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GC-MS Analysis of Bioactive Compounds of Endophytic Fungi *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum*

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Abstract

The present investigation was designed to screen the phytochemical constituents of ethyl acetate extracts of endophytic fungi *Chaetomium globosum, Cladosporium tenuissimum and Penicillium janthinellum* were isolated from the medicinal plants *Passiflora foetida*, *Memecylon edule* and *Justicia adhatoda* respectively. The ethyl acetate extracts were prepared from endophytic fungi and the extracts were subjected to phytochemical analysis by GC-MS. In this study, the mass spectrum of the compounds found in extracts of endophytic fungi was matched with the WILEY8 library and National Institute of Standards and Technology (NIST14) library. The GC-MS analysis of endophytic fungi revealed that the presence of thirty three phytocompounds in *Chaetomium globosum*, thirty four phytocompounds in *Cladosporium tenuissimum* and thirty nine phytocompounds in *Penicillium janthinellum* including the antioxidant and antimicrobial compounds. The major chemical constituents present in the ethyl acetate extracts of endophytic fungi *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* such as 2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)- (13.50%), Bis (2-ethylhexyl) phthalate (12.22%), 2-Propenal, 3-phenyl- (9.39%), n-Hexadecanoic acid (9.35%), N-Didehydrohexacarboxyl-2,4,5-trimethylpiperazine (8.47%), Tetracontane (8.32%), 9,12-Octadecadienoic acid (Z,Z)- (11.97%), 9-Octadecenoic acid, (E)- (10.69%) and (1H-Benzoimidazol-2-yl)-[4-(4-methyl-piperazin-1-yl)-phenyl]-amine (8.62%). So, the present study confirmed that the presence of biologically active compounds in endophytic fungi of medicinal plants, which are used in the traditional system of medicine to treat many diseases. Further study is needed to isolate and characterize the bioactive compounds responsible for therapeutic values.

Keywords: Phytocompounds; Chromatogram; GC-MS analysis; *Chaetomium globosum; Cladosporium tenuissimum; Penicillium janthinellum*

Introduction

Natural products are chemical compounds derived from living organisms. Natural products discovery have played major role in the search for new drugs, and is the most potent source for the discovery of novel bioactive molecules. The role of natural products in discovery of new therapeutic agents can be demonstrated by an analysis of the number and sources of bioactive agents. Plants may serve as a reservoir of large numbers of microorganisms known as endophytes. All plants in natural ecosystems appear to be symbiotic with fungal endophytes [1]. Endophytes are symbionts, often bacteria, fungi or actinomycetes which lead their life inside the plant without causing apparent disease. Many plants have been investigated for endophytic fungi metabolites with beneficial effects to crop plants and many of them also have pesticidal and antimicrobial activity against plant and human pests and pathogens [2]. In many countries, the medicinal plants are used in different ways for the treatment of various diseases. Fungal endophytes residing within the medicinal plants could produce metabolites similar to or with more activity than that of their respective host plants. Medicinal plants have been used for years in daily life to treat disease [3]. India is the richest and most diverse cultural traditions associated with the use of traditional systems of medicine. The traditional medicine in India constitutes with its different components like Ayurveda, Siddha, Unani, Homeopathy and Naturopathy. Nutraceutical is an attempt to accomplish desirable therapeutic outcomes with reduced side effects as compared with synthetic therapeutic agents.

Medicinal plants have been used in the folkloric medicine for the treatment of number of diseases [4]. The identification of bioactive chemical compounds from medicinal plants is important in the pharmaceutical industry for drug development and preparation of therapeutic medicines [5]. Endophytes play vital roles in various aspects of life varying from its effects on host plants to its effects to environmental and human life. Endophytes are capable of synthesizing bioactive agents that can be used by plants for defense against pathogens and/or stimulating plant growth, and other agents have been proven useful for novel drug discovery process. Endophytic fungi are the excellent resources for the secondary metabolites against many diseases. Plants are one of the best resources of endophytic fungi, where they grow within the tissues producing secondary metabolites exhibiting many biological and pharmacological activities without affecting the host plant cells.

Medicinal plants and their endophytes are important resources for discovery of natural products. The herbal drugs are prescribed widely due to their effectiveness, minimal side effects in clinical experience and relatively low cost [6]. Medicinal plants are the resource of drugs in traditional systems of medicine, modern medicines, food supplements,

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folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [7]. The term endophyte includes all organisms that grow inside the plant tissues without causing disease symptoms [8,9]. The endophytic fungi are group of organism having a great biodiversity and they are present in most plant parts including the leaves which have enormous potentials for new pharmaceutical substances. Endophytic fungi play an essential role to provide protection to their host against attack by other pathogens and environmental factors.

Natural products have been used directly as drugs which were low cost and important sources of traditional medicines. They also provided the basic chemical architecture for deriving semisynthetic natural products [10]. So, there is a need to search natural bioactive agents for different pharmaceutical, agriculture, and industrial applications and these should be renewable, ecofriendly and easily obtainable [11]. Since, the discovery of potent antibiotic against Gram positive bacteria, penicillin from culture of fungus *Penicillium notatum* by Fleming in 1929, the search for new drugs from microbial origin started.

Fungi are the most important groups of eukaryotic organisms that are well known for producing many novel metabolites which are directly used as drugs or function as lead structures for synthetic modifications [12-15]. It has been estimated that there may be 1.5 million fungal species, while only about 100,000 species are presently known [16]. Only a few taxa have tested for their biological applications including their ability for drug production and biological control. The importance of endophytes had been demonstrated over a long period as potential sources of pharmaceutical leads, as many of endophytic fungi were reported to produce novel bioactive metabolites such as antimicrobial, anticancer, and antiviral agents. Endophytes are the chemical synthesizers inside plants [17]. Endophytes are capable of synthesizing bioactive agents that can be used by plants for defense against pathogens and/or stimulating plant growth, and other agents have been proven useful for novel drug discovery process.

In the present study, the endophytic fungi *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* were isolated from the leaves of selected medicinal plants *Passiflora foetida, Memecylon edule* and *Justicia adhatoda*, respectively. There are many studies on the endophytic fungi, but no study on screening of phytocompounds of endophytic fungi *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum*. So, the present study was aimed to screen the phytocompounds of ethyl acetate extracts of *Chaetomium globosum, Cladosporium tenuissimum and Penicillium janthinellum* by GC-MS analyses.

