

***IN VITRO* ANTIMITOTIC, ANTIPROLIFERATIVE AND GC-MS STUDIES ON THE METHANOLIC EXTRACT OF ENDOPHYTIC FUNGI, *PENICILLIUM* SPECIES OF *TABEBUIA ARGENTEA* BUR & K. SCH**

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Abstract

The present research was aimed to identify the metabolites in the methanolic extract of endophytic fungi, *Penicillium* species of *Tabebuia argentea* and evaluated the extract, in order to emphasize their *in vitro* cytotoxic activities. The *Penicillium* species extract was evaluated for cytotoxicity and antiproliferative activity and we identified the phytochemicals present in the extract by a GC-MS method. The actively growing onion root cells were inhibited by arresting the cells at various stages of cell cycles. The growth of yeast was also inhibited by inducing necrosis. 18 different phytochemicals were identified in the extract of *Penicillium* species by GC-MS and few of these compounds have significance in anticancer activity. Hence, the endophyte, *Penicillium* species contains very potent phytochemicals depicting a strong cytotoxic activity. Further research is needed to extract the active molecules and to verify their potential anticancer activity.

Rezumat

Prezentul studiu a avut drept scop identificarea într-un extract metanolic a metaboliților din speciile fungilor endofiti de *Penicillium*, dezvoltate pe *Tabebuia argentea*. Activitatea citotoxică și cea antiproliferativă au fost evaluate *in vitro*. S-a identificat compoziția extractului printr-o metodă GC-MS. Extractul a inhibat dezvoltarea rădăcinilor de ceapă prin oprirea ciclului celular în diferite etape. Creșterea drojdiilor a fost de asemenea oprită prin inducere de necroză. Din cei 18 compuși identificați în extractul metanolic, o parte din ei au prezentat activitate anticanceroasă și activitate citotoxică semnificative. Prin urmare sunt necesare cercetări suplimentare pentru a valida activitatea anticanceroasă.

Keywords: *Tabebuia argentea*, endophyte, *Penicillium* sp., antimitotic, antiproliferative, GC-MS

Introduction

Naturally derived products or by products from micro-organisms, plants or animals are considered as natural source for the management of various diseases [1]. In recent years, endophytic micro-organisms are exploited for natural products or novel compounds instead of plants (which are producing important commercial therapeutics). According to several reports, endophytes produce many antibiotics, antiviral, anti-cancer, antioxidant, anti-inflammatory, antidiabetic, anticytotoxicity, immunosuppressive compounds etc. [3].

Tabebuia argentea (Bignoniaceae) is a large and yellow flowering tree and has proven to be a rich source of many organic compounds, especially, of phenolic and polyphenolic nature. The plant is able to produce an anticancer agent, lapachol, which has abilities to interfere with the bioactivities of enzymes like topoisomerases, a group of enzymes that are

essential for cell in the DNA replication [4]. The anti-tumour 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 [5]. Other biological activities of lapachol are antimetastatic activity [6], anti-microbial and antifungal [7], antiviral [8], anti-inflammatory [9], leishmanicidal [10], anti-parasitic [5] and molluscicidal activity [11]. We previously reported the identification of lapachol producing endophytes of *Tabebuia argentea* [11-13]. From our previous reports the endophytic fungal extracts, beside lapachol, contains other phytochemicals, reported as strong antimitotic and antiproliferative in *in vitro* conditions. The present investigation aimed to assess the antimitotic and antiproliferative activity of other endophyte from *Penicillium* sp. and to identify the phytochemicals in the endophytic fungal extract using GC-MS.

Materials and Methods

Plant material collection

Tabebuia argentea was collected in September 2012 from the Shridevi Institute of Engineering & Technology campus, Tumkur, Karnataka, India.

