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Bioactive potential and GC-MS fingerprinting of extracts from endophytic fungi associated with seeds of some medicinal plants

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Abstract: Endophytic fungi are a group of host-associated fungal communities that benefit their hosts. According to the conditions of their specific living environment, plant endophytic fungi produce many bioactive metabolites with different structural features. The bioactive compounds isolated from endophytic fungi, have significant effects on increasing the compatibility of both endophytic fungi and their host plants, such as the tolerance to biotic and abiotic stresses. In addition, some of these metabolites have indicated medicinal and ecological importance. In the present investigation, five fungal endophytes were isolated from the seeds of some medicinal plants. These endophytic isolates were characterized by sequencing of Internal Transcribed Spacer (ITS) regions as *Acremonium* sp. isolated from *Echium amoenum*, *Epicoccum nigrum* from *Rosa canina*, *Fusarium* sp. and *Fusarium equiseti* from *Calendula officinalis* and *Lecanicillium aphanocladii* from *Physalis peruviana*. To screen the phytochemical derivatives of ethyl acetate of these endophytic fungal isolates, the extracts were subjected to phytochemical analysis by gas chromatography-mass spectrometry (GC-MS). GC-MS spectrum of the compounds found in the extracts of the endophytic fungi was matched with the standard compounds present in the WILEY8 library and the National Institute of Standards and Technology (NIST14) library. The GC-MS analysis of extracts from these endophytic fungi revealed the presence of 49 phytocompounds such as 2,4-Di-tert-butylphenol, Hexadecane, Eicosane, Octadecane, Docosane, Nonadecane, Isopropyl myristate, Hexadecanoic acid, Undecane, Methyl stearate and so on. The results of the present study acknowledge that the endophytic fungi of these medicinal plants are the potential source of biologically active compounds and envisage the possible drug discovery using them. In addition, the compounds 14-Beta-H-Pregna and Cyclohexane, 1, 1'-(2-methyl-1,3-propanediyl) bis are reported here for the first time as fungal metabolites.

Keywords: Endophytes, Fungi, Medicinal plants, Secondary metabolites, GC-MS.

INTRODUCTION

Endophytic fungi are a community of fungi that colonize internal tissues of higher plants, asymptomatically (Fouda et al. 2015). Scientific resources consider fungal endophytes as those that live at least part of their life cycle inside diverse plant tissues including leaves, stems, seeds and roots, without any visible damage to their host plants (Fouda et al. 2015). A mutualistic interaction occurs between fungal endophytes and their hosts, the plant provides nutrition and protection for the fungus in one hand, the fungus helps with the competitiveness and growth of the plant by protecting and preserving it against abiotic and biotic stresses on the other hand (Strobel & Daisy 2004). Moreover, plants colonized by endophytes have benefits such as plant growth promotion by helping in the production of growth hormones and secondary metabolites, hence, are generally healthier than those lacking endophytic interaction (Faeth & Fagan 2002). The communication between endophytes and their host plants can also affect the formation of metabolic products in both partners (Faeth & Fagan 2002). It has been revealed that in this mutualistic interactions, fungal endophytes can produce bioactive compounds analogous to plant metabolites and accordingly it has been suggested that, this phenomenon results from co-evolution (Faeth & Fagan 2002). During co-evolution, fungal endophytes gently were

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acclimatized to specific microenvironments using approaches such as the uptake of plant DNA fragments into their genomes or the insertion of their DNA into the host plant genome (Soares et al. 2017). Although many plant species usually host several endophyte species, only a few plants have ever been investigated for their endophytic diversity and biology, therefore, enormous rich potential resources exist for the description of novel fungi (Strobel & Daisy 2004). Some kinds of plant communities such as plants with an ethnobotany background like those used as a medicine, have been selected generally for the isolation of beneficial endophytes (Strobel & Daisy 2004). It has been revealed that endophytic fungi have an affecting role in the quality and quantity of crude drugs through a particular fungus-host interaction in medicinal plants (Faeth & Fagan 2002). Therefore, endophytic fungi are mostly isolated from medicinal herbs to acquire bioactive compounds for remedial activities which are already obtained from host plants (Soares et al. 2017; Musavi et al. 2015).

