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GC-MS Profiling of Secondary metabolites of Endophytic fungi *Chladosporium chalastosporoides* isolated from *Euphorbia laciniata* Panigrahi

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ABSTRACT
Endophytes are the potential sources for new remedies since they are the reservoirs of bioactive secondary metabolites. In the present study, endophytic fungi Chladosporium chalastosporoides isolated from Euphorbia laciniata was investigated. Ethyl acetate extract of the endophytic fungal metabolite was screened by Gas Chromatography - Mass Spectrometry technique. The identified major compounds are 3-Tetradecene, 7-Hexadecene, 1-Dodecanol, 1-Dodecene, 5-Octadecene, 9-Octadecene, 3-Tetradecene, Cyclotetradecane, 1-Tridecene, 3-Tetradecene, 1-Docosene, Cetene, Nonacos-1-ene, 9-Eicosene, 5-Eicosene, 1-Nonadecene, Pentacos-1-ene, 2-Tetradecanol, Oxalic acid, allylpentadecyl ester etc., These findings suggest that potential bioactive compounds could be used in the development of antimicrobial agents that can be used in pharmaceuticals and agrochemicals. Investigation of secondary metabolites from endophytic fungi are can be explored for new drug discovery.
Keywords: Endophytic fungi, *Chladosporium chalastosporoides*, *Euphorbia laciniata*, Bioactive compounds, Gas Chromatography - Mass Spectrometry (GC-MS Profiling).

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INTRODUCTION

Plants used in traditional medicine have played a very important role in the search for new bioactive strains of endophytic fungi, as it is possible that their beneficial characteristics are a result of the metabolites produced by their endophytic community [1]. Endophytic fungi have been reported from various plant species, which contribute to the diversity of microorganisms in innate environment and produce various bioactive compounds and novel metabolites [2,3]. The bioactive metabolites produced by endophytic fungi originate from different biosynthetic pathways and belong to diverse structural groups such as terpenoids, steroids, quinones, phenols, coumarins etc., therefore these are the chemical reservoir for new compounds such as, anticancer, immunomodulatory, antioxidant, antiparasitic, antiviral, antitubercular, insecticidal etc. for use in the pharmaceutical and agrochemical industries.

Endophytic fungi are source for extraction of medicinally important metabolites has been gaining increasing interest. Even then endophytes remain as least explored group of microbes. Metabolites produced by endophytes are being recognized as a versatile arsenal of antimicrobial agents. Endophyte *Taxomyces andreanae* produced bioactive compound like Taxol [4]. Diversity of endophytes 52 diterpenoid compound with anticancer properties, which was first isolated from *Taxus brevifolia* [5]. Endophytes have gained importance as components of screening program for therapeutics. The endophytic fungi are of biotechnological importance as sources of new pharmaceutical compounds. Anticancerous compound Camptothecin identified by *Nothapodytes foetida* and by 22-oxa-(12)-cytochalasins by *Rhinocladiella* sp. endophytic fungi [6,7]. Endophytic bioactive compounds used as agrochemicals viz., Naphthalene and Pestasol Insect repellent compounds isolated by the endophytic fungi *Muscodor vitigenus*, *Drechslera* sp. respectively [8,9].

MATERIAL AND METHODS

Isolation and Identification of Endophytic Fungi

Plant material

Healthy symptomless plant samples were collected from Karigatta hill (12°25′05″N 76°43′17″E) located in the Srirangapatna, Mandya district, Karnataka, India. Samples placed in sterile zipped bags and carried aseptically to the laboratory.

Isolation of endophytic fungi

Samples were washed under running tap water and surface sterilization was carried out by immersion in 70% ethanol for 1 min, 5% sodium hypochlorite for 5 min, 70 % ethanol for 1 min and sterile distilled water for 1 min for two times, then allowed to dry under sterile conditions. The leaf samples were then inoculated on water agar media plates and incubated at 28° C. Individual fungal colonies were transferred onto PDA plates.

Identification of endophytic fungi

The identifications of the endophytic fungi were based on their morphology and mechanism of spore production. The promising fungus was identified by using molecular techniques [10].