Isolation and identification of endophytic fungi

The protocol for isolation followed the method used in other endophyte studies [14] but adjusted for the specific plant tissues used here, under the pilot experiments. The leaves were washed in running tap water for one hour. Fifty segments of leaf from each plant were cut into 2 pieces, of 5 mm each, including a vein (25 samples) and inter-vein (25 samples). 25 segments of branch were then cut randomly to a length of 5 mm. Endophytic fungi were isolated from different parts 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 [15]. Each piece was then placed on malt extract agar (malt extract (20 g/L), rose Bengal (0.033 g/L), chloramphenicol (50 mg/L, agar (15 g/L). All plates were incubated at $26 \pm 2^\circ\text{C}$ in order to allow growth of fungi. After incubation, hyphal tips were cut and transferred to potato dextrose broth (PDB). Half strength PDB was used for subculture and stock culture. Identification was made based on colony and hyphal morphology of the fungal cultures and characteristics of the spores [15-16].

Fungal cultivation and extraction of the metabolite

The fungus, *Penicillium* sp., was isolated from leaf and used for further tests. The fungal endophytes were cultivated on potato dextrose broth (Hi-Media) 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 $26 \pm 2^\circ\text{C}$ for 1 week, with periodical shaking at 150 rpm. After the incubation period, the cultures were taken out, submitted to extraction with methanol and filtered through sterile cheesecloth to remove the mycelia mats.

Preparative column chromatography

Partially purified polyphenolic content was again purified to some extent through preparative column chromatography. Here, column chromatography was carried out in order to remove the traces of endophytic cell mass, precipitated debris and other insoluble compounds. Column chromatography of partially purified polyphenolic content was carried using methanol as the sole eluent. The collected fraction was used for further analysis.

Identification of fungal culture

The fungal strain was identified as *Penicillium* species based on the colony, hyphal and conidial morphology [17].

Mass cultivation of the fungus

For isolation and identification of the metabolites, the PDB was used. The fungus inoculated flasks were incubated at room temperature under static conditions for 21 days.

Extraction and isolation

After incubation, fungal mycelia were separated from liquid culture media and soaked in methanol overnight. The fungal mycelia were macerated by using mortar and pestle for 10 min followed by filtration and exhaustive extraction.

The methanol extract of *Penicillium* species (0.5 g) was separated on silica gel 60 using stepwise elution with n-hexane. The extract sample, in order to be separated, was placed on the top of the column near the end to collect the elute. A methanol extract fraction over Sephadex LH-60 (EtOH) fraction 6 gave the distinguished clear bands.

Antimitotic activity

The method adopted by Shweta *et al.* [18] was used for the determination of the antimitotic activity using *Allium cepa* root with slight modification. *Allium cepa* were collected from the Tumkur vegetable market. *Allium cepa* bulbs were sprouted in water for 24 h at room temperature. The uniform root tips of *Allium cepa* were selected for the study. These roots were dipped in the extract (200 $\mu\text{L/mL}$) for 48 h. Water was used for dilution and lapachol was used as standard. After 48 h, the root tips were fixed in an acetic acid and alcohol mixture (1:3). Squash preparation was made by staining with acetocarmine. Morphology and the number of the cells were microscopically observed (40x). In all, 350 - 400 cells were counted and cells manifesting different stages of mitosis i.e., interphase and prophase (P), metaphase (M), anaphase (A) and telophase (T) were recorded. The mitotic index was calculated using the following formula [18-19].

$$\text{Mitotic index} = \frac{P + M + A + T}{\text{Total cells}} \times 100$$

Antiproliferative activity

The evaluation of the antiproliferative activity of endophytic fungal extract was performed using the yeast *Saccharomyces cerevisiae* model according to Shwetha *et al.* [18].

Yeast inoculum preparation

The yeast was inoculated with sterilized PDB and incubated at 37°C for 24 h and it was referred as seed broth.