Plants and fungi are wealthy sources of thousands of bioactive compounds (Musavi et al. 2015). The bioactive compounds resulting from natural resources can be reviewed and evaluated for pharmacological characterization. The triumph of these resources in finding new and effective drug compounds that can be beneficial for humans was considered further with the discovery of biosynthesis of Taxus-derived, anticancer compound Taxol from endophytic fungi *Taxomyces andreanae* Strobel, A. Stierle, D. Stierle & W.M. Hess and *Pestalotiopsis microspora* (Speg.) G.C. Zhao & Nan Li isolated from *Taxus wallichiana* Zucc. (Stierle et al. 1993). Taxol, arguably the most successful anti-cancer drug of all time, is identified and its source, is the bark of the *Taxus* species (Strobel et al. 1996). Capsaicin, an alkaloid of *Capsicum annuum* L. which has anti-inflammatory, thermogenic, anti-lithogenic, gastro-stimulatory, anti-cancer, anti-diabetic and cardio-protective attributes, has also been produced by *Alternaria alternata* (Fr.) Keissl. isolated from *C. annuum* as an endophytic fungus (Musavi et al. 2015). *Huperzia serrata* Thunb., a tropical medicinal moss, is used for the treatment of Alzheimer's disease and its biologically active alkaloid huperzine A (HupA) was also isolated from its endophytic fungus, *Acremonium* sp. (Musavi et al. 2015). A bioactive natural compound Silymarin which has anti-oxidant, anti-hepatitic, cardioprotective, immunomodulatory, anti-inflammatory, anti-metastatic and hepatoprotective activities, found in *Silybum marianum* (L.) Gaertn fruits, has also been reported from the endophytic fungus *Aspergillus iizukae* Sugiy. isolated from this plant (El-Elimat et al. 2014).

In the present research, the seeds of several important medicinal plants including *Calendula officinalis* L., *Echium amoenum* Fisch & Mey, *Physalis peruviana* L. and *Rosa canina* L. were used for the isolation of endophytic fungi. *Calendula officinalis*, is a medicinal herb that is used in traditional systems of medicine for herpes, scars, ulcers, skin damage and treating wounds, blood purification and frost-bite. Moreover, it is especially used because of its diverse biological activities to treat diseases like gastro-intestinal, gynecological, diabetic, in some cases of burns, analgesic and anti-inflammatory. This plant is wealthy in many pharmaceutic active ingredients like amino acid, flavonoids, glycosides, volatile oil, steroids and carotenoids (Ashwlayan & Verma 2018). *Echium amoenum* is one of the most significant medicinal plants in Iranian traditional medicine and its flowers have been applied as anti-inflammatory, analgesic, demulcent, sedative and anxiolytic in the folk medicine of Iran. The antioxidative stress potential of *E. amoenum* may be due to its bioactive anti-oxidant components, especially rosmarinic acid and flavonoids (Ranjbar et al. 2006). *Physalis peruviana* has a wonderful medicinal value for curing various diseases such as rheumatism, malaria, leukemia, diabetes, asthma, ulcers, hepatitis, dermatitis and cancer (Ramírez et al. 2013). The fruit of *P. peruviana* was exhibited to have both anti-oxidant and anti-inflammatory activities and contains high levels of vitamin A, vitamin B-complex and vitamin C (Pardo et al. 2008). *Rosa canina* contains various vitamins (especially vitamin C), carotenoids, polyphenols, fatty acids and carbohydrates. Moreover, there is evidence of anti-bacterial, anti-diabetic, anti-cancer and anti-obesity properties of this medicinal plant (Selahvarzian et al. 2018). The current study aimed to identify potential bioactive metabolites of five endophytic fungi associated with seeds of the above-mentioned medicinal plants

MATERIALS AND METHODS

Sample collection and isolation of endophytic fungi

Plants and seeds of medicinal plants *C. officinalis*, *E. amoenum*, *P. peruviana* and *R. canina* were collected from Mazandaran Province in Iran during the years 2018–2019 and transferred to the laboratory in sterile Zipper bags in portable cool chambers (4 °C). Endophytic fungal isolations were carried out aseptically from healthy seeds as follows: after primarily washing with tap water, seed samples were processed for surface sterilization with 75% ethanol for 1 min, 3% sodium hypochlorite for 5 min, 70% ethanol for 30 s and, then washed three times with sterile distilled water. Dried plant seeds were cut into 2–3 pieces under sterile conditions and were plated on Petri dishes containing potato dextrose agar (PDA; Merck, Darmstadt, Germany). The plates were incubated at 25 ± 1 °C for 7–12 days and were checked daily. Fungal hyphae and fruiting structures emerging from the plated seeds were subcultured on water agar (WA) media and maintained as the pure culture at 4 °C for further use. The effectiveness of the seed surface sterilization method was evaluated by placing individual seeds onto Petri dishes

containing PDA medium and the plates were incubated at 25 ± 1 °C for 7–12 days.