Materials and Methods

Collection of plant material

Mature healthy and disease free leaves of medicinal plants *Passiflora foetida, Memecylon edule* and *Justicia adhatoda* were collected from the natural habitats of Narthamalai hills at Pudukkottai District, Tamil Nadu, India during the months of January and February 2014. The collected plant was identified by Rev. Dr. S. John Britto, Director, Rapinet Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli, Tamilnadu, India. The leaf samples from each plant were placed separately in sterile polythene bags and stored in an icebox. The stored samples in chilled condition were used for the isolation of endophytic fungi within 48 hrs of collection.

Isolation of endophytic fungi

Isolation of endophytic fungi was carried out by the modified method of Hallman et al. [18]. The collected leaf samples were washed with mild detergent and thoroughly with running tap water to remove the soil particles and adhered debris and then finally washed with sterile distilled water. The leaf samples were subjected to surface sterilization with 70% ethanol for 1 min. For further surface sterilization and to remove adhering microorganisms, the leaf samples were immersed in 4% sodium hypochlorite for 3 min. The leaf samples were then rinsed with 70% ethanol for 1 min. Finally the leaf samples were rinsed with deionised water and blot dried on sterile tissue paper. The leaf samples were cut into 5-10 × 5-10 mm in size using a sterile scalpel. The leaf explants were cultured in petridishes containing Potato Dextrose Agar (PDA) medium supplemented with 100 µg/mL of streptomycin to suppress bacterial growth. Petri dishes were sealed with parafilm and incubated at 27 ± 2°C for 15 days under dark condition and monitored every day. Fungi growing out of the plant explants were subcultured on separate PDA plates and maintained at 4°C. Fungi were identified in their sporulation state by staining with Lactophenol blue. The fungal isolates were identified based on the colony colour, morphology, hyphal structure, spore size and spore bearing structures and compared with standard manuals of endophytic fungi [19-22].

Preparation of fungal extracts

The fungal endophytes *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* were mass cultured by placing agar blocks of actively growing pure culture (5 mm in diameter) in 250 mL Erlenmeyer flasks containing 100 mL of potato dextrose broth. The flasks were incubated at 27 ± 2°C for 14 days with periodical shaking at 150 rpm. After incubation period, the cultures were taken out and used for the preparation of fungal extracts. The ethyl acetate extracts of endophytic fungi *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* were prepared according to the modified method of Raviraja et al. [23]. After mass cultivation of endophytic fungi, the cultures were filtered through four layers of sterile cheese cloth to separate the mycelial mats. Then the culture filtrate was extracted with equal volume of the filtrate and ethyl acetate were taken in separating funnel and shaken vigorously for 15 min. The solutions were then allowed to stand, the cell mass got separated and the organic phase of solvents so obtained, were collected. The solvent ethyl acetate was evaporated and the resultant residue was dried in vacuum evaporator to yield the crude extract (Culture filtrate extract). Mycelial mats were also used for the preparation of ethyl acetate extract followed the same procedure by taking mats instead of filtrate (Mycelial mat extract). After evaporation, the dried extracts of culture filtrate and mycelial mat were equally mixed. The ethyl acetate extracts of *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* were subjected to GC-MS analysis.

GC-MS analysis

The GC–MS analysis was carried out for the screening of phytocompounds present in the ethyl acetate extracts of endophytic fungi *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum*. GC-MS analysis was performed at University Science Instrumentation Centre, AIRF, Jawaharlal Nehru University, New Delhi in GC-MS QP2010 Ultra (Shimadzu, Kyoto, Japan) system with head space sampler (AOC-20s) and auto injector (AOC-20i), equipped with mass selective detector, having ion source temperature of 230°C, interface temperature of 270°C, a solvent cut time of 4.50 min threshold of 1,000 eV and mass range of 40 to 650 m/z. Compounds were separated using a elite-1 fused silica capillary column, having a dimension of 30 × 0.25 mm Id × 0.25 µm df, composed of 5% diphenyl

95% dimethyl poly siloxane, operated in the electron impact mode with ionization energy of 70 eV. The split mode was used at a ratio of 10:1. The temperature of the injector was initialized to 260°C, having a split injection mode. The temperature was programmed from 60°C (3 min hold), then increased to 250°C at a ramp rate of 10°C/min (2 min hold) and further increased to 280°C at a ramp rate of 15°C/min (19 min hold). Helium (99.999%) was used as the carrier gas at a linear flow velocity of 40.1 cm/s. The debit of helium gas vector was fixed to 16.3 mL/min, with a total flow of 1. 21 mL/min. The volume of injected sample was 1.0 µL of extract.

Identification of phytocompounds

The mass spectrum of compounds in the sample was obtained by electron ionization. The components were identified by comparison of their mass spectral fragmentation patterns with those data provided in WILEY8 library and National Institute of Standard and Technology (NIST14) library. The identification was assumed when a good match of mass spectrum and RI was achieved. Interpretation of mass spectra of the ethyl acetate extracts of endophytic fungi *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* were conducted using the database of WILEY8 and NIST14 libraries. The spectrum of the compound was compared with the spectrum of WILEY8 and NIST4 library databases. The identity of the spectra above 95% was needed for the identification of compounds. The name, molecular weight and structure of the compounds of the ethyl acetate extracts of endophytic fungi *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area with the total area. The spectrum of the unknown component was compared with the spectrum of the component stored in the WILEY8 and NIST14 libraries.

Results

The phytochemical constituents of ethyl acetate extracts of endophytic fungi *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* were identified by GC-MS analyses and the results were presented in the Tables 1-3. The GC-MS chromatogram of ethyl acetate extracts of *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* revealed that the presence of various compounds with corresponding peaks at different retention times Figures 1-3. The molecular formula, molecular weight, peak area percent, retention time, nature of the compound and biological activities of phytocompounds of ethyl acetate extracts of *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum* were represented in the Tables 1-3.

The phytocompounds 2-Pentanol, 2,3-dimethyl-; Dodecane, 4,6-dimethyl-; Hexadecane, 2,6,10,14-tetramethyl-; Heneicosane; Hexadecane; Tetradecanoic acid; 9,12-Octadecadienoic acid (Z,Z)-; Octadecanoic acid; Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester; Bis(2-ethylhexyl) phthalate and Octadecanoic acid, 2,3-dihydroxypropyl ester were commonly present in all three ethyl acetate extracts of endophytic fungi *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum*. The phytocompounds such as 3-Ethyl-3-methylheptane; Bicyclo[3.1.0] hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-; Dodecane, 2,6,11-trimethyl-; D-Carvone; Undecane, 3,8-dimethyl-; Caryophyllene; Acetic acid, cinnamyl ester; (7-Bromobicyclo[4.1.0] hept-7-yl)phenylmethanol; Erythro-9,10-Dibromopentacosane; Ergosterol and Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1) were identified in the ethyl acetate extract of *Chaetomium globosum*. The phytocompounds 5-Isopropyl-2-methylbicyclo[3.1.0]hex-2-ene; Propane, 1,1,3-triethoxy-; 2,6-Octadienal, 3,7-dimethyl-, (Z)-; 2-Propenal, 3-phenyl-; Thymol; 2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-; 5-Allyl-2-methoxyphenol; Dodecanoic acid; n-Hexadecanoic acid; 9-Octadecenoic acid, (E)- and Decanedioic acid, bis(2-ethylhexyl) ester were commonly present in the ethyl acetate extracts of *Chaetomium globosum* and *Penicillium janthinellum*.