Determination of cell viability

The cell viability assay was performed with 2.5 mL PDB and 0.5 mL yeast inoculum in four separate test tubes. In the first test tube distilled water, in second test tube quercetin (Sigma-Aldrich) as standard (1 mg/mL), in the third and fourth test tubes endophytic extract (10 mg/mL respectively) were added. All tubes were incubated at 37°C for 24 h. In

the above cell suspension, 0.1% methylene blue dyes was added in all tubes and were observed under a low power microscope. The number of viable cells (those that do not stain and look transparent with oval shape while dead cells get stained and appear blue) were counted in 16 chambers of the hemocytometer and calculated the average number of cells. The percentage of cell viability was calculated using the formula [19].

$$\% \text{ cytotoxicity} = \frac{\text{No. of dead cells}}{\text{No. of viable cell} + \text{No. of dead cells}} \times 100$$

GC-MS analysis

The endophytic fungal bioactive crude extract was separated into various fractions by column chromatography. The column was packed with silica gel (mesh 60 - 120) and run with n-hexane: EtOH (8:2). The earlier stated 6th fraction showed a clear band in TLC and was selected for GC-MS analyses that were performed by the Central Instrumentation Department, Indian Institute of Sciences (IISc), Bangalore. GC-MS measurements were performed with a Shimadzu instrument equipped with GC: Aligent 7890 A, MS: MS detector 5975C, Ionization for MS: Electron Impact Ionization, Mass Analyzer: Quadrupole, Software: Data Analysis, Library: Nist 2008, column: HP 5 ms, Dimensions: 30 m (L) x 0.25 mm (ID) x 0.25 μ m film thickness, the initial temperature was 0 to 40°C for 2 min hold time, room temperature was 100°C to 310°C for 10 min is the hold time, total time is 34 min, carrier gas was helium, flow (mL/min) was 1.0, split flow: 1 mL/min, injection volume: 1 μ L, Scan mass range: 30 m/z - 600 m/z and polarity +Ve. GC-MS performed based on the database having more patterns. The spectrum of the unknown compound was compared with the spectrum of the known compounds in the library.

Results and Discussion

Figure 1 presents the flowering habitat at *T. argentea*. The Figure 2A shows the different endophytic fungal species from the cut end part of the leaves of *T. argentea*, the arrow showing the *Penicillium* species (Figure 2B). The isolated pure culture of *Penicillium* species was deposited in conical flasks containing potato dextrose broth. Totally, 12 different endophytic fungal species were identified from three different parts (leaf, stem and bark) of *T. argentea* and were reported in a previous study [12]. The conical flasks showed actively grown mycelium of *Penicillium* species in potato dextrose broth medium (Figure 3). Partially purified methanolic extract of *Penicillium* sp. from leaves were carried out under preparative column chromatography. Each 20 mL of purified extract for every 20 min was collected from chromatography column.



Figure 1.

Habitat of *Tabebuia argentea*

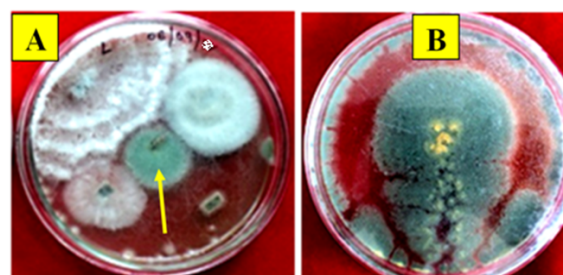


Figure 2.

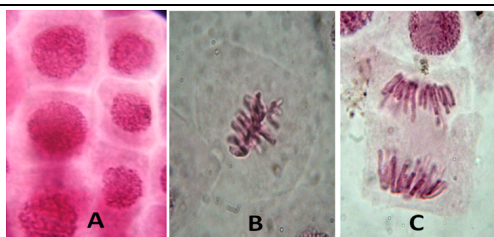
A) Different endophytes of *Tabebuia argentea* and
B) Pure culture of *Penicillium* sp.



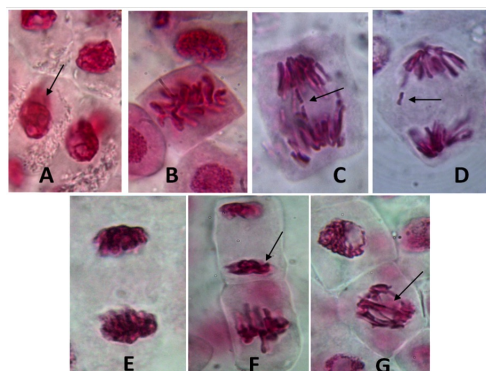
Figure 3.

