration of 1×10^8 cfu/ml. Harvested fungi and bacteria were used for *in vitro* inhibition assay.

Isolation and identification of endophytic fungi

The protocol for isolation follow methods used in other endophyte study (Rungjindamai et al., 2008; Oses et al., 2008; Theantana et al., 2009) with slight modifications. The plant tissues were washed in running tap water for one hour. Fifty segments of leaves from each plant were cut into 5 mm 2 pieces, including a vein (25 samples) and intervein (25 samples). 25 segments of branches were then cut randomly to a length of 5 mm. Endophytic fungi were isolated from the bark of the plant (25 segments). Twenty five segments (5 mm long) were cut from the stems and the roots. The total 150 segments of plant material were treated by triple surface sterilization techniques (Bussaban et al., 2001). Each piece was then placed on malt extract agar (malt extract 20 g/l), rose Bengal (0.033 g/l), chloromphenicol (50 mg/l; agar 15g/l). All plates were incubated at 26±2°C until mycelium grew out; hyphal tips were cut and transferred to potato dextrose agar (PDA). Half strength PDA was used for subculture and stock culture. Identification was based on colony, hyphal morphology of the fungal cultures and characteristics of the spores (Ellis, 1971; Barnett and Hunter, 1972).

Fungal cultivation and extraction of metabolites

The fungal endophytes were cultivated on Potato Dextrose Broth (Himedia, Germany) by placing agar blocks of actively growing pure culture (3 mm diameter) in 250 ml Erlenmeyer flasks containing 100 ml of the medium. The flasks were incubated at 26 ± 2°C for 1 week, with periodical shaking at 150 rpm. After the incubation

Table 1. Isolation of endophytes from *Tabebuia argentea* on PDA media.

Types of endophytes	Leaves		Bark	Stem	Root	Petiole
	Vein	Inter-vein				
Chaetomium crispatum	+	+	+	-	-	-
Trichoderma sp.	-	-	-	-	+	-
Colletotrichum gleosporioides	-	-	+	+	+	-
Alternaria alternata	+	+	+	+	+	+
Aspergillus niger	+	+	+	+	+	+
Aspergillus flavus	+	+	-	-	-	+
Cladosporium cladosporioides	+	+	+	-	-	+
Fusarium oxysporum	+	+	+	+	+	-
F. solani	+	+	+	+	-	-
Sterile mycelium						
SIETSMF1	+	+	-	+	+	+
SIETSMF1	-	-	-	-	-	+
SIETSMF1	-	-	+	+	+	+
SIETSMF1	+	+	+	+	-	-

Experiments were repeated thrice for each sample, + = presence, - = absence, SIETSMF- Shridevi Institute of Engineering and Technology, Biotechnology, sterile mycelial fungi.

period, the cultures were taken out and filtered through sterile cheesecloth to remove the mycelia mats. The fungal metabolites from different endophytic mycelial mats were extracted by using ethyl acetate solvent extraction. Equal volume of the filtrate and solvents were taken in a separating funnel and was shaken vigorously for 10 min. The solution was then allowed to stand, the cell mass got separated and the solvent so obtained, was collected. All solvents were evaporated and the resultant compound was dried in vacuum evaporator using $MgSO_4$ to yield the crude extract (Raviraja et al., 2006).

Solvents

Identification of the phytochemical active substances was carried out using different solvents (Table 1), at 5 g/ 15 ml (W/V).

Phytochemical screening

Test for saponins

1 ml aliquots of the various plant extracts were combined with 5 ml water which is at 60°C, then, shaken for 2 min, as saponins are known to possess frothing activity, the volume of froth produced in this experiments was observed and recorded every 10 min for a period of 30 min (Bandoni et al., 1976).

Test for phenolic compounds

1 ml of test solution was treated with 10% ethanolic ferric chloride. Phenolic compounds were considered present when a colour changes to blue green (Bandoni et al., 1976).

Test for anthraquinones

The Borntrager test was used for the detection of anthraquinones. 2 ml of the test sample was shaken with 4 ml of hexane. The upper lypophilic layer was separated and treated with 4 ml of dilute ammonia.

If the lower layer changed to violet pink, it indicates the presence of anthraquinones (Bandoni et al., 1976).

Test for steroids

1 ml of the respective plant extract was treated with three drops of acetic anhydride and one drop of concentrated sulphuric acid. A colour change from deep green, turning to brown indicated the presence of sterols (Bandoni et al., 1976).