The phytocompounds such as Acetic acid, 1-methylpropyl ester; Glycerin; 2,5-Furandione, dihydro-3-methyl-; 2-Propanone, 1-phenyl-; 2,6-Dimethyl-6-nitro-2-hepten-4-one; Tetradecane; 1,3-Dioxane, 2,2-dimethyl-5-trimethylsilyloxy-; D-Mannitol, 1,2:5,6-bis-O-(1-methylethylidene)-; N-Didehydrohexacarboxyl-2,4,5-trimethylpiperazine; l-(+)-Ascorbic acid 2,6-dihexadecanoate; 6-Octadecenoicacid,(Z)-;1H-Pyrazole,4-(trimethylsilyl)-;Pentacosane; Bicyclo[2.2.1]heptane, 2-(phenylmethyl)-; 2-Thiophenecarboxamide, N-[2-(4-ethylphenoxy)ethyl]-; Tetracontane; Hexatriacontane; Sebacic acid, di(2-propylpentyl) ester; Aflatoxin d1 and Tetrapentacontane were present in the ethyl acetate extracts of *Cladosporium tenuissimum*. The phytocompounds Eicosane; Ergosta-5,7,22-trien-3-ol, (3.beta.,22e)- and Hexadecanoic acid, methyl ester were commonly present in the ethyl acetate extracts of *Cladosporium tenuissimum* and *Penicillium janthinellum*.

The phytocompounds such as D-Limonene; Gamma – Terpinene; 2-[2-({[Tert-butyl(dimethyl)silyl]oxy}methyl)-2-oxiranyl]; Octadecyl octyl ether; Sorbitol; 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione; Cyclopentadecanone, 2-hydroxy-; Heptadecanoic acid; 4-(4-Oxo-1,2,3,4,6,7,12,12b-octahydropyrido[2,1-a]-.beta.-carbolin-12b-yl)butanoic; 3-Methoxy-12-methyl-D-homoestra-1,3,5(10),8,14-pentaen-17a-one; Octahydrochrom-4,5-dione, 4',8'-epoxy-; Benzoic acid, 4-(pentafluoropropionylamino)-; Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22E)- and (1H-Benzoimidazol-2-yl)-[4-(4-methyl-piperazin-1-yl)-phenyl]-amine were present in the ethyl acetate extracts of Penicillium janthinellum. The biological activities of phytocompounds of ethyl acetate extracts of *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* mentioned in the Tables 1-3 are based on the Phytochemical and Ethnobotanical Databases created by Dr. Duke's of the Agricultural Research Service/ USDA.

Discussion

A number of modern drugs have been isolated from natural sources. Plant derived substances have recently become a great interest owing to their versatile applications. There is a growing awareness in correlating the active principles from the medicinal plants with their biological activities [24]. In this study, the medicinal plants *Passiflora foetida, Memecylon edule* and *Justicia adhatoda* were selected based on the medicinal important and used in the treatment of many diseases. Mature healthy and disease free leaves of medicinal plants *Passiflora foetida, Memecylon edule* and *Justicia adhatoda* were used for isolation of endophytic fungi. The endophytic fungi *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* were isolated and used for GC-MS analysis. GC-MS is an instrumental technique, comprising a Gas Chromatograph [GC] coupled with a Mass Spectrometer [MS] and it is an unanimously accepted method for the analysis of phytoconstituents of herbal medicines, due to their sensitivity, stability and high efficiency [25,26]. The development of pharmaceuticals begins with identification of active principles, detailed biological assays and dosage formulations followed by clinical studies to establish safety, efficacy and pharmacokinetic profile of the new drugs

Name of the compound	Molecular formula	MW	RT	Peak area %	Nature of compound	Activity*
5-Isopropyl-2-methylbicyclo[3.1.0]hex-2-ene	$C_{10}H_{16}$	136	5.617	1.16	Monoterpene	Antioxidant, Antimalarial, Antimicrobial and Herbicidal
3-Ethyl-3-methylheptane	$C_{10}H_{22}$	142	7.980	0.48	Alkane	Antifungal
Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-	$C_{10}H_{180}$	154	8.076	0.46	Monoterpene alcohol	Anti-inflammatory, Sedative, Anticancer, Antitumor, Antibacterial, Antiflu, Nematicide, Insecticide, Pesticide, Herbicide, Immunomodulator, Antiobesity, Detoxicant, Expectorant and Photosensitizer
Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	8.247	4.92	Alkane	Nf
Dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	212	8.760	0.44	Alkane	Nf
2-Pentanol, 2,3-dimethyl-	$C_7H_{16}O$	116	10.213	0.76	Alkyl alcoholic	Nf
2,6-Octadienal, 3,7-dimethyl-, (Z)-	$C_{10}H_{16}O$	152	10.988	0.87	Aromatic	Antimicrobial
D-Carvone	$C_{10}H_{14}O$	150	1.115	0.96	Terpenoids	Antioxidant
Undecane, 3,8-dimethyl-	$C_{13}H_{28}$	184	11.333	0.28	Alkane	Nf
2-Propenal, 3-phenyl-	C_9H_8O	132	11.541	9.39	Aromatic aldehyde	Antimicrobial and Anti-inflammatory
Thymol	$C_{10}H_{14}O$	150	11.737	2.91	Essential oil	Antibacterial, Antifungal, Vermifuge
2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-	$C_{10}H_{14}O$	150	11.873	13.50	Cyclic compound	Nf
Dodecane, 4,6-dimethyl-	$C_{14}H_3O$	198	12.192	0.40	Alkane	Nf
5-Allyl-2-methoxyphenol	$C_{10}H_{12}O_2$	164	12.657	4.46	Aromatic	Antiseptic and Anesthetic
Caryophyllene	$C_{15}H_{24}$	204	13.694	0.36	Bicyclic sesquiterpene	Antinociceptive, Antidepressant, Neuroprotective, Anxiolytic and Anti-alcoholism
Acetic acid, cinnamyl ester	$C_{11}H_{12}O_2$	176	13.880	0.61	Aromatic	Anticholesterolemic, Antifungal, Antihyperglycemic and Antimalarial
(7-Bromobicyclo[4.1.0]hept-7-yl) phenylmethanol	$C_{14}H_{17}BrO$	280	14.360	0.15	Cyclic bromo compound	Nf
Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	282	14.426	0.61	Alkane	Nf
Heneicosane	$C_{21}H_{44}$	296	14.992	0.54	Hydrocarbon	Antibacterial and Pheromone
Dodecanoic acid	$C_{12}H_{24}O_2$	200	15.244	0.60	Fatty acid	Cancer preventive and Insectifuge
Hexadecane	$C_{16}H_{34}$	226	15.790	0.38	Aliphatic hydrocarbon	Antibacterial, Antifungal and Antioxidant
Tetradecanoic acid	$C_{14}H_{28}O_2$	228	17.563	6.71	Myristic acid	No activity
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	19.652	9.35	Fatty acid	Anti-inflammatory, Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic, Hemolytic, 5-Alpha reductase inhibitor and Larvicidal
9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	21.297	3.52	Fatty acid	Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic and Anticoronary
9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282	21.352	4.25	Fatty acid	Antihyperglycemic, Immunosuppressant, Alzheimer's disease treatment, Antipruritic, Antiprotozoal , Antineurogenic and Anti-inflammatory
Octadecanoic acid	$C_{18}H_{36}O_2$	284	21.558	2.18	Fatty acid	Cancer preventive and Insectifuge
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	25.181	5.71	Fatty acid ester	Hemolytic, Pesticide and Antioxidant
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	25.395	12.22	Diester of phthalic acid	Antifungal
Erythro-9,10-Dibromopentacosane	$C_{25}H_5OBr_2$	508	26.003	0.50	Bromo compound	Nf
Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358	27.072	3.26	Fatty acid ester	Nf
Decanedioic acid, bis(2-ethylhexyl) ester	$C_{26}H_{50}O_4$	426	27.858	0.52	Fatty acid ester	Nematicidal , Anticancer, Antioxidant and Antimicrobial
Ergosterol	$C_{28}H_{44}O$	396	34.279	2.27	Sterol	Antitumor
Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	$C_{42}H_{63}O_3Pi$	646	39.747	2.78	Phenolics	Nf