Mass culture of endophyte - *Penicillium* sp. of
Tabebuia argentea

The antimutagenic assay revealed that the *Penicillium* species extract showed good inhibition of meristematic cells, and also inhibition of the cell growth in different stages of the cell cycle, due to various toxicity levels of the extract. Normal mitotic phases of interphase, metaphase and anaphase were mentioned in Figure 4a. The antimutagenic activity of the endophytes extract from *Penicillium* sp. showed the chromosomal, nucleolar and cellular abnormalities, arrest of cells at interphase and prophase with large nucleoli, chromosomal stickiness at metaphase, chromosomal fragments and bridge at anaphase, displacement of telomeric end at anaphase, chromosomal clumping at telophase, and metaphase and chromosomal bridge at anaphase and nucleolar opening at inter-phase (Figure 4b).

**Figure 4a.**

Normal mitotic phases, A) interphase, B) metaphase, C) anaphase

**Figure 4b.**

Antimitotic activity of the endophyte extract - *Penicillium* sp. isolated from *Tabebuia argentea* leaves: nucleolar, chromosomal and cellular abnormalities,

- A) Arrest of cells at interphase and prophase with large nucleoli, B) Chromosomal stickiness at metaphase, C) Chromosomal fragments and bridge at anaphase, D) Displacement of telomeric end at anaphase, E) Chromosomal clumping at telophase, F) Chromosomal clumping at telophase and metaphase, G) Chromosomal bridge at anaphase and nucleolar opening at interphase.

Table I

Antimitotic activity of endophyte- *Penicillium* sp. extract in *Allium cepa* root tips

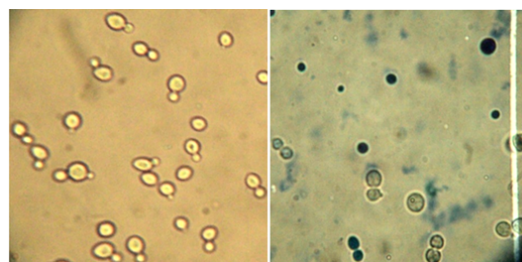
Sample	Concentration	Mitotic index
Control	Distilled water	96.4
<i>Penicillium</i> sp.	10 mg/mL	14.7
Lapachol (Std)	1 mg/mL	12.2

The *Penicillium* species mitotic index was found to be 14.7 and the standard lapachol have shown 12.2, whereas the untreated control showed 96.4 (Table I). The *Penicillium* species extract was evaluated using *Saccharomyces cerevisiae* for its antiproliferative activity and has showed potent inhibition of yeast cell growth. The number of dead cells was calculated. The *Penicillium* species extract inhibited the yeast cell growth by 79.9%, compared to the standard lapachol 86.5%. This result confirms the existence of potent phytochemicals in the extract and their cytotoxic activity on yeast cells (Table II). The dead cells have a dark colour due to their fragmentation, compared to normal cells (no colour) (Figure 5). The *Penicillium* species exhibited necrosis type of cell death of yeast and the necrosis was sequentially photographed (Figure 6). These results indicated that *Penicillium* species extract acts on growth inhibition of yeast cells.

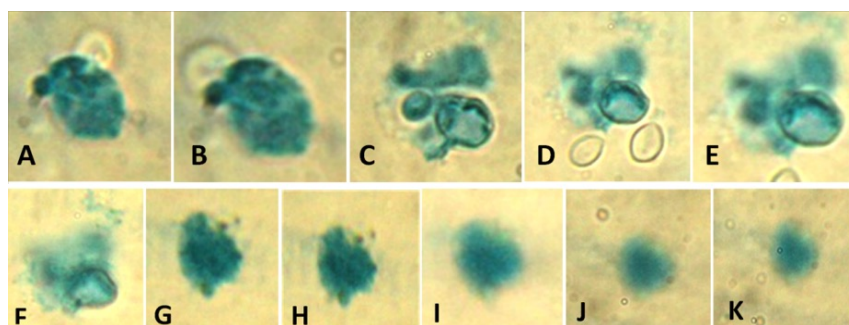
Table II

In vitro antiproliferative activity of endophyte - *Penicillium* sp. extract in yeast cells