DNA isolation, PCR amplification and sequencing

Mycelium was scraped from the surface of fungal cultures growing on PDA using a sterile scalpel blade and DNA was extracted according to the protocol of Möller et al. (1992). The DNA samples were diluted 50–100 times in preparation for further DNA amplification reactions. The primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) were used to amplify part of the internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS) of the nrRNA operon. The polymerase chain reaction (PCR), mixture and amplification condition were performed in a final volume of 25 µl as explained in Bakhshi & Braun (2022). The PCR products were sequenced with the amplified primers at a commercial sequencing service provided at Microsynth Company (Balgach, Switzerland). Obtained sequences were assembled with MEGA v. X software and consensus sequences were manually generated from the forward and reverse sequences. The consensus regions of sequences were compared with the reference sequences using the BLAST algorithm and submitted to the NCBI GenBank nucleotide database (http://www.ncbi.nlm.nih.gov.blast). The obtained sequences from GenBank and the novel generated sequences during this study were aligned and subjected to construct a phylogenetic tree using MrBayes v. 3.2.6 as elucidated by Bakhshi & Braun (2022). *Melampsora caprearum* (DC.) Thüm. (KU550034) was used as the outgroup taxon.

Extraction of metabolites

The metabolites were extracted according to the method of Siddiquee et al. (2012) as follows. The pure fungal colonies were cultured onto 50 ml potato dextrose broth (PDB; Q-LAB) media. After 10 days of incubation, extraction was performed by adding 20 ml Ethyl acetate ($C_4H_8O_2$) to the broth culture in Erlenmeyer flasks and the mixture was incubated at 4 ºC for 10 min. Then, flasks were shaken for 30 min at 120 rpm. The Erlenmeyer flasks were placed in a stationary state to form two phases for 60 min. Taken the upper phase was transferred to a clean 15 ml falcon tube. The supernatant was spined for 10 min, with 4000 rpm, at room temperature in the centrifuge. Once again, it was taken in the upper aqueous phase and was transferred to a clean 50 ml falcon tube. Then, the supernatant (metabolites and $C_4H_8O_2$) was separated from the liquid culture and evaporated using a rotary evaporator at 45 ºC. The residue was dissolved in one ml methanol (CH_4OH), filtered through a 0.2 µm syringe filter (Millipore) and stored at 4 ºC for 24 h before injection to GC-MS. The chemical composition of the ethyl acetate fractions was analyzed by GC-MS.

GC-MS analysis of the compounds

An Agilent Technologies 7890 A gas chromatograph (GC) connected to a 5975 C inert mass spectrometer (MS) detector (Agilent, USA) was applied for the detection of secondary metabolites from the crude extract of fungal isolates. Furthermore, an HP-5MS fused silica capillary column (Hewlett-Packard, 30 m* 0.25 mm i.d., 0.25 µm film, cross-linked to 5% phenyl methyl siloxane stationary phase) was used. The software Chemstation (Hewlett-Packard, version A.01.01) was used to control entire system. Electron impact mass spectra were documented at 70 electron voltage and ultra-high pure He (99.999%) gas was applied as the carrier gas at a flow rate of 1 mL/min. The injection volume was 1 µL and all the injections were conducted in a split-less mode. The temperature of the injector and detector was 250 and 280 °C, respectively. The column oven temperature was firstly set at 50 °C for 5 min, then enhanced to 260 °C (ramp: 4 °C/min) and held for 5 min (Vandendool & Kratz 1963). The compounds found in the extracts of the fungi were recognized by computer matching their GC-MS spectral patterns with the WILEY8 library and the National Institute of Standards and Technology (NIST14) library.