Abbreviation: RT: Retention Time; MW: Molecular Weight; Nf: Not found
*Dr. Duke's Ethnobotanical databases

Table 1: List of identified phytocompounds of ethyl acetate extracts of endophytic fungus *Chaetomium globosum* by GC-MS analysis

Name of the compound	Molecular formula	MW	RT	Peak area %	Nature of compound	Activity*
Acetic acid, 1-methylpropyl ester	$C_6H_{12}O_2$	116	5.350	0.37	Ester	Nf
Glycerin	$C_3H_8O_3$	92	6.241	0.59	Alcoholic	Antimicrobial and Antiseptic
2,5-Furandione, dihydro-3-methyl-	$C_5H_6O_3$	114	7.314	0.59	Furanone	Nf
2-Propanone, 1-phenyl-	$C_9H_{10}O$	134	9.223	0.21	Acetone	Nf
2,6-Dimethyl-6-nitro-2-hepten-4-one	$C_9H_{15}NO_3$	185	9.467	0.34	Nitro compound	Nf
2-Pentanol, 2,3-dimethyl-	$C_7H_{16}O$	116	10.205	2.48	Alcoholic	Antimicrobial
Dodecane, 4,6-dimethyl-	$C_{14}H_3O$	198	11.521	0.31	Alkane	Nf
Tetradecane	$C_{14}H_3O$	1413	13.269	0.23	Alkane	Nf
Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	282	14.442	0.66	Diterpenoid alkane	Antimicrobial and antioxidant
Eicosane	$C_{20}H_{42}$	282	15.114	0.46	Alkane	Antitumour
Hexadecane	$C_{16}H_{34}$	226	15.787	0.39	Aliphatic hydrocarbon	Antibacterial, Antifungal and Antioxidant
1,3-Dioxane, 2,2-dimethyl-5-trimethylsilyloxy-	$C_9H_{20}O_3Si$	204	16.025	0.30	Silicon	Nf
D-Mannitol, 1,2:5,6-bis-O-(1-methylethylidene)-	$C_{12}H_{22}O_6$	262	16.780	1.51	Alcoholic sugar	Nf
Tetradecanoic acid	$C_{14}H_{28}O_2$	228	17.549	1.61	Myristic acid	Larvicidal, Repellent, Cosmetic and Topical medicine preparations for skin diseases
Heneicosane	$C_{21}H_{44}$	296	18.039	0.37	Alkane	Anti-inflammatory, Antiasthmatics, Urine Acidifiers and Antimicrobial
N-Didehydrohexacarboxyl-2,4,5-trimethylpiperazine	$C_{13}H_{22}N_2O$	222	18.739	8.47	Nitro compound	Nf
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	19.320	0.48	Fatty acid ester	Hypercholesterolemic, Lubricant, Antimicrobial, Flavor, Cosmetic and Perfumery
l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	19.643	3.99	Vitamin C derivative	Antioxidant, Antiscorbutic, Antiinflammatory, Antinociceptive, Antimutagenic and Wound healing
9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	21.291	0.59	Fatty acid	Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic and Anticoronary
6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282	21.344	0.42	Fatty acid	Antihyperglycemic, Antipruritic, Antiprotozoal, Antineurogenic and Antiinflammatory
Octadecanoic acid	$C_{18}H_{36}O_2$	282	21.557	1.02	Fatty acid	Antimicrobial
1H-Pyrazole, 4-(trimethylsilyl)-	$C_6H_{12}N_2Si$	140	21.616	0.96	Heterocyclic nitrocompound	Nf
Pentacosane	$C_{25}H_{52}$	352	21.937	1.84	Aliphatic hydrocarbon	Antibacterial
Bicyclo[2.2.1]heptane, 2-(phenylmethyl)-	$C_{14}H_{18}$	186	22.298	2.05	Cyclic hydrocarbon	Nf
2-Thiophenecarboxamide, N-[2-(4-ethylphenoxy)ethyl]-	$C_{15}H_{17}NO_2S$	275	22.701	0.93	Sulphur compound	Nf
Tetracontane	$C_{40}H_{82}$	562	25.084	8.32	Aliphatic hydrocarbon	Antibacterial
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	25.177	2.47	Fatty acid ester	Hemolytic, Pesticide, Flavor and Antioxidant
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	25.394	16.93	Phthalic acid	Antimicrobial and Antioxidant
Hexatriacontane	$C_{36}H_{74}$	506	26.004	6.37	Alkane	Nf
Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358	27.069	2.05	Fatty acid ester	Anti-inflammatory and CNS depressant activity
Sebacic acid, di(2-propylpentyl) ester	$C_{26}H_{50}O_4$	426	27.857	0.19	Diester of *sebacic acid*	Plasticizers
Aflatoxin d1	$C_{16}H_{14}O_5$	286	27.913	6.61	Toxin	Mutagenic and Carcinogenic
Tetrapentacontane	$C_{54}H_{11}O$	758	29.080	1.54	Alkane	Nf
Ergosta-5,7,22-trien-3-ol, (3.beta.,22e)-	$C_{28}H_{44}O$	396	34.297	1.26	Steroid	Antifungal and Antibacterial