Preparation of crude extracts

The isolated fungal endophyte was cultivated in a 500 ml conical flask containing 200ml of sterilized PDB and incubated at 28°C for 28 days until the stationary phase was reached. After incubation, the cultures were filtered through muslin cloth. Afterwards, the supernatant was mixed with an equal volume of ethyl acetate (1:1 v/v) in a separating funnel and mixed thoroughly for 15-20 min. then ethyl acetate phase with metabolites was collected in a conical flask [11] and this was repeated twice followed by evaporation using a Rotary flash evaporator and stored in vials for further use.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The ethyl acetate fungal extract was subjected to GCMS analysis to analyze various metabolites present in it. The Clarus 680 GC was used in the analysis employed a fused silica column, packed with HP-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250μm df) and the components were separated using Helium as carrier gas at a constant flow of 2 ml/min. The injector temperature was set at 280°C during the chromatographic run. Individual peaks of compounds were assigned by comparing their RI (retention indices) and MS (mass spectra) by computer matching against NIST (National Institute of Standards and Technology) library [12].

RESULTS AND DISCUSSION

A total of 11 endophytes were isolated from *Euphorbia laciniata.* Among them *C. chalastosporoides* exhibited an effective antimicrobial activity against bacterial and fungal strains. Hence, *C. chalastosporoides* was selected for secondary metabolite analysis.

There are many studies on endophytic fungi from various plants; this is the first report on endophytic fungi *C. chalastosporoides* secondary metabolite screening from *Euphorbia laciniata* Panigrahi.

GC-MS profiling of metabolites from ethyl acetate culture filtrate of *C. chalastosporoides*

In this study ethyl acetate was used as extraction solvent since it is the most efficient method for obtainment of fungal secondary metabolites. The ethyl acetate extract of *C. chalastosporoides* was characterized and identified by GC-MS analysis. The interpretation on mass spectrum GC-MS was conducted using the database of National institute standard and technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The active principles with their molecular formula, molecular weight, exact mass and NIST numberare represented in Fig.1 and Table 1.

The GC-MS analysis of endofungal extract revealed that the presence of 3-Tetradecene, 7-Hexadecene, 1-Dodecanol, 1-Dodecene, 5-Octadecene, 9-Octadecene, Cyclotetradecane, 1-Tridecene, 3-Tetradecene, 1-Docosene, Cetene, Nonacos-1-ene, 9-Eic9-Eicosene, 5-Octadecene, 5-Eicosene, 3-Octadecene, 1-Nonadecene, 3-Eicosene, 1-Docosene, Pentacos-1-ene, 7-Hexadecene, 2-Tetradecanol, 2-Dodecanol, Pentacos-1-ene, Oxalic acid- allyl pentadecyl ester, Oxalic acid- allyl hexadecyl ester. (Table 1 and 2). Most of the identified compounds possessed many biological properties.

Whereas the GC-MS analysis of ethyl acetate extract of *Cladosporium cladosporioides* isolated from *Cymbopogon martinii* (Roxb.) has shown the presence of important compounds like hexadecane, octadecane, eicosane, 2,4-ditert-butyl-phenol, hexadecanal, heptadecanal, tricosane , geraniol, geranyl acetate and cubenol [13]. Dodecene was obtained as a major compound from an endophytic fungus *Fusarium solani* isolated from *Taxus baccata* bark [14]. 1-Hexadecene, Phenol, 2, 4-bis (1,1- dimethyl ethyl) and eicosene was reported in ethyl acetate fraction of the fungi *Monochaetia kansensis* as main compounds identified by GCMS [15].

The latex of *Argemone ochroleuca* which also contain cyclohexasiloxane, dodecamethyl- (5.607%) could be a good source of antifungal agent against *Drechslerahalodes* and *Candida* sp. [16]. Cycloheptasiloxane, tetradecamethyl is used as anticaking and skin conditioning agent reported in ethyl acetate extract of the *Aspergillus tamari* [17]. Gas chromatography-mass spectrometry was utilized to identify 14 volatile organic compounds released by *Gymnascella thermotolerans* which having antifungal and anticancer properties [18].

Recent discoveries in the area of these secondary metabolites from endophytic fungi has risen the promise towards using such organisms for the production ofbioactive substances of medicinal value. In this study, the GC-MS profiling of secondary metabolites from *Euphorbia laciniata* from *C. chalastosporoides* provided crucial bioactive compounds. This comprehensive analysis serves as a foundation for further investigation and the development of therapeutically important compounds.