Sample	Concentration	% of cytotoxicity
Control	Distilled water	0.0
<i>Penicillium</i> sp.	10 mg/mL	79.7
Lapachol (Std)	1 mg/mL	86.5

**Figure 5.**

In vitro antiproliferative activity of endophyte - *Penicillium* sp. extract in yeast cells, A. control, B. endophytic extract treated yeast cells (stained cells are dead cells with cell debris)

**Figure 6.**

Sequential process of cell necrosis in yeast cells treated by extract of endophyte - *Penicillium* sp. isolated from *Tabebuia argentea* leaves

The methanolic extracts of *Penicillium* species have showed 18 detectable peaks in GC-MS and the identified compounds were: phenol,2,4-bis(1,1-dimethylethyl) (1); 12-Azabicyclo[9.2.2] pentadeca-1(13), 11,14-trien-13-ylamine (2); 1H-2-Benzopyron-1-one, 3,4-dihydro-8-hydroxy-6-methoxy-3-methyl-(R)-(isocoumarin) (3); 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl)ester (4); Hexadecanoic acid, methyl ester (5); Phthalic acid, methyl octyl ester (6); 10,13-Octadecadienoic acid, methyl ester (7); 10, 13-Octadecadienoic octyl methyl ester (8); unknown compound 1 (9); unknown compound 2 (10); Phenol,2,4-bis(1-phenylethyl) (11); Bis(2-ethylhexyl) pththalate (12); Phenol, 2,4-bis(1-phenylethyl) (13); Phenol, 2,6-bis(1,1-dimethylethyl)-4-

[(4—hydroxy-3-5-dimethylphenyl)methyl] (14); 1,2-Benzenedicarboxylic acid, diisooctyl ester (15); Naphthalene, decahydro-1, 8a-methyl-7-(1-methylethyl)-[1R(1.apha.,4a.beta.,7.beta.,8a.alpha.)] (16); 5-dimethylamino-2-nitrosophenol (17); 6-Isopropenyl-4, 8a-dimethyl-4a, 5,6,7,8,8a-hexahydro-1H-nphthalen-2-one (18); (Figure 7 and Table III). Based on the retention time, the compounds were identified by comparison with the standard peaks available in the library of IISc, Bangalore, India. The known 16 compounds have been reported as cytotoxic compounds by inhibiting various types of neoplastic cell lines and some of the compounds are potent anticancer agents [20-32].