Cardiac glycosides

1 ml of sample solution was mixed with 1 ml of glacial acetic acid then treated with one drop of 5% ethanolic ferric chloride solution. 1 ml of concentrated sulphuric acid was carefully poured down the sides of test tube.

The appearance of a brownish ring between the two layers with lower acidic layer turning blue green upon standing indicates the presence of cardiac glycosides (Bandoni et al., 1976).

Test for lapachol identification

Dried endophytic extract and flower extract of *T. argentea* was extracted with ethyl acetate. 1 g of the endophytic and flower

extract was re-crystallized in petroleum ether and benzene (80:60) and heated at 139°C to 140°C for 5 min. 2 ml of ferric chloride solution was added and observed for the color change (Thomson, 1987).

Antimicrobial activity of lapachol extract

All endophytes extracts was screened for its antifungal and antibacterial activity by paper disc method (Wang and NG, 2001; Girish et al., 2006). Spore suspension of different test fungi/different bacteria was spread over the medium and autoclaved Whatman filter paper discs) (4 mm in diameter) were placed equidistantly from the rim of the plate that were pre treated with fungicides (as control) and all endophytic extract aseptically. A 10 µλ (20 µg) aliquot of all endophytic extract in sterile Tris-HCl buffer (0.01) pH 7.0 was applied to a disc and only Tris-HCl buffer and 1% of BSA was added to the control disc. Bavistin, an antifungal agent (0.2%, W/V) was used as the positive control. Petri plates were incubate at 26 ± 2°C until mycelia growth surrounded peripheral discs containing the control and generated crescents of inhibition around discs with antifungal samples. The bacteria cultured plates were incubated at 36 ± 2°C for 48 h.

A transparent ring around the paper disc signifies antimicrobial activity. The inhibition zone was measured from the edge of the disc to the inner margin of the surrounding pathogens. For each treatment, three replicates were used. An average of three independent reading for each microorganism was used.

Effect of endophytes extracts on fungal morphology

To evaluate the effect of endophytes extracts on fungal hyphae, mycelia mats adjacent to the discs were harvested at regular intervals from day 2 to 5 after application of test solution (20 µg of extract). The samples were squashed and stained with lactophenol cotton blue and observed under bright field microscope with an integrated camera (Leitz Wetzlar, Germany 513594) at a magnification of 40 × 12.5.

Antioxidant activity

Antioxidant activity of extracts was carried out using ferric ion reducing antioxidant power (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay.

FRAP assay

FRAP reagents was freshly prepared by mixing 25 ml acetate buffer (300 mM, pH 3.6), 0.5 mL 2,4,6-tris (2-pyridyl)-S-triazine (TPTZ) solution (10 mM TPTZ in 40 mM/l HCl) and 2.5mL $FeCl_3$ (20 mM) water solution.

Each sample (150 µl) (0.5 mg/ml) dissolved in methanol was added to 4.5 mL of freshly prepared FRAP reagent and stirred. After 5 min, absorbance was measured at 593 nm, using FRAP working solution as blank (Szőllősi and Szőllősi Varga, 2002; Tomic et al., 2009).

A calibration curve of ferrous sulfate (100 to 1000 µmol/l) was used and results were expressed in µmol Fe^{2+}/mg dry weight extract. The relative activity of the samples was compared to L-ascorbic acid.

DPPH radical assay

The effect of endophytic extracts on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi (2005). DPPH solution was freshly prepared by dissolving 24 mg DPPH in 100 ml methanol, stored at -20°C before use. 10 µl of the sample was added to 140 µl distilled water and allowed to react with 2850 µl of DPPH reagent (190 µl reagent + 2660 µl distilled water) for 24 h in the dark condition. Absorbance was measured at 515 nm. A linear standard curve between, 25 to 800 µM ascorbic acid, was obtained and expressed in µm AA/g fresh mass. Additional dilution will be needed if the DPPH value measured is over the linear range of the standard curve; mix 10 ml of stock solution in a solution of 45 ml of methanol, to obtain an absorbance of 1.1 ± 0.02 units at 517 nm using spectrophotometer (Katalinic et al., 2006). All determinations were performed in triplicate. The percentage inhibition of DPPH radical by the samples was calculated according to formula of Yen and Duh (1994):

$$\% \text{ inhibition} = [\{Abs_{control} - Abs_{sample}\}/Abs_{control}] \times 100,$$

Where $Abs_{control}$ is the absorbance of the DPPH radical + ethyl acetate, Abs_{sample} is the absorbance of DPPH radical+ sample extract/standard.