RESULTS AND DISCUSSION

Identification of endophytic fungal isolates

The isolates were identified with the DNA sequence analysis of the ITS region and based on the ITS phylogenetic tree, were assigned to five species as *Acremonium* sp., *Epicoccum nigrum* Link, *Fusarium equiseti* (Corda) Sacc., *Fusarium* sp. and *Lecanicillium aphanocladii* Zare & W. Gams (Fig. 1). The detailed identification of these endophytic fungi with their Genbank accession numbers are presented in Table 1. There are many studies on endophytic fungi from various plants. This is the first report of endophytic fungi associated with seeds of the medicinal plants *C. officinalis*, *E. amoenum*, *P. peruviana* and *R. canina*.

Identified compounds through GC-MS

According to the results of GC-MS analysis, a total of 49 compounds, including 1-Docosanol, 1-Hexanol 2-ethyl, 2,4-Di-tert-butylphenol, 2-Coumaranone, (2'R)-2-(2'-hydroxypropyl)-4-methoxyl-1,3-benzenediol, 14-Beta-H-Pregna, Adipic acid, Benzenepropanoic acid, Bromotetradecane, Cyclohexane,1,1'-(2-methyl-1,3-propanediyl) bis, Cyclonerolidol, Di-2-ethylhexylphtalate, Di-epi-alpha-cedrene (1H-3a,7-Methanoazulene), Diisooctyl phthalate, Di-n-decylsulfone, Dioxomorpholine, Docosane, Dodecane, Dotriacontane, Eicosane, Heneicosane, Heneicosene, Hentriacontane, Heptacosane, Heptadecane, Heptadecanoic acid, Hexacosane, Hexadecane, Hexadecanoic acid, Isopropyl myristate, Lauric acid, Methyl stearate, N-ethyl-1,3-dithioisoindoline, Nonadecane, Octadecane, Octadecene, Pentacosane, Pentadecane, Pentatriacontane, Propanediol, Tetradecane, Tetradecyl ester, Tetracosan, Tetratetracontane, Trichoacorenol, Tridecane, Tritriacontane, Undecane and Zaragozic acid were identified in this study. Of these 15, 18, 18, 31 and 7 compounds were identified in culture filtrates of the endophytic fungi *Acremonium* sp., *E. nigrum*, *F. equiseti*, *Fusarium* sp.

and *L. aphanocladii* respectively. Some metabolites were produced similarly by several species such as Diisooctyl phthalate and Octadecane and some were produced exclusively in one species like Adipic acid and Dioxomorpholine in *Fusarium* sp., 2-Coumaranone in *F. equiseti*, Cyclonerolidol, 1-Docosanol, Pentatriacontane, Heptadecanoic acid, Trichoacorenol and Octadecene in *Acremonium* sp. and, 14-Beta-H-Pregna in *E. nigrum*. Details of the various secondary metabolites produced by each fungus are presented in Table S1. The retention time and area of the compounds under the described conditions in the GC-MS section are shown in Tables 2 to 6 and GC–MS chromatograms of the ethyl acetate extract of isolates are presented in Figs. 2 to 6.

Table 1. Identification of endophytic fungi isolated from seeds of some medicinal plants using ITS primers.

Endophytic fungal isolates	**Host source**	**Collection accession number**	**GenBank accession number**
Acremonium sp.	*Echium amoenum*	AAH5-1	OQ162344
Epicoccum nigrum	*Rosa canina*	AAH13-3	OQ162360
Fusarium equiseti	*Calendula officinalis*	AAH9-1	OQ162352
Fusarium sp.	*Calendula officinalis*	AAH9-2	OQ162353
Lecanicillium aphanocladii	*Physalis peruviana*	AAH4-1	OQ162343

Table 2. Identification of most abundant bioactive compounds from *Acremonium* sp. extract isolated from *E. amoenum* by GC–MS.