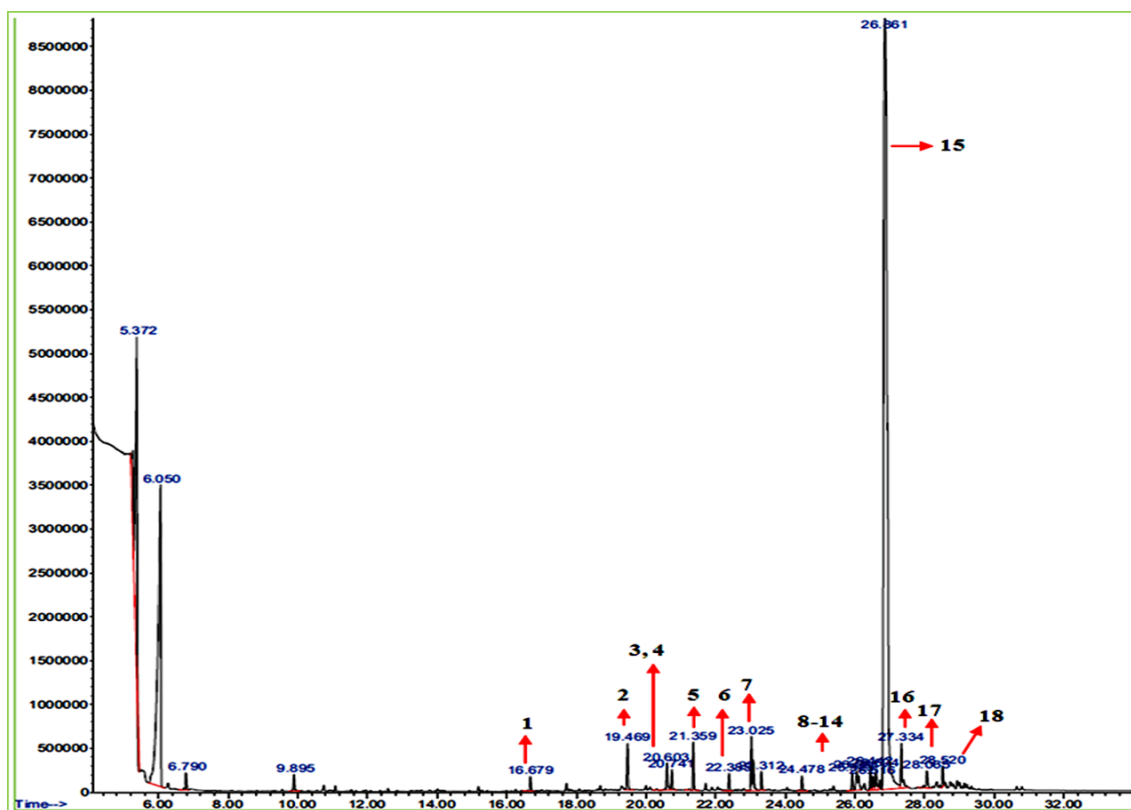


Figure 7.

GC-MS total ion chromatogram of endophyte - *Penicillium* sp. isolated from *Tabebuia argentea* leaves

Table III

Identified phytochemicals in *Penicillium* species extract and their synonymous, identified based on retention time in GC-MS

Peak No	Retention time (min)	Extracted ionic peaks	Identified compound name	Synonyms
1	16.681	191.1, 57, 206.1, 192.1, 41	Phenol, 2,4-bis(1,1-dimethylethyl)-	1. Phenol, 2,4-di-tert-butyl-; 2. 2,4-Di-tert-butylphenol; 3. 2,4-di-t-Butylphenol; 4. 1-Hydroxy-2,4-di-tert-butylbenzene; 5. 2,4-Bis(1,1-dimethylethyl)phenol; 6. 2,4-Bis(tert-butyl)phenol; 7. 2,4-tert-butylphenol; 8. 2,4-bis(1,1'-dimethylethyl)phenol;
2	19.469	121, 91, 41, 93, 77	12-Azabicyclo[9.2.2] pentadeca-1(13), 11,14-trien-13-ylamine	12-azabicyclo[9.2.2]pentadeca-1(13),11,14-trien-13-amine;