Abbreviation: RT: Retention Time; MW: Molecular Weight; Nf: Not found
*Dr. Duke's Ethnobotanical databases

Table 2: List of identified phytocompounds of ethyl acetate extracts of endophytic fungus *Cladosporium tenuissimum* by GC-MS analysis

[27]. More than 20,000 bioactive metabolites are of microbial origin with various bioactive properties [28]. So, there is a great opportunity to utilize fungal endophytes as a new source for production of reliable and novel antimicrobial and antioxidant agents. These bioactive compounds could be a promising way to solve the problem of microbial resistance to commonly used drugs and meet the emergency demand of discovering highly effective, low toxicity, and environmental friendly antibiotics in future.

The gas chromatogram of ethyl acetate extracts of *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* showed that the relative concentration of various compounds getting

Name of the compound	Molecular formula	MW	Peak area %	RT	Nature of compound	Activity*
5-Isopropyl-2-methylbicyclo[3.1.0]hex-2-ene	$C_{10}H_{16}$	136	1.32	5.612	Monoterpene	Antibacterial, Antioxidant, Antimalarial, Antimicrobial and Herbicidal
D-Limonene	$C_{10}H_{16}$	136	0.24	7.553	Monoterpenoid	Antitumor and Anticarcinogenic
Gamma – Terpinene	$C_{10}H_{16}$	136	0.25	8.069	Monoterpene	Anti-inflammatory, Antioxidant, Antimicrobial and Anti-proliferative
Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	2.10	8.242	Ether	Nf
2-Pentanol, 2,3-dimethyl-	$C_7H_{16}O$	116	0.58	10.211	Alcoholic compound	Nf
2,6-Octadienal, 3,7-dimethyl-, (Z)-	$C_{10}H_{16}O$	152	0.21	10.983	Aroma compound	Antimicrobial
2-Propenal, 3-phenyl-	C_9H_8O	132	2.43	11.533	Aldehyde	Antioxidant and Antimicrobial
Thymol	$C_{10}H_{14}O$	150	0.85	11.733	Phenol	Antibacterial, Antifungal and Vermifuge
2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-	$C_{10}H_{14}O$	150	4.27	11.866	Cyclic hexane	Nf
Dodecane, 4,6-dimethyl-	$C_{14}H_3O$	198	0.38	12.187	Alkane	Nf
5-Allyl-2-methoxyphenol	$C_{10}H_{12}O_2$	164	1.27	12.651	Oxy compound	Anti-inflammatory, Antimicrobial and Antinioceptive
2-[2-({[Tert-butyl(dimethyl)silyl] oxy}methyl)-2-oxiranyl]	$C_{13}H_{26}O_4Si$	274	0.69	14.217	Silicon	Nf
Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	282	0.48	14.422	Diterpenoid alkane	Antimicrobial and Antioxidant
Octadecyl octyl ether	$C_{26}H_{54}O$	382	0.13	14.776	Ether	Nf
Eicosane	$C_{20}H_{42}$	282	0.63	14.988	Alkane	Antitumour
Dodecanoic acid	$C_{12}H_{24}O_2$	200	0.54	15.237	Saturated fatty acid (Capric acid)	Nf
Hexadecane	$C_{16}H_{34}$	226	0.23	15.787	Aliphatic hydrocarbon	Ovulation inhibitor, Antianginal, Kidney function stimulant and Cognition disorders treatment
Tetradecanoic acid	$C_{14}H_{28}O_2$	228	1.62	17.545	Saturated fatty acid (Myristic acid)	Antioxidant, Lubricant, Hypercholesterolemic, Cancer preventive and Cosmetic
Heneicosane	$C_{21}H_{44}$	296	0.29	18.036	Alkane	Anti-inflammatory, Antiasthmatics, Urine acidifiers and Antimicrobial
Sorbitol	$C_6H_{14}O_6$	182	1.71	18.612	Alcoholic sugar	Antibacterial, Cryoprotective and Cosmetic agent
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	0.08	19.167	Keto acid	Nf
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.30	19.316	Fatty acid ester	Antioxidant, Decrease blood cholesterol and Anti-inflammatory
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	10.87	19.656	Saturated fatty acid (Palmitic acid)	Antioxidant, Nematicide, Pesticide, Hypocholesterolemic, Lubricant, Antiandrogenic, Hemolytic, 5-Alpha reductase inhibitor and Antipsychotic
Cyclopentadecanone, 2-hydroxy-	$C_{15}H_{28}O_2$	240	0.49	20.412	Cyclic Compound	Nf
Heptadecanoic acid	$C_{17}H_{34}O_2$	270	0.50	20.615	Fatty acid	Antioxidant, Antifungal and Surfactant
9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	11.97	21.310	Unsaturated fatty acid (Linoleic acid)	Hypocholesterolemic, 5-Alpha reductase inhibitor, Antihistaminic, Insectifuge, Antieczemic and Antiacne
9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282	10.69	21.363	Unsaturated fatty acid	Antifungal, Antitumour, Antibacterial, Antipruritic, Antiprotozoal, Antineurogenic and Antiinflammatory
Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.53	21.561	Saturated fatty acid (Stearic acid)	Antibacterial, Soap Lubricant and Cosmetics
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330	3.05	25.175	Fatty acid ester	Hemolytic, Pesticide, Flavor and Antioxidant
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	9.83	25.392	Diester of phthalic acid	Antifungal
4-(4-Oxo-1,2,3,4,6,7,12,12b-octahydropyrido[2,1-a]-.beta.-carbolin-12b-yl)butanoic	$C_{19}H_{22}N_2O_3$	326	0.58	25.563	Pyrrole compound	Nf
3-Methoxy-12-methyl-D-homoestra-1,3,5(10),8,14-pentaen-17a-one	$C_{21}H_{24}O_2$	308	0.22	25.851	Steroid	Nf
Octahydrochrom-4,5-dione, 4',8'-epoxy-	$C_{18}H_{28}O_4$	308	0.34	25.997	Oxy compound	Nf
Octadecanoic acid, 2,3- dihydroxypropyl ester	$C_{21}H_{42}O_4$	358	2.36	27.076	Fatty acid ester	Phospholipase A2 inhibitor
Benzoic acid, 4-(pentafluoropropionylamino)-	$C_{10}H_6F_5NO_3$	283	5.79	27.222	Tricyclic fluoro compound	Nf
(1H-Benzoimidazol-2-yl)-[4-(4-methyl-piperazin-1-yl)-phenyl]-amine	$C_{18}H_{21}N_5$	307	8.62	27.436	Imidazole	Nf
Decanedioic acid, bis(2-ethylhexyl) ester	$C_{26}H_{50}O_4$	426	0.47	27.857	Ester	Nf
Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22E)-	$C_{28}H_{42}O$	394	0.55	30.049	Steroid	Antibacterial and Cytotoxicity
Ergosta-5,7,22-trien-3-ol, (3.beta.,22e)-	$C_{28}H_{44}O$	396	5.83	34.278	Steroid	Antibacterial