Compounds	**R_T min**	**Area (**Maximum**)**	**Compounds**	**R_T min**	**Area (**Maximum**)**
2,4-Di-tert-butylphenol	28.951	608147	Dotriacontane	39.837	806720
Hexadecane	31.374	1943814	Pentatriacontane	40.516	558901
Trichoacorenol	33.299	7344440	Eicosane	40.673	3121508
Cyclonerolidol	35.302	7344154	Heptadecanoic acid	44.600	1812040
Octadecane	35.504	997711	Docosane	46.156	1884389
Heneicosane	36.013	446423	Eicosane	50.292	2033097
Isopropyl myristate	37.465	6407491	Diisooctyl phthalate	53.332	652290683

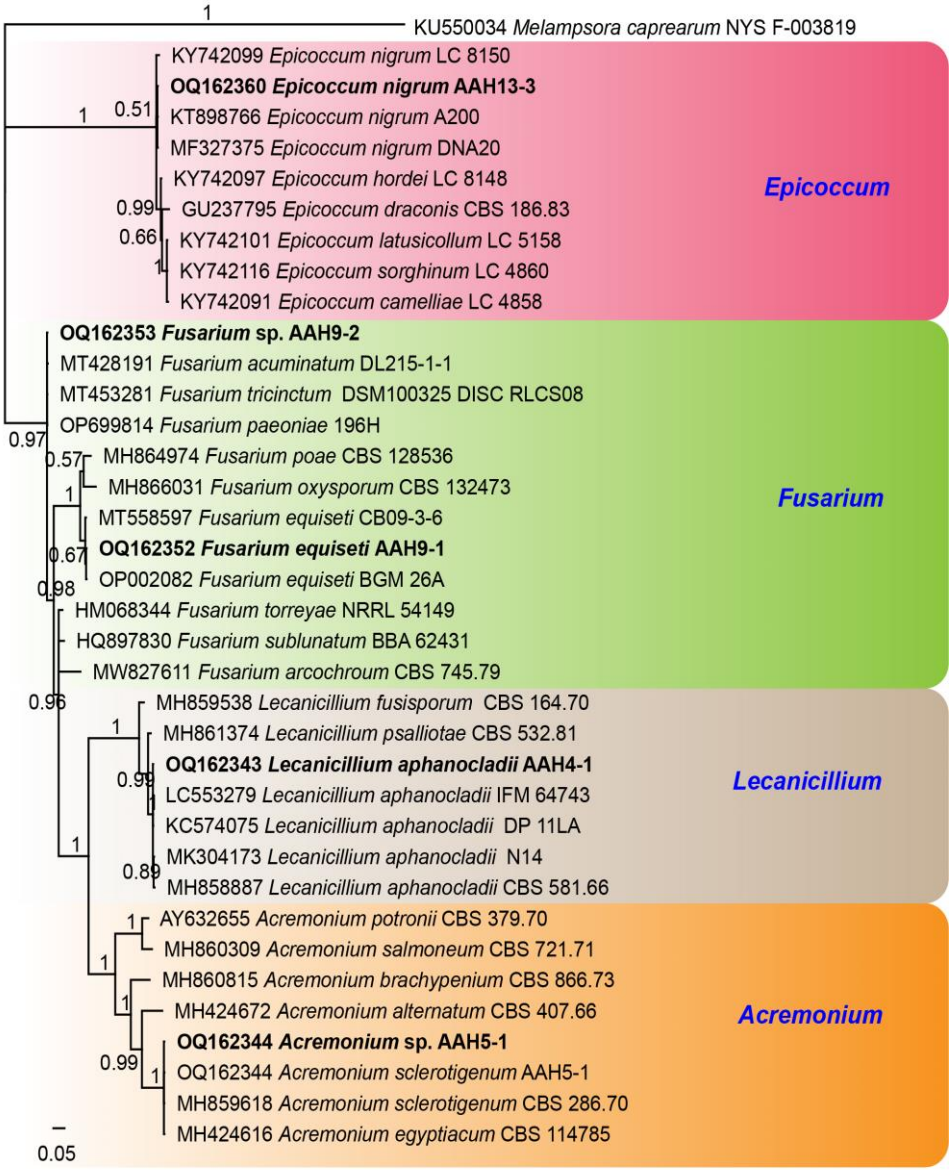

Fig. 1. Phylogenetic tree inferred by Bayesian analysis of the ITS sequence alignment using MrBayes v.3.2.6. The scale bar indicates 0.05 expected changes per site. The tree was rooted to *Melampsora caprearum* (KU550034). The isolates of this study are shown in bold.

Table 3. Identification of most abundant bioactive compounds from *E. nigrum* extract isolated from *R. canina* by GC–MS.