Peak No	Retention time (min)	Extracted ionic peaks	Identified compound name	Synonyms
3	20.601	164, 208, 165, 190, 78	1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-6-methoxy-3-methyl-, (R)-	1. (3R)-8-Hydroxy-6-methoxy-3-methyl-3,4-dihydro-1H-isochromen-1-one; 2. (3R)-8-Hydroxy-6-méthoxy-3-méthyl-3,4-dihydro-1H-isochromén-1-one; 3. 1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-6-methoxy-3-methyl-, (3R)- ; 4. 1H-2-Benzopyran-1-one, 3,4-dihydro-8-hydroxy-6-methoxy-3-methyl-, (R)- ; 5. Isocoumarin, 3,4-dihydro-8-hydroxy-6-methoxy-3-methyl-, (R)-(-)- ; 6. (3R)-8-Hydroxy-6-methoxy-3-methyl-3,4-dihydro-1H-isochromen-1-one; 7. (R)-(-)-6-methoxymellein; 8. (R)-6-methoxymellein; 9. 2,4-Dihydro-8-hydroxy-6-methoxy-3-methyl-1H-2-benzopyran-1-one;
4	20.744	148.9, 57, 41, 150, 104	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	1. Phthalic acid, diisobutyl ester; 2. Diisobutyl phthalate; Hexaplas M/1B; 3. Isobutyl phthalate; Palatinol IC; 4. Diisobutylester kyseliny ftalové; 5. 1,2-Benzenedicarboxylic acid, di(2-methylpropyl) ester; 6. 1,2-Benzenedicarboxylic acid, 1,2-bis(2-methylpropyl) ester; 7. Bis(2-methylpropyl) phthalate; 8. Isobutyl-o-phthalate; 9. di-2-methylpropyl phthalate;
5	21.358	74, 87, 43, 55, 41	Hexadecanoic acid, methyl ester	1. Palmitic acid, methyl ester; 2. n-Hexadecanoic acid methyl ester; 3. Methyl hexadecanoate; 4. Methyl n-hexadecanoate; 5. Methyl palmitate; 6. Acide hexadecanoïque methyl ester;
6	22.387	163, 148, 70, 181, 77	Phthalic acid, methyl octyl ester	1. 1,2-Benzenedicarboxylic acid methyl octylester; 2. 1,2-Benzenedicarboxylic acid, octyl methyl ester; 3. Methyl octyl phthalate;
7	23.027	67, 81, 55, 95, 41	10,13-Octadecadienoic acid, methyl ester	1. Methyl (10E,13E)-10,13 octadecadienoate; 2. methyl (10E,13E)-octadeca-10,13-dienoate;
8	23.312	74, 87, 43, 55, 41	Octadecanoic acid, methyl ester	1. Methyl stearate; 2. Methyl octadecanoate; 3. Octadecanoic acid, methyl ester; 4. Stearic acid, methyl ester; 5. Stearic acid methyl ester; 6. Methyl n-octadecanoate; 7. n-Octadecanoic acid methyl ester;
9	24.476	87, 148.9, 41, 45, 43	Unknown compound	Unknown;
10	25.925	287.1, 302.1, 209, 105, 165	Unknown compound	Unknown;
11	26.054	287.1, 302.1, 209, 105, 165	Phenol, 2,4-bis(1-phenylethyl)-	1. 2,4-Bis(1-phényléthyl)phenol; 2. 2,4-Bis(α-méthylbenzyl) phenol; 3. Phenol, 2,4-bis(1-phenylethyl)- ; 4. 2,4-Bis(1-phenylethyl)phenol;