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

Identification of endophytes

Out of 13, 7 fungal species were reported from leaves, bark, stem and root whereas petiole exhibited only 6 fungal endophytic species. *A. alternata* and *A. niger* were present in all the incubated plant part. 4 sterile mycelium, were also obtained from all incubated parts at different range. Sterile mycelium1 (SIETSMF1) was identified from each plant organ except bark. Whereas sterile mycelium 2 (SIETSMF2) was found only in petiole (Table1).

Phytochemical screening

Phytochemical analysis of ethyl acetate solvent extract revealed the presence of saponins, phenolic compounds, anthraquinones, steroids, cardiac glycosides and tannins. Extracts from cultures of all (13 different species) endophytic fungi gave a wide variety of biological activities. *A. alternata* has showed presence of all compounds (of saponins, phenolic compounds, anthraquinones, steroids, cardiac glycosides and tannins), whereas *A. niger* and plant extracts also have all the phytochemical except tannins. The presence of anthraquinones was observed

in only two endophytes (*A. alternata* and *A. niger*) and flower extract (Table 2).

Lapachol identification test

1 g of the endophyte and flower extract was re-crystallized in petroleum ether and benzene (80:60) and heated at 139 to 140 °C for 5 min. 2 ml of ferric chloride solution was added and observed for colour change. Yellow colour confirmed the presence of quinoned compounds (Naphthoquinone). Naphthoquinone was identified in *A. alternata*, *A. niger* and in plant extract. Other endophytes did not show the presence of naphthoquinone presence (Table 3).

Antimicrobial activity of endophytic extracts

We selected two endophytes for antimicrobial activity which have lapachol present in them. Only two endophytes (*A. niger* and *A. alternata*) and plant extract was used for antimicrobial activity. *A. alternata* and *A. niger* exhibited antimicrobial activity against some bacteria species. The endophytes were effective against all tested bacteria (Table 4).

Antioxidant capacity and phenolic contents of the endophytes

Table 5 shows the difference in total antioxidant capacity (FRAP) between selected endophytes (*A. niger* and *A. alternata*) and plant extract. It was observed that FRAP is varied in all tested materials, compared with ascorbic acid. It was observed that ethyl acetate extract of *A. niger*, *A. alternata* and plant at a concentration of 0.1 mg/ml, the scavenging activity of the endophytes reached at high concentration. Figure 1 shows the dose response curve of DPPH radical scavenging activity of ethyl acetate extract of two endophytes (*A. niger* and *A. alternata*) and plant extracts (all parts; bark, wood, leaf and stem powder). Values of *A. alternata* extracts had high radical scavenging activity when compared with *A. niger* extracts activity. The total Phenolic content of the metabolites of two endophytic strains (*A. niger* and *A. alternata*) and host plant extract are shown in Table 5. *A. niger* had the highest total phenolic content (TPC). The phenolic compounds in the endophytic fungi may have contributed significantly to their antioxidant activity.

DISCUSSION

We identified all the endophytes based on morphology and conidial characteristics. Most of the endophytic fungi were identified by morphological characteristic, which was consistent with the other reports from different hosts (Cao et al., 2004; Coombs, 2002; Verma et al., 2007, 2009). Endophytic fungi are reported as ubiquitously higher plant, which has been investigated for their endophytic microbial complement (Carroll, 1988; Gond et al., 2007). The endophytes have made greater interest in the use reservoir of natural bioactive compounds that they (host) produced (Faeth and Hammon 1997; Hawksworth, 1991). Endophytic fungal species are now considered as exciting novel sources for obtaining new bioactive compounds and have been reported from several hosts (Cai et al., 2004; Verma et al., 2009; Castillo et al., 2007).

Phytochemical analysis was carried out on all endophytes. The presence of phytochemicals within endophytes can be potential source for medicinal and industrial use. The presence of phytochemicals in endophytes is an indicator that they can be potential source of precursors in the development of synthetic drugs (Castillo et al., 2007; Jack and Okorosaye-Orubite, 2008; Segismundo et al., 2008). Phytochemical analysis is carried out in plant species but only few reports are available in endophytes (Tan and Zou, 2001; Huang et al., 2007).