Compounds	**R_T min**	**Area (Maximum)**	**Compounds**	**R_T min**	**Area (Maximum)**
14-. Beta.-H-Pregna	4.128	11135152	Hexadecanoic acid	39.951	3590075
1-Hexanol, 2-ethyl	12.337	84562	Tetradecane	40.516	458115
Hexadecane	31.379	1061704	10-Heneicosene	41.943	595219
Tridecane	34.404	548231	Methyl stearate	44.610	1672585
Octadecane	36.775	2824435	Docosane	44.776	1202468
Dodecane	36.947	1125497	Pentacosane	46.167	1288079
Isopropyl myristate	37.471	3916314	Heptadecane	49.280	652177
Eicosane	39.847	1204447	N-ethyl-1,3-dithioisoindoline	53.410	886316

Table 4. Identification of most abundant bioactive compounds from *F. equiseti* extract isolated from *C. officinalis* by GC-MS.

Compounds	**R_T min**	**Area (**Maximum**)**	**Compounds**	**R_T min**	**Area (**Maximum**)**
2-Coumaranone	19.974	981915	Hexadecanoic acid	39.946	3862236
Hexadecane	31.374	2428833	Undecane	40.516	615460
Docosane	34.399	561295	Tetracosane	40.854	898019
Octadecane	35.514	850768	Heptacosane	40.999	520550
Heptadecane, 3-methyl-	36.018	611280	Eicosane	41.679	3437782
Nonadecane	36.775	4951791	Methyl stearate	44.600	1827009
Cyclohexane,1,1'-(2-methyl-1,3-propanediyl) bis	36.936	1227276	Heneicosane	44.771	1264392
Isopropyl myristate	37.471	6735356	Hexacosane	46.161	2011737
Hentriacontane	39.842	1022517	Diisooctyl phthalate	50.021	351547623

Table 5. Identification of most abundant bioactive compounds from *Fusarium* sp. extract isolated from *C. officinalis* by GC–MS.

Compounds	**R_T min**	**Area (**Maximum**)**	**Compounds**	**R_T min**	**Area (**Maximum**)**
2,4-Di-tert-butylphenol	28.951	1417127	Bromotetradecane	39.847	849959
Dioxomorpholine	29.957	14833923	Hexadecanoic acid	39.942	6833887
Dioxomorpholine	30.990	1697320	Benzenepropanoic acid	40.340	1182369
Hexadecane	31.369	3458348	Heneicosane	40.345	602905
Adipic acid	32.106	3896169	Dotriacontane	40.859	1323393
Di-epi-alpha-cedrene	32.246	1580966	Heptacosane	40.994	219254
Zaragozic acid	32.775	637333	Eicosane	41.673	3632902
Di-n-decylsulfone	34.155	662795	Tetratetracontane	41.004	510019
Docosane	34.394	1172734	Tetradecyl ester	41.674	3632902
Hentriacontane	35.317	1073510	Octadecene	41.943	929945
Pentadecane	35.509	1345791	Isobutyl methyl phthalate	42.467	2904871
Nonadecane	36.771	5485589	Hentriacontane	44.776	2317927
n-Tetracosanol-1	36.936	1602275	Heptadecane	46.162	2127231
Propanediol	36.941	1343047	Heneicosane	49.275	872730
Isopropyl myristate	37.465	7793088	Tritriacontane	50.297	1942157
Lauric acid	38.373	5957526	Diisooctyl phthalate	53.283	484429839

Table 6. Identification of most abundant bioactive compounds from *L. aphanocladii* extract isolated from *P. peruviana* by GC–MS.

Compounds	**R_T min**	**Area (**Maximum**)**	**Compounds**	**R_T min**	**Area (**Maximum**)**
Hexadecane	31.379	3077182	Methyl stearate	44.605	3332974
Octadecane	36.775	4673430	Docosane	46.167	1867239
Isopropyl myristate	37.476	6204609	Hexatriacontane	50.297	1854631
Hexadecanoic acid, methyl ester	39.946	6263271	Diisooctyl phthalate	53.275	414283706