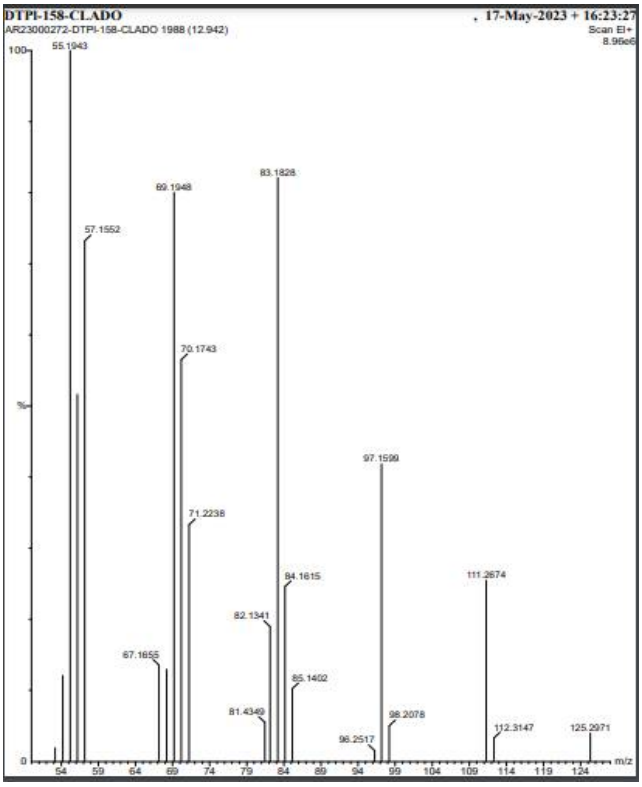


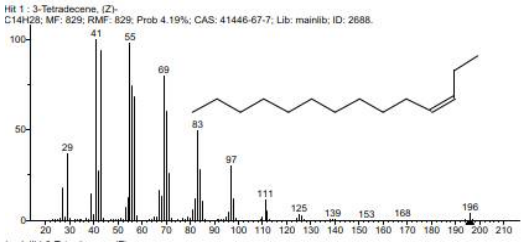
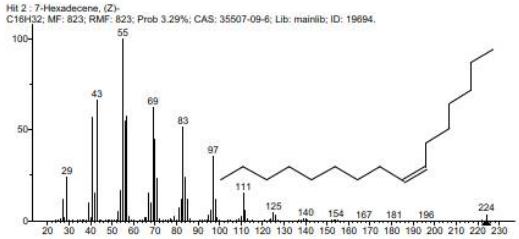
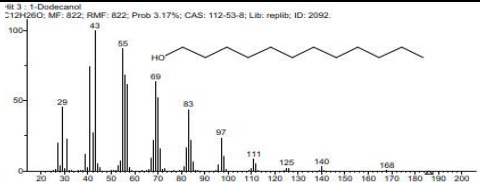
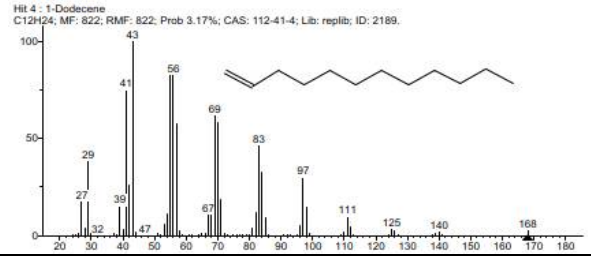
Figure 1. GC Chromatogram showing different compounds present in Ethyl acetate culture filtrate of *C. chalastosporoides.*