Naturally occurring naphthoquinone such as lapachol are widely distributed in nature and have been found in bacteria, fungi, higher plants, animals and fungi; *Penicillium notatum* (Otten and Rosazza, 1983). The lapachol is a natural naphthoquinone from the *Tabebuia* species. *A. alternata* and *A.niger* revealed the presence of naphthoquinone (natural lapachol), which was first identified in the plants (Paterno, 1882; Salmon-Chemin et al., 2001). Here, we first identified the naphthoquinone (lapachol) producing endophytes in the world.

Antimicrobial activity of *A. niger* and *A. alternata* showed significant effect on different Gram positive and negative bacteria and on different fungi. These endophyetes can reduce the growth of the harmful bacteria or fungi by different mode of action. Our results correlated with the findings of other reports (Verma et al., 2009; Wiyakrutta et al., 2004; Corrado et al., 2004; Li et al., 2008; Ramasamy et al., 2010) and they reported the antimicrobial activity of endophytes. The fungal cell wall protects the organism against a hostile environment and relies on signal for invasion and infection of a likely plant, animal or human host. The cell wall of fungi is synthesized at each hyphal apex in a complex assembly sequence. Several classes of antifungal proteins are involved in inhibition of the fungal cell wall or disruption of cell wall structure and/or function and other perturb fungal membrane structure, resulting in cell lysis (Selitrennikoff, 2001). Examination by bright field microscopy showed

Table 2. Phytochemical analysis for the different endophytic fungal extract (ethyl acetate solvent).

Types of endophytes	Saponins	Phenolic compounds	Anthraquinones	Steroids	Cardiac glycosides	Tannins
Chaetomium crispatum	-	-	-	+	+	-
Trichoderma sp.	+	+	-	-	-	-
Colletotrichum gleosporioides	+	+	-	-	+	-
Alternaria alternata	+	+	+	+	+	+
Aspergillus niger	+	+	+	+	+	-
Aspergillus flavus	+	+	-	-	-	-
Cladosporium cladosporioides	-	+	-	+	+	-
Fusarium oxysporum	-	+	-	-	+	-
F. solani	-	+	-	+	+	+
Sterile mycelium						
SIETSMF1	+	+	-	-	-	+
SIETSMF1	+	+	-	+	+	+
SIETSMF1	+	-	-	-	-	+
SIETSMF1	+	-	-	-	+	+
Plant parts	+	-	+	+	+	-

+: Presence; -: absence, repeated the experiments three times for each replicates, SIETSMF- Shridevi Institute of Engineering and Technology, Biotechnology, sterile mycelial fungi.

Table 3. Identification of lapachol from different endophytes using different procedures.

Source	Test 1	Test 2
Chaetomium crispatum	-	-
Trichoderma sp.	-	-
Colletotrichum gleosporioides	-	-
Alternaria alternata	+	+
Aspergillus niger	+	+
Aspergillus flavus	-	-
Cladosporium cladosporioides	-	-
Fusarium oxysporum	-	-
F. solani	-	-
Sterile mycelium		
SIETSMF1	-	-
SIETSMF1	-	-
SIETSMF1	-	-
SIETSMF1	-	-
Plant parts	+	+

+: Presence, -: absence, repeated the experiments three times for each replicates. SIETSMF- Shridevi Institute of Engineering and Technology, Biotechnology, sterile mycelial fungi.

inhibition of mycelia growth and sporulation. Morphologically, in endophytic mycelia, crude extract and plant extract treated fungus, a dramatic shrinkage of hyphal tips was observed. This result is comparable to the antimicrobial proteins isolated from *Withania somnifera* was affected on mycelia shrinkage leads to cell

Table 4. Zone of inhibition (in mm) of antimicrobial activity from endophytic extract by disc diffusion method.

