

Research Article

Antibacterial Activity, Phytochemical and Enzyme Analysis of Crude Extract of Endophytic Fungus, *Alternaria* Sp. Isolated from an Ethanobotanical Medicinal Plant *Tridax procumbens*.

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ABSTRACT

An endophytic fungus TP-1 was isolated by using potato dextrose agar medium from ethanobotanical medicinal plant *Tridax procumbens*. The isolated endophytic fungi TP-1 was identified as *Alternaria* sp. based on morphological, microscopic and reproductive characters. The crude solvent extract of TP-1 showed broad antibacterial activity and showed the presence of most of phytochemical compounds. Further, the endophyte shows the presence of enzyme protease, naringinase, cellulose and chitosanase. The present study will be useful for both industrial and pharmaceutical applications.

Keywords: Antibacterial activity, phytochemical analysis, enzyme analysis, endophytic fungi, *Alternaria* sp.

INTRODUCTION

Plants form the basis of all sophisticated traditional medicine system that has been in existence for thousands of years¹ but, the advent of drug resistance in human pathogenic bacteria has prompted a search for more and better antibiotics^{2,3}. So, there is indisputable need for new bioactive compounds to control human diseases caused by pathogenic organisms.

Molecules derived from natural products, particularly those products of plants and microbes have an excellent record of providing novel chemical compounds for the development of new pharmaceutical products⁴. The probability of obtaining a novel compound is higher from a novel source. So we selected endophytic fungi as potential sources and accessed for its biological activities. The term endophyte was coined by the German scientist Heinrich Anton De Bary (1884), and is used to define fungi or bacteria occurring inside plant tissues without causing any apparent symptoms in the host⁵. Endophytes have mutualistic relations to their hosts, often protects plant against herbivores, insect attack or tissue invading pathogens⁶. Endophytes have been found in every plant species and it is population approximated to be around a million or more⁷. Hence the present study deals with the antibacterial activity, phytochemical and enzyme analysis of the endophytic fungi isolated from *Tridax procumbens* medicinal plant.

MATERIALS AND METHODS

Source of endophytic fungi

The plant material of *Tridax procumbens* was collected in Gulbarga University Campus Kalaburgi, India. A mature

and healthy leaves was collected in a sterile bag and brought laboratory for the isolation of endophytic fungi.

Isolation of endophytic microorganism

The isolation of endophytic microorganism from *Tridax procumbens* was carried out by using standard method⁸. The fungi grown out tissue were brought in to pure culture on potato dextrose agar (PDA) plates and preserved on PDA agar slants at temperature 4°C for further work. The isolated endophytic fungi were identified based on morphological, microscopic and reproductive characters.

Fermentation and extraction of secondary metabolites

Fresh mycelia of endophytic fungi were transferred in to 500 ml Erlenmeyer flask containing 200 ml of potato dextrose broth and kept under shaking condition at 30°C for 12 days. The fungal culture was filtered to separate mycelia and fermented medium. The obtained medium was re-suspended into various solvents such as methanol, chloroform, ethyl acetate and petroleum ether. The solvent phase was separated and collected in to a watch glass and air dry in sterile condition. The dried extracts were re-dissolved in the same solvents and stored at 4°C for further use.

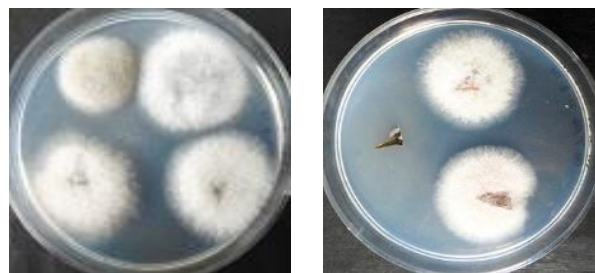


Figure 1: Isolation photos of endophytic fungi

Antibacterial activity

Antibacterial activity of extract were analysed by disc diffusion method. The crude solvent extracts of the endophytic fungi were screened against six pathogenic bacteria such as *E. coli*, *Salmonella typhi*, *Bacillus* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumonia*. The test bacterial strains were grown in nutrient broth. The lawn of bacteria (0.5 McFarland) was made on Muller Hinton Agar (MHA) with the aid of sterile cotton swabs⁹. Sterile filter paper discs were impregnated with 10µl of crude extract. The discs were dried and placed on the test organisms. The plates were incubated at 37°C for 24 h. The plates were examined for the zone of inhibition and zone diameter was measured in mm.

Preliminary qualitative screening of phytochemicals

The crude fermented broth extracted in various solvents such as methanol, chloroform, ethyl acetate and petroleum ether was checked for the presence of alkaloid, flavonoids, phenols, cardiac glycosides, terpenoids, saponins, steroids, and tannins^{10,11}.

Screening for enzymes

The endophytic fungi isolated from *Tridax procumbens* was investigated the production of enzymes by assessing the digestion of suspended or dissolved substrate in agar plates¹². The enzyme such as amylase, cellulase, lipase, protease, laccase, naringinase, L- Asparaginase and chitosanase were screened^{12,15}.