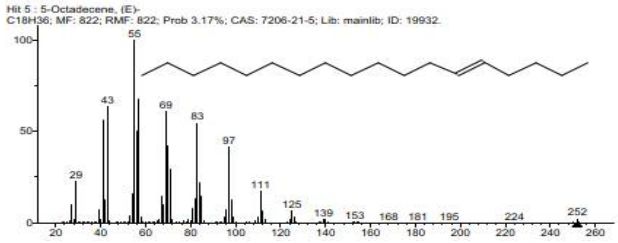
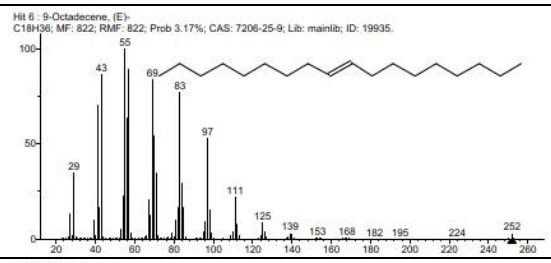
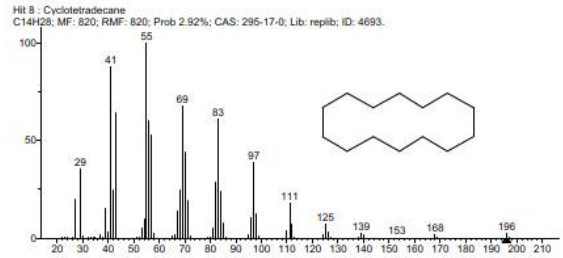
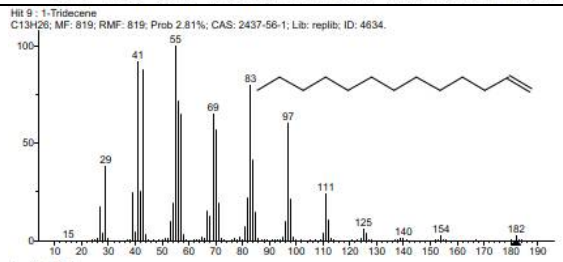
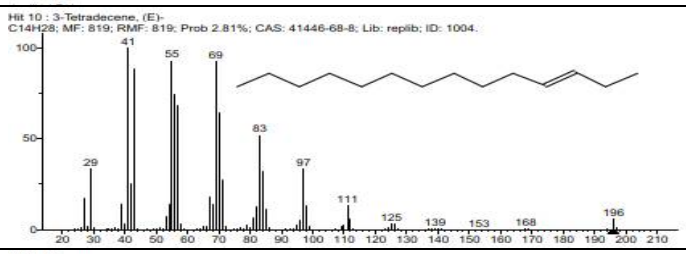
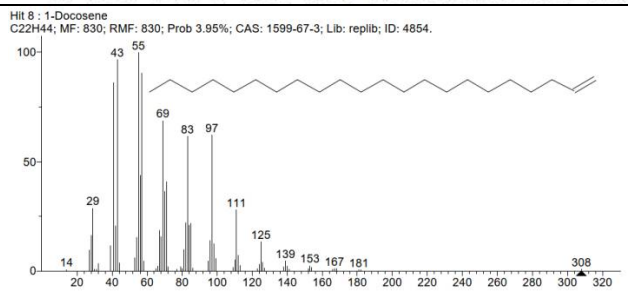
Table 1: Compounds identified in the crude ethyl acetate extract of*Cladosporium chalastosporoides* by GC-MS analysis