Microorganisms	Endophytes			
	Aspergillus niger	***Alternaria alternata***	***Fusarium oxysporum***	**Plant**
Bacterial pathogens				
Klebsiella pneumonia	3.2±0.57[ab]	3.4±0.57[a]	0.8±0.03[a]	2.1±0.45[a]
Escherichia coli	4.1±0.57[a]	3.4±0.57[a]	0.0±0.01[a]	3.4±0.57[a]
Staphylococcus aureus	3.4±0.57[a]	2.8±0.57[b]	0.0±0.01[a]	2.8±0.57[b]
Pseudomonas aeruginosa	2.1±0.45[b]	2.6±0.45[a]	0.6±0.03[a]	0.4±.03[a]
Pseudomonas fluorescens	1.3±0.35[b]	1.9±0.35[a]	1.1±0.35[b]	2.2±0.45[a]
Clavibacter michiganensis sub sp. *michiganensis*	2.8±0.57[b]	3.3±0.57[a]	1.6±0.35[a]	3.1±0.57[a]
Xanthomonas oryzae pv. *oryzae*	2.4±0.45[a]	2.9±0.57[ab]	0.9±0.10[a]	2.8±0.57[b]
Xanthomonas axanopodis pv. *malvacearum*	1.8±0.35[a]	1.4±0.35[b]	0.4±0.03[b]	2.2±0.45[a]
Fungal pathogens				
Aspergillus flavus	4.6±0.57[a]	5.2±0.57[a]	2.1±0.45[a]	3.6±0.577[a]
A. niger	3.1±0.57[ab]	3.4±0.57[ab]	1.8±0.45[b]	3.2±0.577[b]
A. nidulans	1.8±0.35[a]	1.4±0.35[a]	0.8±0.35[b]	2.6±0.577[c]
A. flaviceps	1.2±0.35[b]	1.9±0.35[a]	1.2±0.35[a]	2.2±0.45[a]
Alternaria carthami	2.5±0.45[a]	3.2±0.57[ab]	2.1±0.45[a]	3.6±0.577[a]
A. helianthi	2.8±0.57[b]	3.4±0.57[a]	1.4±0.45[c]	2.9±0.577[abc]
Cercospora carthami	1.7±0.35[a]	1.1±0.35[b]	0.7±0.3[b]	3.4±0.577[ab]
Fusarium solani	2.2±0.45[b]	2.8±0.57[b]	2.1±0.45[a]	3.4±0.577[ab]
F. oxysporum	4.2±0.57[a]	3.9±0.57[a]	2.1±0.45[a]	3.8±0.577[a]
F. verticilloides	2.8±0.57[b]	2.6±0.45[a]	2.1±0.45[a]	3.4±0.577[ab]
Nigrospora oryzae	1.8±0.35[a]	2.3±0.45[a]	1.8±.45[b]	2.6±0.577[d]

+: Presence; -: absence, repeated the each experiments three times for each replicates.

Table 5. Total antioxidant activity of the endophytes and plant extract of *T. argentea*.

Extracts	FRAP (µmol/l)	PAC
ethyl acetate extract of flower	5276±0.01[a]	3.8±0.01 [a]
ethyl acetate extract of AA	4693±0.03[a]	2.5±0.02 [b]
ethyl acetate extract of AN	4189±0.03[c]	2.6±0.02 [b]
ascorbic acid	4401±0.03[b]	2.7±0.02 [b]

AN- *Aspergillus niger*, AA-*Alternaria alternate*. Data is three replicates of each sample. Phenolic antioxidant coefficient calculated as the ratio FRAP (µM/l)/ total phenols (µMCE/l).

wall disruption and inhibited the growth (Girish et al., 2006) and in our findings the endophytic fungal extracts also shown disruption of cell wall of the fungi tested.

Antioxidants are thought to be highly oxygen specific-mediated tissue impairments. It has been reported that many antioxidants compounds posses anti-inflammatory, anti-atherosclerotic, antitumor, anti-mutagenic, anti-carcinogenic, antibacterial or antiviral activities to a greater or lesser extent (Cai et al., 2004). Ethyl extract of *A. niger* had showed highest antioxidant activity compared to *A. alternata* and plant extract in FRAP method. Some antioxidant compounds isolated from endophytic fungi and antioxidant activities have also been reported (Harper et al., 2003; Song et al., 2005).

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Baumann et al., 1979). *A. alternata* has shown slightly better DPPH ntioxidant activity as compared to *A. niger*. Many of the endophytes (*Phyllosticta* sp., *Xylaria* sp., *Pestalotiopsis* sp., *Chaetomium* sp., etc) have antioxidant compounds