Peak No	Retention time (min)	Extracted ionic peaks	Identified compound name	Synonyms
12	26.443	148.9, 57, 166.9, 71, 43	Bis(2-ethylhexyl) phthalate	1. Phthalic acid, bis(2-ethylhexyl) ester; 2. Bis(2-ethylhexyl) 1,2-benzenedicarboxylate; 3. Di(ethylhexyl) phthalate; 4. Di(2-ethylhexyl) phthalate; 5. Dioctyl phthalate; 6. Octyl phthalate; 2-Ethylhexyl phthalate; 7. Phthalic acid di(2-ethylhexyl) ester; 8. di-iso-Octyl phthalate; 9. Di(2-ethylhexyl) o-phthalate; 10. Di-sec-octyl phthalate; 11. Di(2-ethylhexyl)orthophthalate; 12. Bis(2-ethylhexyl) o-phthalate; 13. 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) esterBis(2-ethylhexyl)ester phthalic acid; 14. 1,2-Benzenedicarboxylic acid, 1,2-bis(2-ethylhexyl) ester;
13	26.514	287.1, 302.1, 105, 288.1, 209	Phenol, 2,4-bis(1-phenylethyl)-	1. 2,4-Bis(1-phényléthyl)phenol; 2. 2,4-Bis(α-methylbenzyl) phenol; 3. Phenol, 2,4-bis(1-phenylethyl)- ; 4. 2,4-Bis(1-phenylethyl)phenol;
14	26.604	95, 81, 91, 55, 107	Phenol, 2,6-bis(1,1-dimethylethyl)-4-[(4-hydroxy-3,5-dimethylphenyl)methyl]-	1. Mesitol; 2. alpha.4-(3,5-di-tert-butyl-4-hydroxyphenyl)- ; 3. 4-[(3,5-ditert-butyl-4-hydroxyphenyl)methyl]-2,6-dimethylphenol;
15	26.863	149, 167, 57.1, 71.1, 43.1	1,2-Benzenedicarboxylic acid, diisooctyl ester	1. Diisooctyl phthalate; Hexaplas M/O; 2. Isooctyl phthalate; 3. Flexol plasticizer diop; 4. Phthalic acid, bis(6-methylheptyl) ester; 5. Phthalic acid, diisooctyl ester; 6. Bis(6-methylheptyl) phthalate;
16	27.335	55, 95, 81, 41, 43	Naphthalene, decahydro-1,8a-dimethyl-7-(1-methylethyl)-, [1R-(1.alpha.,4a.beta.,7.beta.,8a.alpha.)]-	1. 4.beta.H,5.alpha.-Eremophilane; 2. 10.alpha.-Eremophilane; 3. 7-Isopropyl-1,8a-dimethyldecahydronaphthalene; 4. 1,8a-dimethyl-7-(propan-2-yl)decahydronaphthalene; 5. 1,8a-dimethyl-7-propan-2-yl-2,3,4,4a,5,6,7,8-octahydro-1H-naphthalene; 6. Naphthalene, decahydro-1,8a-dimethyl-7-(1-methylethyl)-, [1R-(1.alpha.,4a.beta.,7.beta.,8a.alpha.)]- ;
17	28.066	95, 194.1, 81, 149, 55	5-diethylamino-2-nitrosophenol	5-(Diethylamino)-2-nitrosophenol
18	28.519	149, 43, 55, 122, 95	6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	1. 6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-2(1H)-naphthalenone; 2. 4,8a-dimethyl-6-prop-1-en-2-yl-1,4a,5,6,7,8-hexahydronaphthalen-2-one.

Many anticancer agents isolated from endophytes are primarily tested *in vitro* using cytotoxicity assays using different model organisms. For *in vitro* cytotoxicity activity, testing the onion root tip method, yeast inhibition, mung bean growth, shrimp and silkworm toxicity methods are being used. From our earlier report [12] we identified 12 different fungal endophytic species from three parts of the plant, *T. argentea*. In the present study, we have chosen leaf endophytic fungi from *Penicillium* species, and further, the extract was prepared in methanol thus being evaluated for the *in vitro* cytotoxicity and the extract compounds were identified using GC-MS. The *Penicillium* species extract had shown potent antimitotic activity by inhibiting actively growing onion roots, the activity was 14.7,

close to the one exerted by the standard drug, lapachol (12.2). Hence, the *Penicillium* species extracts have a toxic activity by inhibiting different stages of cell division, thus ultimately inhibiting the root growth. The root length also decreased. The extract exhibited arrests of cells at interphase and prophase with large nucleoli, chromosomal stickiness, bridges and fragments, displacement and clumping of chromosomes were observed. These results were confirmed by other research studies published in literature [12, 13, 33].

A potent antiproliferative activity was noticed for *Penicillium* species extract by inhibiting the growth of yeast cells. The extract showed 79.7% of growth inhibition, whereas the standard had shown 86.5%. The extract caused death of yeast cells by necrosis.

Conclusions

The current study supports the therapeutic potential of endophytic phytochemicals as they exhibited strong *in vitro* cytotoxicity activities. Performing the GC-MS analysis of *Penicillium* species extracts, with potent phytochemicals, we may conclude that they induce cytotoxic mechanism. The results indicated that the endophytic fungi from *Penicillium* species had a very good natural ingredient that possesses strong cytotoxicity. Further studies are required to identify the exact phytochemicals responsible for these activities and that can be studied as potential anticancer agents.

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Conflict of interest

The authors confirm that this article content has no conflict of interest.

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