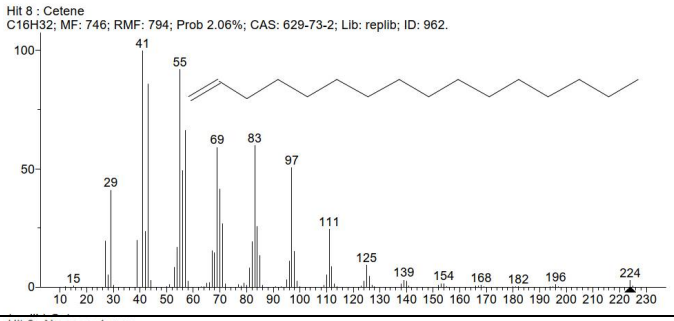
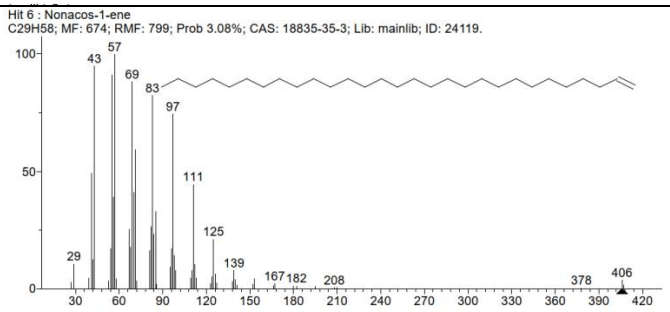
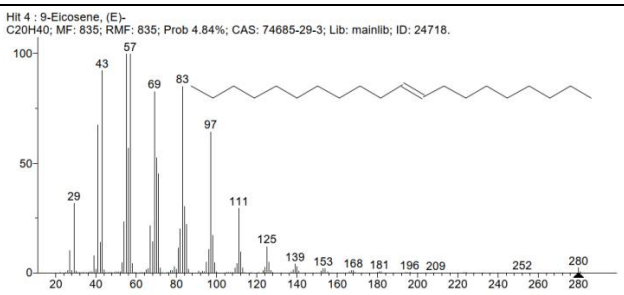
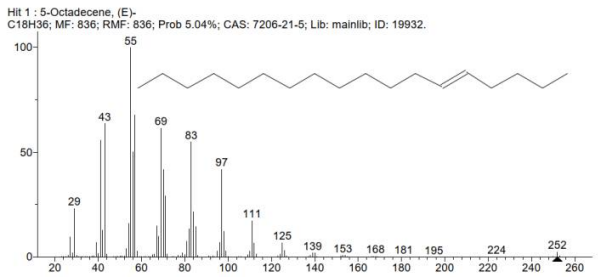
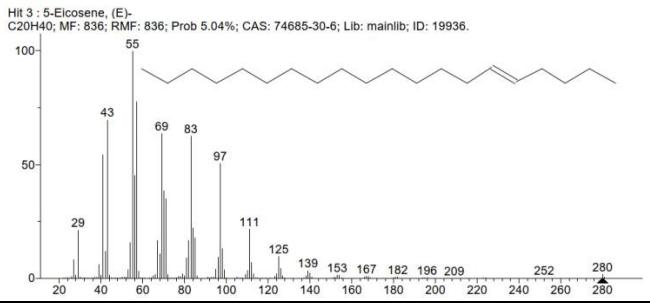
Sl. No	Systemic name	Molecular formula	Molecular weight	ID#	Exact mass	NIST#
1	3-Tetradecene	C14H28	196	2688	196.219	62806
2	7-Hexadecene	C16H32	224	19694	224.250	62796
3	1-Dodecanol	C12H26O	186	2092	186.198	63858
4	1-Dodecene	C12H24	168	2189	168.187	61826
5	5-Octadecene	C18H36	252	19932	252.281	62810
6	9-Octadecene	C18H36	252	19935	252.281	62809
7	Cyclo tetradecane	C14H28	196	4693	196.219	61052
8	1-Tridecene	C13H26	182	4634	182.203	107768
9	3-Tetradecene	C14H28	196	1004	196.219	62795
10	1-Docosene	C22H44	308	4854	308.344	113878
11	Cetene	C16H32	224	962	224.250	118882
12	Nonacos-1-ene	C29H58	406	24119	406.453	406.453
13	9-Eicosene	C20H40	280	24718	280.313	62815
14	5-Octadecene	C18H36	252	35120	252.281	62811