RESULTS

Isolation of endophytic fungi

A total of 6 endophytic fungi designated as TP1-TP6 were isolated (Fig-1 and 2). The cultural characteristics of all isolates were studied and presented in the (Table-1).

Screening of antibacterial activity

The crude extract of six endophytic fungi were screened for antibacterial activity against pathogenic bacteria such as *E. coli*, *Salmonella typhi*, *Bacillus* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumonia* by using four different solvents such as methanol, chloroform, ethyl acetate and petroleum ether. The antibacterial activity of different solvents extract obtained from the six endophytic fungi showed promising results, amongst all the isolated endophytic fungi, TP- 1 showed superior activity which was selected for the further study.

The crud ethyl acetate extract from TP- 1 showed encouraging result with highest zone of inhibition (Fig-3). The TP- 1 ethyl acetate extract showed a zone of inhibiting from 7 mm to 21 mm (for different test pathogens) with largest zone of inhibition of 21 mm against *E. coli*. The methanol extract showed zone of inhibition ranged from 8 mm to 16 mm and largest zone of inhibition was 16 mm against *E. coli*. The TP-1 chloroform extract showed zone of inhibition ranged from 8 mm to 12 mm and largest zone of inhibition was 12 mm against *Staphylococcus aureus*. Further all antibacterial activity was mentioned in Table-2.

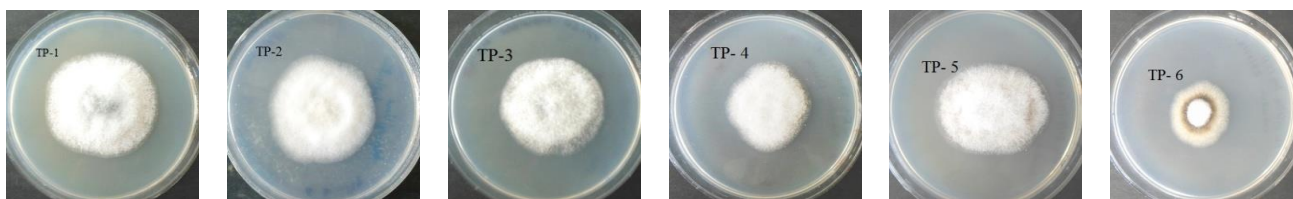


Figure 2: Subculture photos of endophytic fungi

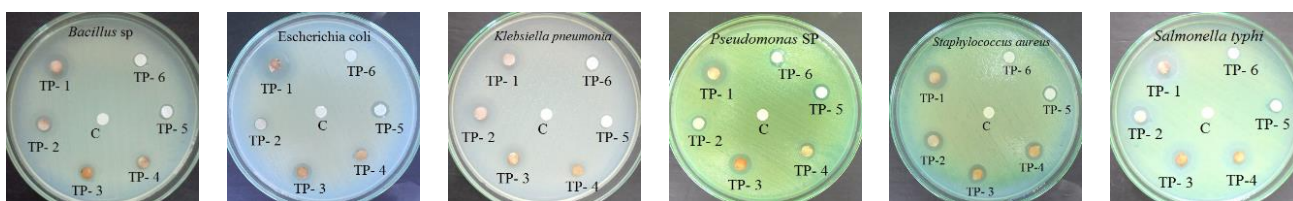


Figure 3: Screening of endophytic fungi for antibacterial activity by ethyl acetate extract

Table 1: Cultural characteristics of endophytic fungi

Colony No.	Media	Colony characters					
		Size	Texture	Surface color	Pigmentation	Topography	Sporulation
TP-1	PDA	Large	Cottony	Whitish orange	Light orange	Raised	Heavy
TP-2	PDA	Large	Velvety	White	NO pigment	Concentric	Heavy
TP-3	PDA	Large	Cottony	Creamish orange	Light orange	Hilly	Heavy
TP-4	PDA	Medium	Cottony	White	NO pigment	Flat	Medium
TP-5	PDA	Large	Cottony	Whitish orange	NO pigment	Flat	Heavy
TP-6	PDA	Small	Cottony	Centre white with brown margin	NO pigment	Concentric	Poor

Table 2: Zone of inhibition (in mm) of crude extracts of endophytic fungi by disc diffusion method (- = Indicates the no zone of inhibition).

Pathogenic bacteria	Fungal crude extracts			Petroleum ether			Methanol			Ethyl acetate			Chloroform											
	TP	TP	TP	TP	TP	TP	TP	TP	TP	TP	TP	TP	TP	TP	TP									
<i>Bacillus</i> sp.	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6						
<i>E. coli</i>	10	-	8	-	9	-	10	-	8	9	-	18	8	10	8	8	8	-	8					
<i>Klebsiella pneumoniae</i>	8	10	10	8	-	8	16	8	-	10	-	8	21	-	10	8	10	-	8					
<i>Pseudomonas aeruginosa</i>	12	8	9	-	-	-	12	8	9	-	10	-	16	12	12	9	-	8	9					
<i>Staphylococcus aureus</i>	15	-	-	8	-	10	8	-	-	10	12	10	20	8	14	8	10	9	-	8				
<i>Salmonella typhi</i>	13	-	10	8	-	-	12	-	10	-	8	-	18	11	11	10	8	-	12	8	10	9	-	-
	16	-	-	8	9	8	12	-	-	10	-	8	20	10	10	8	-	-	-	8	12	-	-	8