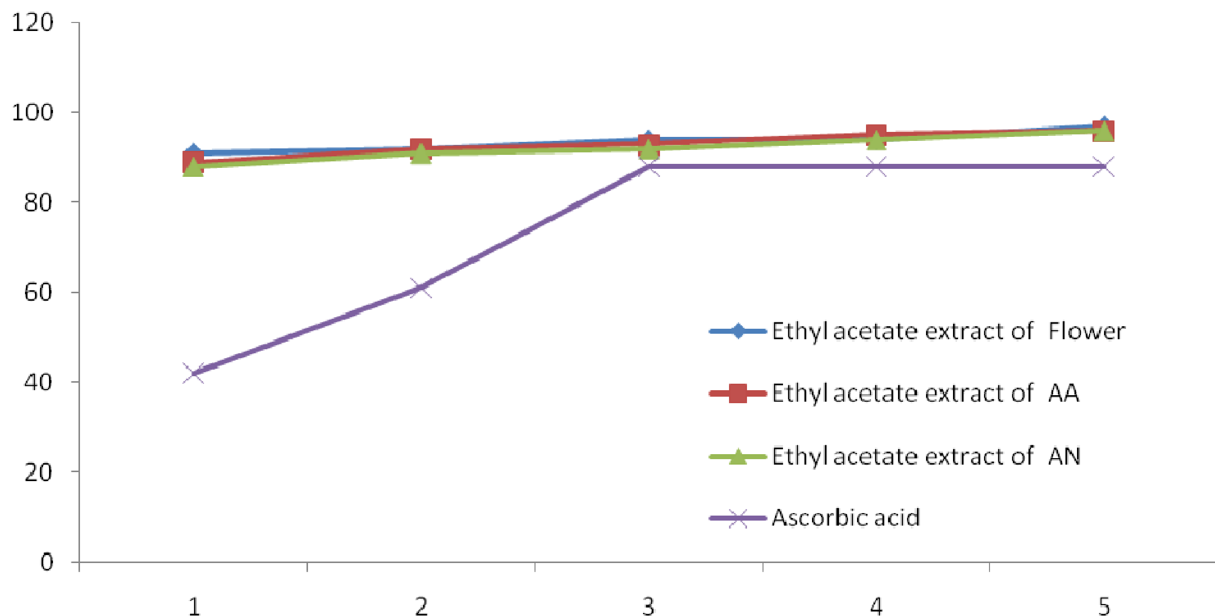

Figure 1. DPPH scavenging activities of the extracts of endophytes and plant extracts of *T. argentea*. AA- *Alternaria alternata,* AN- *Aspergillus niger*, 1- ethyl acetate extract of flower, 2- ethyl acetate extract of AA, 3- ethyl acetate extract of AN, 4- ascorbic acid, experiment was done thrice for all replicates (sample).

and also proved as strong antioxidants. Our results are in agreement with the previous studies with other endophytes of different host (Duan et al., 2003; Srinivasan et al., 2010).

The Phenolic compounds (for example, phenolic acids and their derivatives, flavonoids and Phenolic terpenoids) and certain volatile and aliphatic constituents identified in the present study might be responsible to some extent for total antioxidant properties of the host plant and the isolated endophytic fungi. In our study, the two tested endophytes (*A. niger* and *A. alternata*) showed total phenolic content value. Similar results were observed in many endophytes and they showed antioxidant activity and showed high range of phenolic content (Cai et al., 2004; Shan et al., 2005; Surveswaran et al., 2007; Huang et al., 2007). Some antioxidant compounds isolated from endophytic fungi and their antioxidant activities have also been reported (Harper et al., 2003; Song et al., 2005; Cai et al., 2004).

Endophytic extract showed antimicrobial activity by inhibiting all tested bacteria and fungi at different range. The endophytic extracts possess antioxidant activity and also serve as free radical inhibitor or scavenger or as primary antioxidants. These tested endophytes also yielded naphthoquinone (natural lapachol) from *T. argentea.* Lapachol which was isolated from *Tabebuia impetiginosa* and *Tabebuia avellanedae* exhibited the anticancer activity against different types of cancer (Lee et al., 2005; Kim et al., 2007). The endophytes has showed the presence of different phytochemicals, saponins (Kanna and Kannabiran, 2008), phenolic compounds (Pelczar et al. 1988; Lai et al., 2010), steroids (Kalyoncu et al., 2009), cardiac glycosides (Ahmed et al., 2005), tannins (Kaur and Arora, 2009; Zhang and Lin, 2008) and napthoquinone (Lim et al., 2007; Vinothkumar et al., 2010) and they are known to possess strong antimicrobial and antioxidant activity. A lot of attention is devoted to natural (plants and microbial) sources of antioxidant and antimicrobial agents, our results suggests that a possible use of two endophytes (*A. niger* and *A. alternata*) as a source of natural antimicrobial and antioxidant agents could be a valuable candidates for new agents for antimicrobial, antioxidant and anticancer purposes.

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