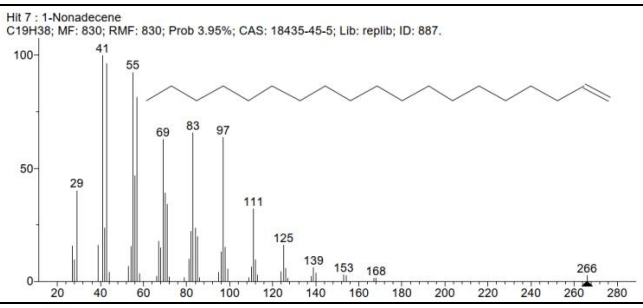
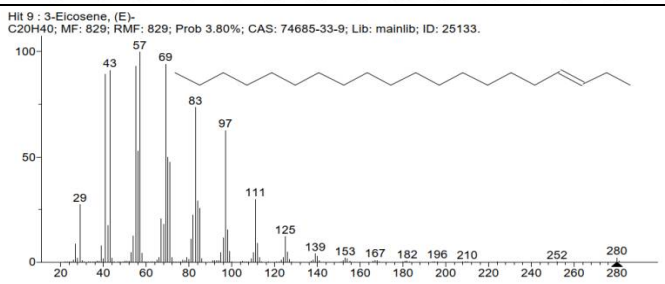
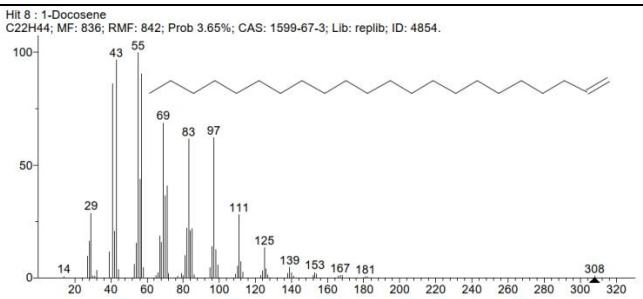
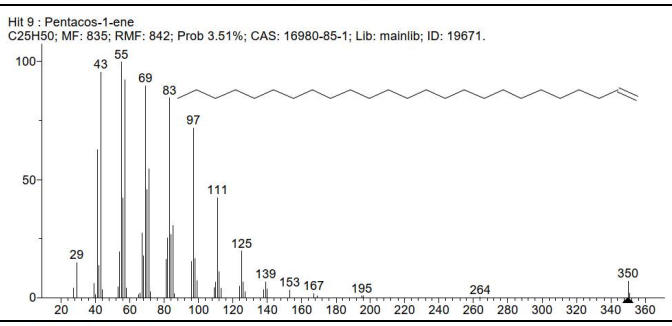
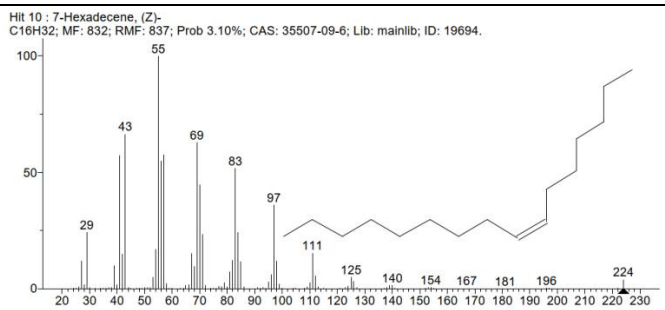
15	5-Eicosene	C20H40	280	19936	280.313	62816
16	1-Nonadecene	C19H38	266	887	266.297	113626
17	3-Eicosene	C20H40	280	25133	280.313	62838
18	1-Docosene	C22H44	308	4854	308.344	113878
19	Pentacos-1-ene	C25H50	350	19671	350.391	412723
20	7-Hexadecene	C16H32	224	19694	224.250	62796
21	2-Tetradecanol	C14H30O	214	3997	214.229	113687
22	2-Dodecanol	C12H26O	186	16758	186.198	114096
23	Pentacos-1-ene	C25H50	350	19671	350.391	412723
24	Oxalic acid, allyl pentadecyl ester	C20H36O4	340	2466	340.261	309243
25	Oxalic acid, allyl hexadecyl ester	C21H38O4	354	2467	354.277	309244