Abbreviation: RT: Retention Time; MW: Molecular Weight; Nf: Not found
*Dr. Duke's Ethnobotanical databases

Table 3: List of identified phytocompounds of ethyl acetate extracts of endophytic fungus *Penicillium janthinellum* by GC-MS analysis

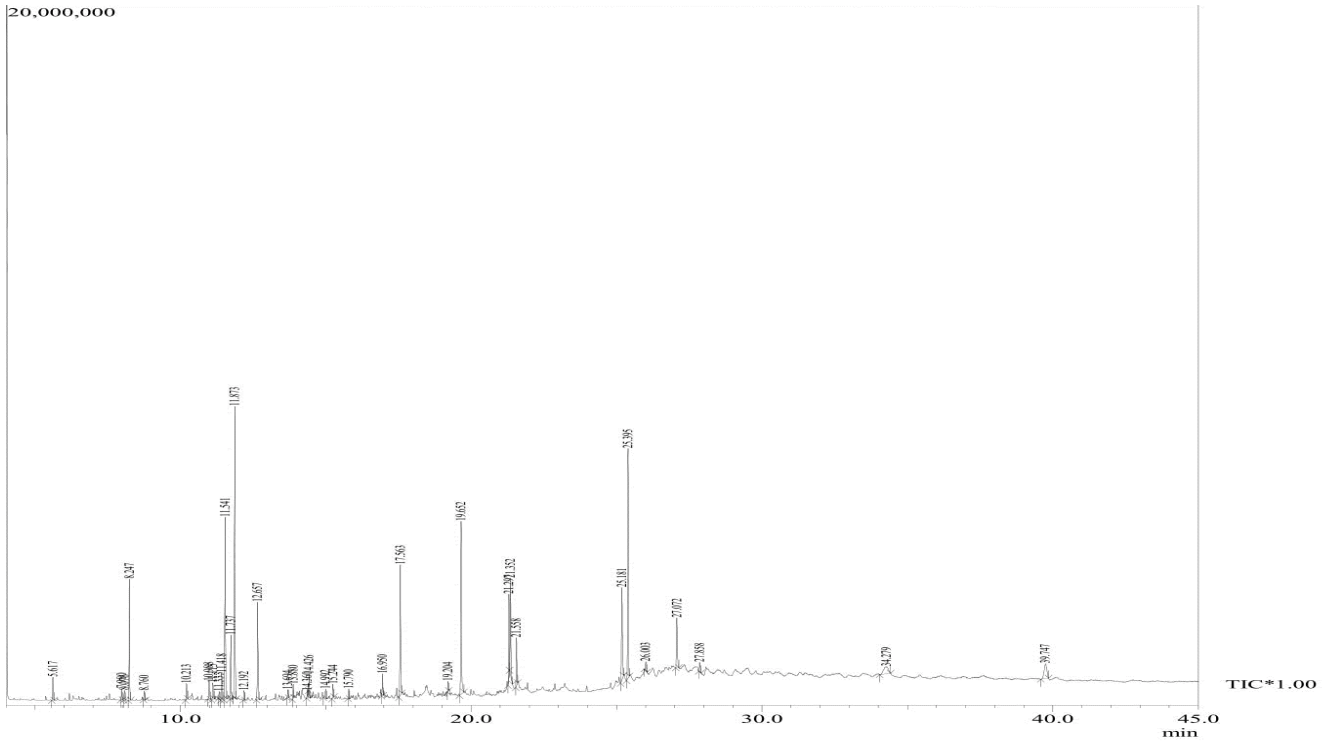

Figure 1: GC-MS Chromatogram of ethyl acetate extract of *Chaetomium globosum*

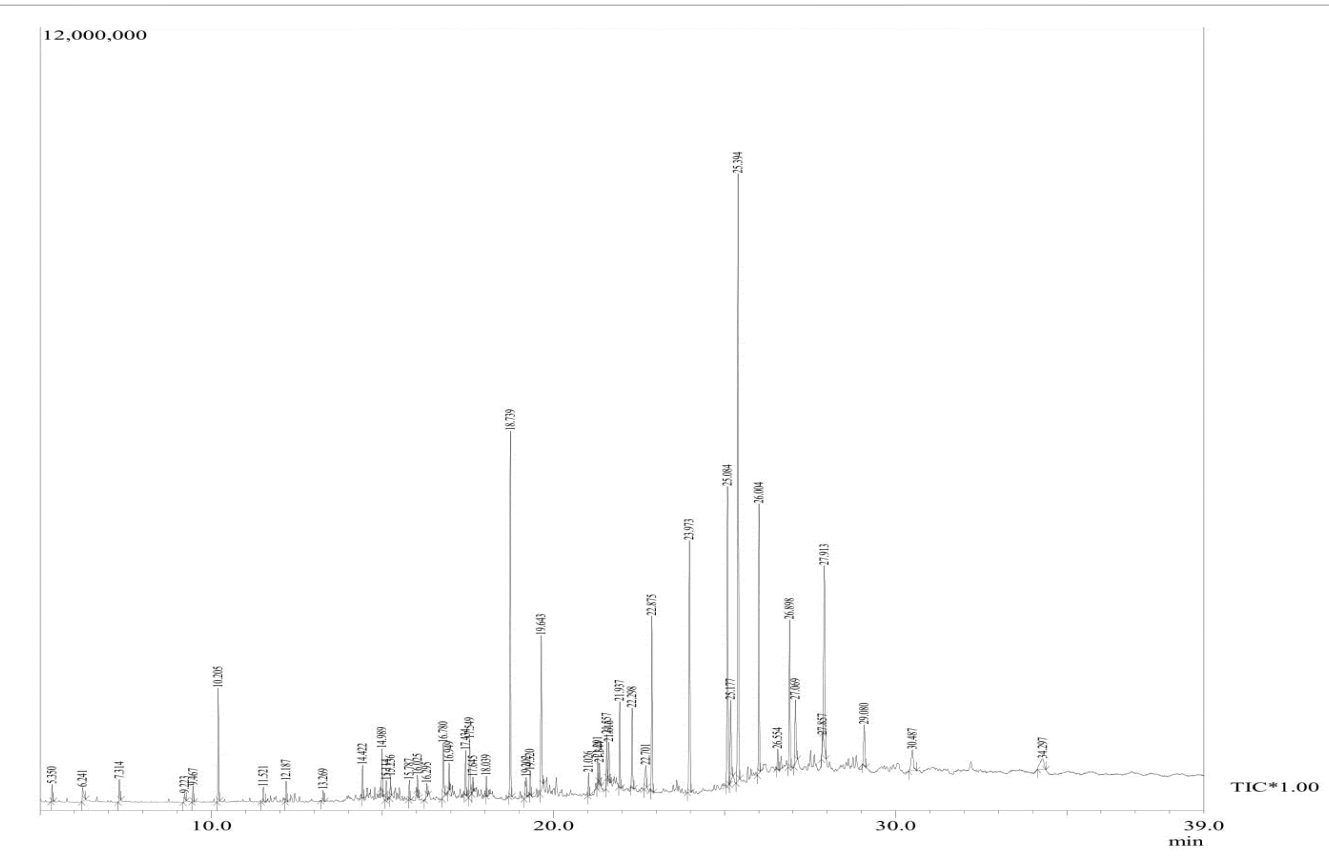

Figure 2: GC-MS Chromatogram of ethyl acetate extract of *Cladosporium tenuissimum*

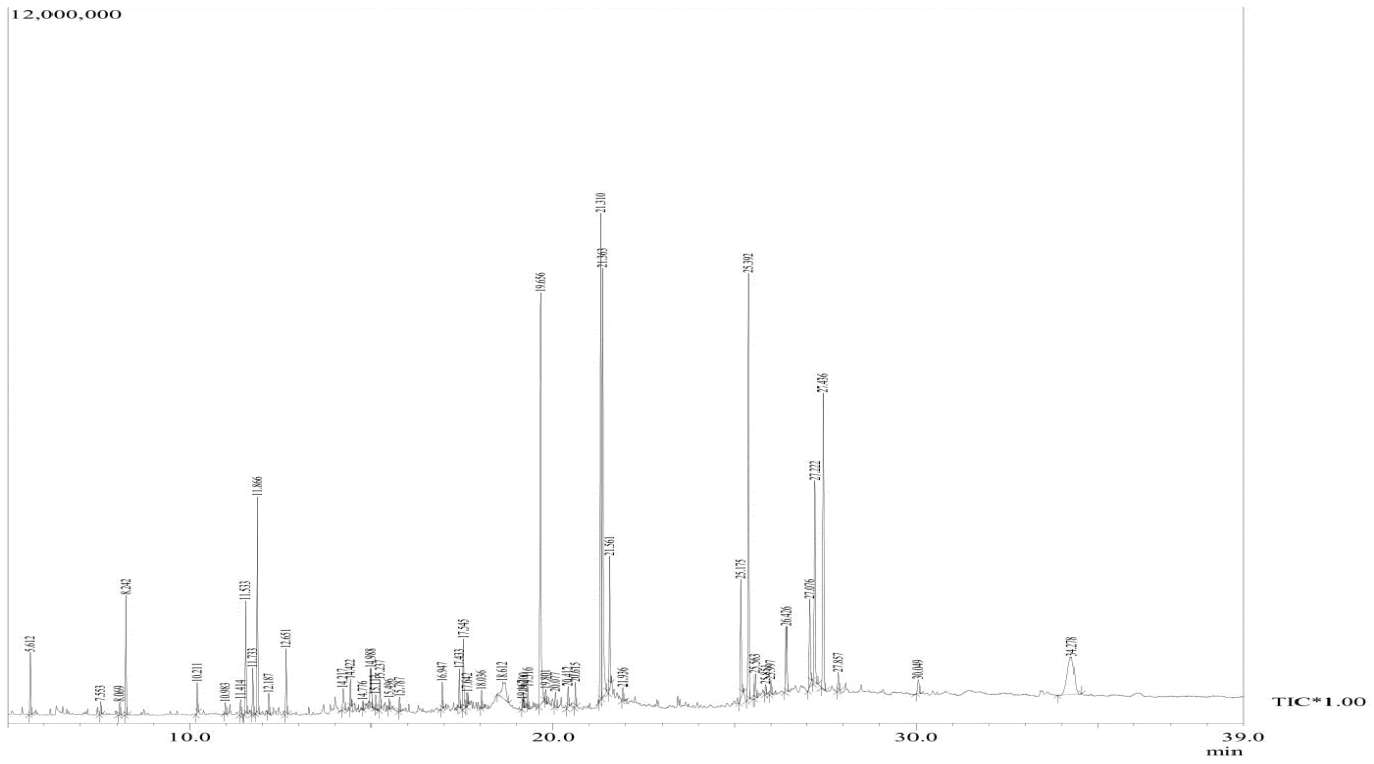

Figure 3: GC-MS Chromatogram of ethyl acetate extract of *Penicillium janthinellum*

eluted at different retention time. The height of the peak indicates that the relative concentrations of the component present in the ethyl acetate extracts of *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum*. The mass spectrometer analysis was used to identify the nature and structure of the compounds in the ethyl acetate extracts of *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* eluted at different retention time. The mass spectra are the fingerprints of phytocompounds of *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum*, which were identified by using the WILEY8 and NIST14 library. Similarly, the bioactive compounds of Dolichandrone atrovirens [29] and Indian seagrasses [30] were analysed by GC-MS and the mass spectra of the compounds were matched with NIST library.