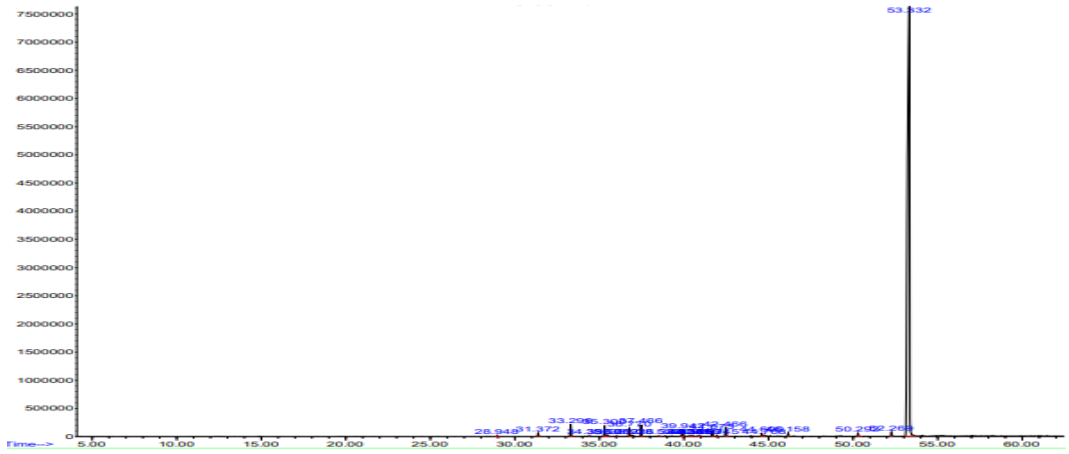

Fig. 2. Chromatogram of the active extract from *Acremonium* sp. showing major volatile compounds by GC–MS.

Fig. 3. Chromatogram of the active extract from *E. nigrum* showing major volatile compounds by GC–MS.

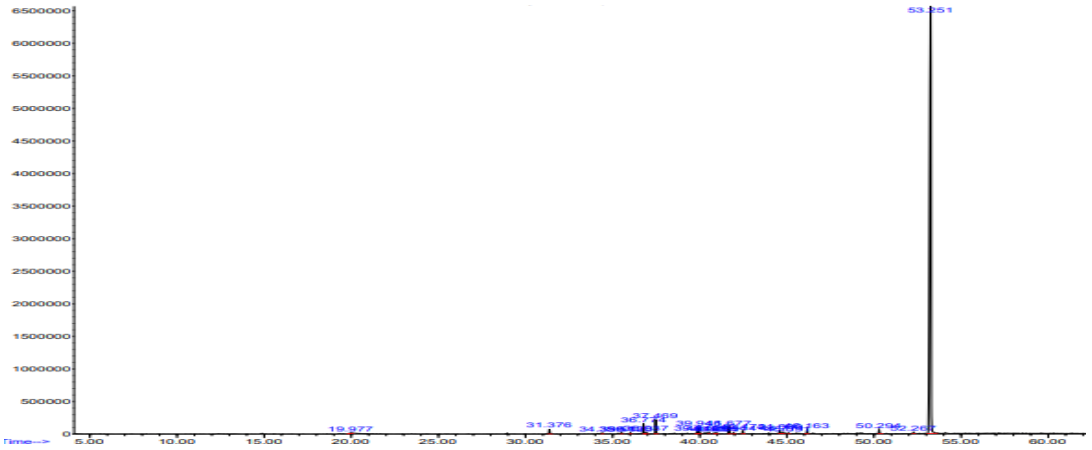

Fig. 4. Chromatogram of the active extract from *F. equiseti* showing major volatile compounds by GC–MS.