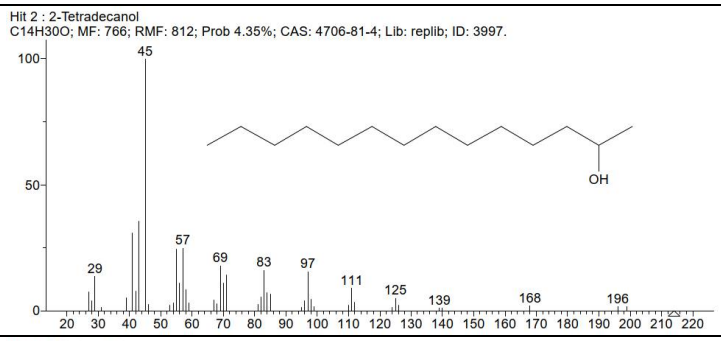
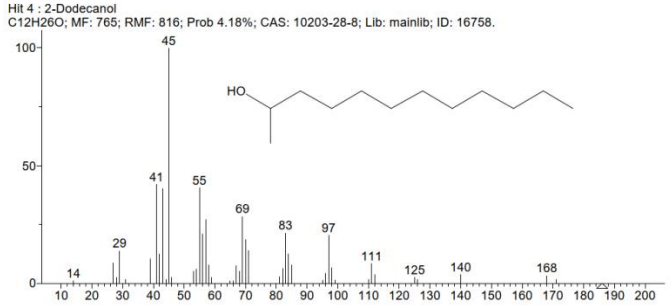
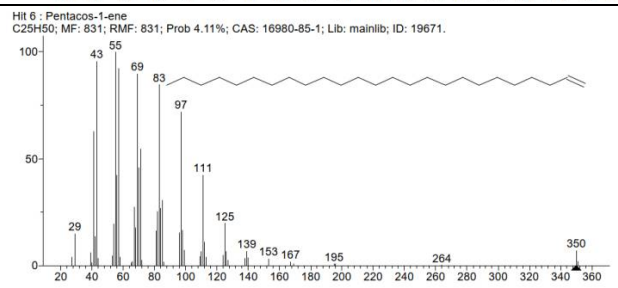
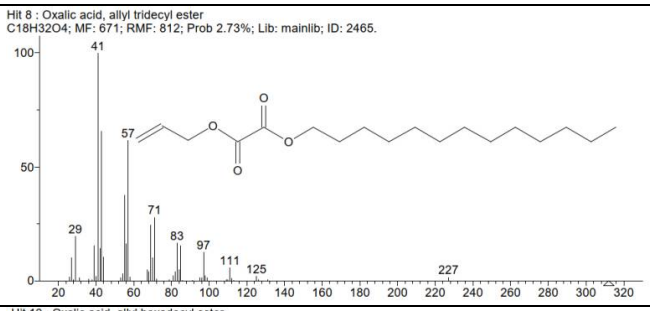
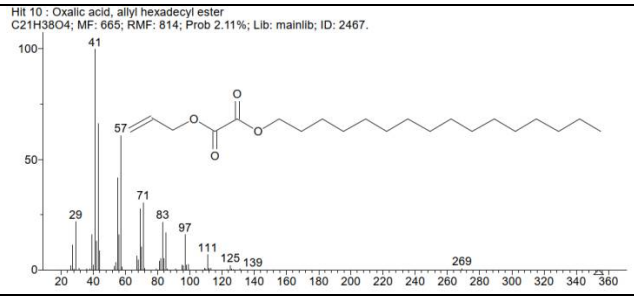
Table 2: Mass spectrometric analysis of compounds with their structure

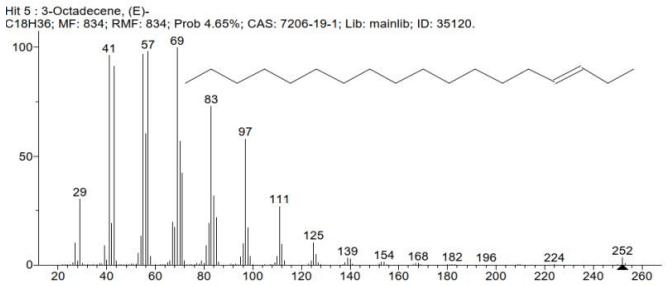
Sl no	Name of the compound	Full scan mass spectrometric analysis of compounds with structure
1	3-Tetradecene	
2	7-Hexadecene	
3	1-Dodecanol	
4	1-Dodecene	
5	5-Octadecene	

		
6	9-Octadecene	
7	Cyclotetradecane	
8	1-Tridecene	
9	3-Tetradecene	
10	1-Docosene	

11	Cetene	
12	Nonacos-1-ene	
13	9-Eicosene	
14	5-Octadecene	
15	5-Eicosene	

16	1-Nonadecene	
17	3-Eicosene	
18	1-Docosene	
19	Pentacos-1-ene	
20	7-Hexadecene	

21	2-Tetradecanol	
22	2-Dodecanol	
23	Pentacos-1-ene	
24	Oxalic acid, allyl pentadecyl ester	
25	Oxalic acid, allyl hexadecyl ester	

26	3-Octadecene	

CONCLUSION

Studies revealed that isolated fungal endophytes are great sources of various metabolites biosynthesized by the plants. From the above study it becomes clear that *C. chalastosporoides* produces various secondary metabolites in its ethyl acetate culture filtrate which are reported to have antimicrobial activity. Studies are in progress to purify these pharmaceutically important compounds. Hence, further investigation is required to identify and elucidate the bioactive constituents which have different biological activities and they can be used for human and environmental benefits.

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