Identification of TP-1

The potential isolate TP-1 which was used for the investigations was identified as *Alternaria* sp. based on the

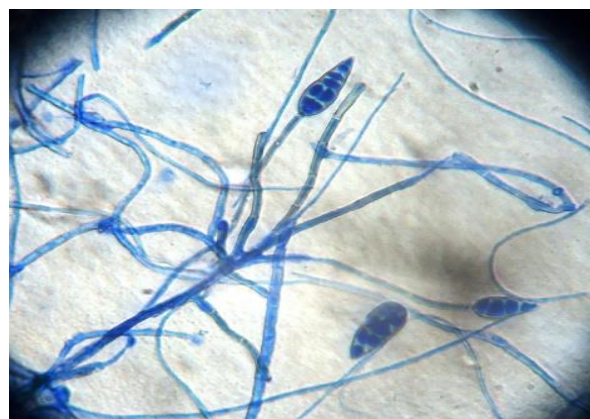


Figure 4: Microscopic image of endophytic fungi TP- 1

morphological, microscopic and reproductive characters (Fig-4).

Phytochemical analysis

The qualitative phytochemical analysis of the crude extract of endophytic isolate TP- 1 was performed using four different solvents such as methanol, chloroform, ethyl acetate and petroleum ether. The ethyl acetate extract showed good result compared to the other solvent. The crud extract from ethyl acetate contain alkaloids, flavonoids, cardiac glycosides, terpenoids and steroids where as phenols, saponins and tannins were absent. The crud extract from methanol contains flavonoids and cardiac glycosides but alkaloids, terpenoids, sterioids, phenols, saponins and tannins were absent. The crud extract from chloroform contain alkaloids and tannins but flavonoids, cardiac glycosides, terpenoids, sterioids, phenols, and saponins were absent. The crud extract from petroleum ether extract shows positive for flavonoids, cardiac glycosides and sterioids but alkaloids, tannins, terpenoids, phenols, and saponins were absent (Table-3).

Screening for enzymes

The endophytic fungi TP-1 have the ability to produce protease, naringinase, cellulose and chitosanase but unable to produce such as amylase, laccase, lipase and L-asparaginase.

DISCUSSION

In India, medicinal plants have long been used for the treatment of various diseases. Medicinal plants provide a special environment for endophytes. Many previous reported endophytic fungi with novel and bioactive natural products are obtained from medicinal plants^{16,17}.

In this study, endophytes were isolated and characterized from *Tridax procumbens*. *Tridax procumbens* is spreading annual herb and widely distributed in the Indian environment but unfortunately it is one of the neglected plants and have been used traditionally for treatment of various diseases¹⁸. Most of the endophytes isolated in this study belong to the phylum Deuteromycota and Ascomycetes. The endophytic fungi TP-1 mainly belong to the Ascomycetes. The endophytic fungi TP-1 was selected as the result shown was good. The endophytic fungi TP-1 was identified as *Alternaria* sp. based on the morphological, microscopic and reproductive characters.

Table 3: Phytochemicals screening (+ = Indicates the presence, - = Indicates the absence)

Test	Results			
	Petroleum ether	Methanol	Ethyl acetate	Chloroform
Alkaloid	-	-	+	+
Flavonoids	+	+	+	-
Phenols	-	-	-	+
Cardiac glycosides	+	+	+	-
Terpenoids	-	-	+	-
Saponins	-	-	-	-
Steroids	+	-	+	-
Tannins	-	-	-	-

The antibacterial activity was carried out for the endophytic fungi. Amongst all the isolated endophytic fungi TP- 1 crud extract from ethyl acetate showed encouraging result with good zone of inhibition in all pathogenic bacteria compared to the other solvent. The TP- 1 crud extract from ethyl acetate inhibiting a zone of inhibition ranged from 7 mm to 21 mm and largest zone of inhibition is 21 mm against *E. coli*. Our results similar to many earlier result¹⁹.

The phytochemical analysis was carried out for the crude extract of TP- 1 using four different solvents such as methanol, chloroform, ethyl acetate and petroleum ether. The ethyl acetate extract showed positive result for alkaloid, flavonoids, cardiac glycosides, terpenoids and steriods. The methanol extract showed positive result for flavonoids and cardiac glycosides. The chloroform extract showed positive result for alkaloid and tannins. The petroleum ether extract showed positive result for flavonoids, cardiac glycosides and steroids.

There are many bioactive compounds were observed in our endophyte TP- 1 which are similar to many available reports^{20,21}.

CONCLUSIONS

TP- 1 has ability to produce various secondary metabolites which will be useful for various human ailments. So it could be recommended as an organism of pharmaceutical importance. However isolation of individual secondary metabolite constituents and subjecting it to biological activity will definitely give fruitful results. However, further studies will need to be undertaken to ascertain fully its bioactivity, toxicity profile effect on the ecosystem and agricultural products.

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