In this study, the GC-MS analyses showed that the presence of phytocompounds in ethyl acetate extracts of endophytic fungi *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum*. The important biologically active phytocompounds Bicyclo[3.1.0]hexan-2-ol,2-methyl-5-(1-methylethyl)-, (1.alpha.,2. alpha.,5.alpha.)-; Caryophyllene; n-Hexadecanoic acid; 9,12-Octadecadienoic acid (Z,Z)-; 9-Octadecenoic acid, (E)- and Decanedioic acid, bis(2-ethylhexyl) ester were identified in the extract of *Chaetomium globosum*. The phytocompounds including Tetradecanoic acid; Tetradecanoic acid; Heneicosane; Hexadecanoic acid, methyl ester; l-(+)-Ascorbic acid 2,6-dihexadecanoate; 9,12-Octadecadienoic acid (Z,Z)-; 6-Octadecenoic acid, (Z)- and Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester were present in the ethyl acetate extracts of *Cladosporium tenuissimum*. There are many phytocompounds were identified in the ethyl acetate extracts of *Penicillium janthinellum* including 5-Isopropyl-2-methylbicyclo[3.1.0]hex-2-ene; Gamma-Terpinene; 5-Allyl-2-methoxyphenol; Hexadecane; Tetradecanoic acid; Heneicosane; Hexadecanoic acid, methyl ester; n-Hexadecanoic acid; Heptadecanoic acid; 9,12-Octadecadienoic acid (Z,Z)-; 9-Octadecenoic acid, (E)- and Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester.

The nature of identified bioactive compounds of *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* are phenolic compounds, flavonoids, fatty acids, sugar alcohols, terpenoids, aldehydes, steroids, nitro compounds and aroma compounds. The prediction of the biological activities of phytocompounds was identified in the ethyl acetate extracts of *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* by applying the Duke's ethnobotanical databases. In the Duke's ethnobotanical databases, the identified phytocompounds are reported to possess antifungal, antimalarial, antioxidant, antibacterial, antitumor, anti-inflammatory, hypocholesterolemic, anticancer, diuretic, antihyperglycaemic, nematicide, insecticide, antihistaminic, antieczemic, antiacne, antiarthritic, anticoronary, antiprotozoal, antineurogenic, hemolytic and analgesic activities. So, the presence of above mentioned phytocompounds in the ethyl acetate extracts of *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum* may be responsible for controlling diseases, when people are using the leaves of plants *Passiflora foetida, Memecylon edule* and *Justicia adhatoda* as medicine.

Similarly, many researchers reported that the phytocompounds of endophytic fungi with various pharmacological properties were isolated from medicinal plants. The endophytic fungus *Curvularia lunata* isolated from *Niphates olemda*, was found to produce cytoskyrins, which showed antibacterial activity, and is considered as a potential anticancer

agent [31,32]. An endophytic fungus *Phoma medicaginis* associated with medicinal plants *Medicago sativa* and *Medicago lupulina*, yielded the antibiotic brefeldine A, which also initiated apoptosis in cancer cells [33]. Ergoflavin is a dimeric xanthene, belonging to the class of ergochromes, and was described as a novel anticancer agent isolated from an endophytic fungi growing in the leaves of medicinal plant *Mimusops elengi* [34]. Secalonic acid D was isolated from the mangrove endophytic fungus and showed high cytotoxicity by inducing leukemia cell apoptosis [35]. About 1500 fungal metabolites had been reported to show antitumor and antibiotic activity [36] and some have been approved as drugs. Endophytes produce bioactive metabolites that may be involved in the host-endophyte relationship [37], and may serve as potential sources of novel natural products for exploitation in medicine, agriculture, and industry [38,39]. The presence of phytocompounds is varied in between the ethyl acetate extracts of endophytic fungi *Chaetomium globosum*, *Cladosporium tenuissimum* and *Penicillium janthinellum* from the leaves of medicinal plants *Passiflora foetida, Memecylon edule* and *Justicia adhatoda*. The difference in endophytes with their metabolic profile and biological activity might be related to the chemical difference of host plants [40]. This depends on the environment, and shows the importance of studying host endophytes relationships, and the effect of host plants on endophytic metabolites production. So, the endophytic fungi have been proven useful for novel drug discovery as suggested by the chemical diversity of their secondary metabolites.

The beneficial roles of these bioactive phyto-compounds may be useful in the pharmaceutical and food industries for the production of drugs. Based on the ethno-botanical database, the compounds such as furan, phenols, flavonoids and fatty acid esters possess antioxidant, antibacterial, antimicrobial, anti-inflammatory, anti-proliferative, anticancer, antitumor, antidiabetic, antiarthritic and antimalarial activities [41,42]. Similarly, in the present study, the phytocompounds furanose, phenols, flavonoids, alkaloids, terpenoids, steroids and fatty acid esters were observed in the ethyl acetate extracts of *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum*. So, the presence of these compounds in *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* implies a possibility of exhibiting pharmacological activities.

The researchers were reported many of secondary microbial metabolites which showed potent pharmaceutical application against various diseases [43,44]. In the United States of America, more than 50% of prescribed drugs are natural products or semisynthetic derivatives. In addition, a number of chemicals used in crop protection are also of natural origin [45]. Thus natural sources make a very significant contribution to the health care like the medicinal drugs from fungi such as the antibiotic penicillin from *Penicillium* sp., the immunosuppressant cyclosporine from *Tolypocladium inflatum* and *Cylindrocarpon lucidum*, the antifungal agent griseofulvin from *Penicillium griseofulvum*, the cholesterol biosynthesis inhibitor lovastatin from *Aspergillus terreus*, and beta lactam antibiotics from various fungal taxa, has shifted the focus of drug discovery from plants to microorganisms. Suryanarayanan et al. discussed many fungal secondary metabolites with various chemical structures and their wide ranging biological activities. The fungal secondary metabolites and their synthesis in fungi were reported [46].

Many endophytic fungi have been reported to produce novel metabolites of biological interest, such as antibacterial, antifungal, antiviral, anti-inflammatory, and antitumor compounds belonging to the alkaloids, steroids, flavanoids and terpenoids derivatives [47,48]. Similarly, the results of GC-MS profile of ethyl acetate extracts of endophytic fungi *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* showed that the presence of many biologically active phytocompounds including phenolic compounds and flavonoids. So, the production of natural metabolites from endophytic fungi may also help to protect the natural resources and to satisfy the requirement of drugs *via* production of plant derived natural metabolites by fermentation.

Conclusion

The results of this study confirmed that the presence of biologically active phytocompounds in ethyl acetate extracts of fungal endophytes *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum* by GC-MS analyses, which may also be responsible for the pharmacological properties of the host medicinal plants *Passiflora foetida, Memecylon edule* and *Justicia adhatoda*. The endophytic fungi from medicinal plants are easily cultured in the laboratory for production of secondary metabolites through fermentation process instead of harvesting plants and affecting the environmental biodiversity. So, this study may be useful for pharmaceutical industry to produce biologically active phytocompounds from endophytic fungi. The further study is needed to identify the exact active compound possess therapeutic value and it may be helpful to find new drug for treating diseases.

Conflict of Interest

The authors have declared that there is no conflict of interest exists.

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