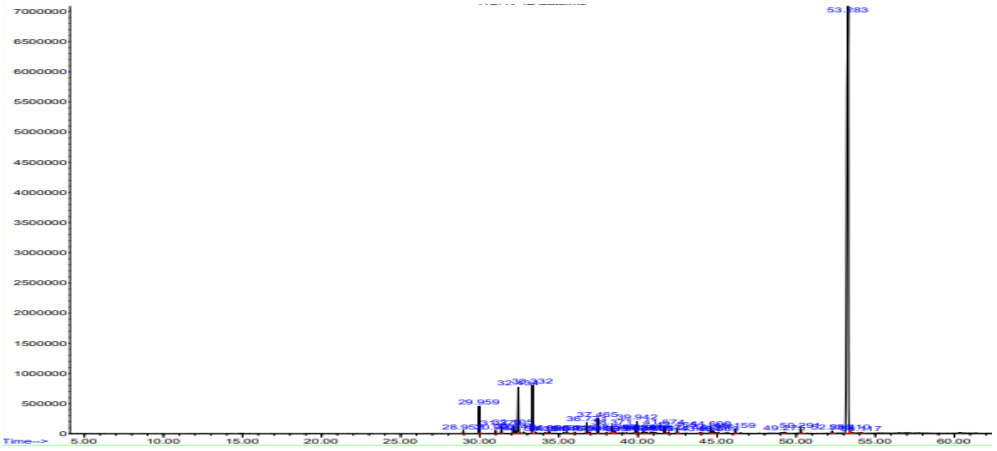

Fig. 5. Chromatogram of the active extract from *Fusarium* sp. showing major volatile compounds by GC–MS.

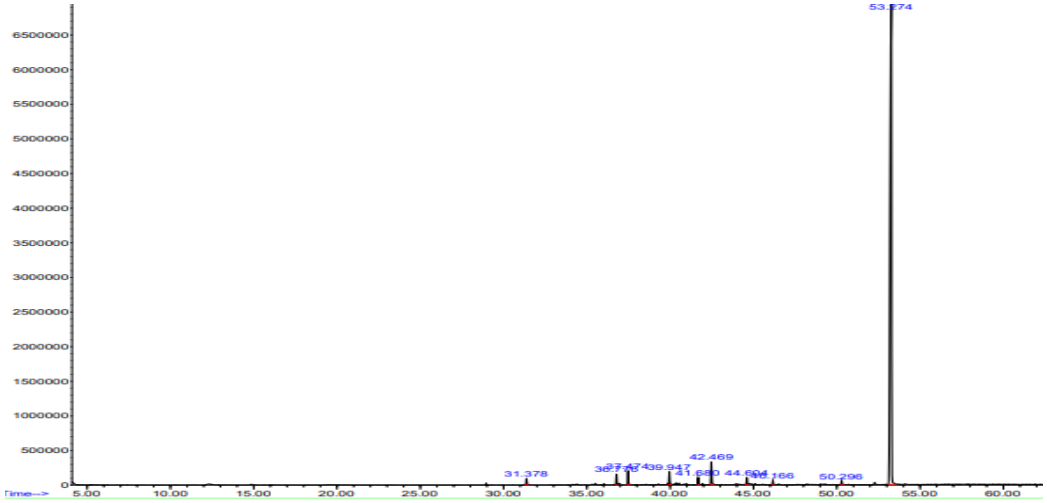

Fig. 6. Chromatogram of the active extract from *L. aphanocladii* showing major volatile compounds by GC–MS

Potential biological activities of compounds identified in this study

A diverse range of biological activities has been reported in the literature for different compounds identified in this research. To give some examples, Hexadecanoic acid which was produced by all the fungal endophytes studied in this research has been confirmed that displays a wide range of biological activities such as antifungal, antioxidants, hypocholesterolemic prevention and anticancer effects (Anisha & Radhakrishnan 2017). This metabolite was mainly found in the endophytic fungus *Fusarium* sp. obtained from the well-known medicinal plant *Zingiber officinale* Roscoe which is commonly used in traditional medicine (Anisha & Radhakrishnan 2017). Diisooctyl phthalate is another compound that was produced by all the fungal endophytes studied here. Abdel-Motaal et al. (2022), stated that analysis of GC-MS of extracts from three fungal endophytes including *Aspergillus terreus* Thom., *Fusarium solani* (Mart.) Sacc. and *Penicillium verrucosum* Dierckx, isolated from the medicinal plant *Solenostemma argel* (Delile) Hayne., indicated the presence of Diisooctyl phthalate as the main compound. Kalavathi et al. (2017) documented that Diisooctyl phthalate derived from an endophytic fungus, *Penicillium* sp., isolated from the medicinal plant *Tabebuia argentea* (Bureau & K.Schum.) Britton, displayed anti-diabetic activity. Isopropyl myristate which was produced in this study exclusively by *Fusarium* sp. isolated from *Calendula officinalis* has been shown that depicts cytotoxic activity against different cancer cell lines (Mo & Lim 2005). Pelo et al. (2021) declared that *Aureobasidium pullulans* (de Bary & Löwenthal) G. Arnaud, *Cladosporium* sp., *Fusarium* sp., *Hyalodendriella* sp., *Penicillium chrysogenum* Thom. and, *Phialophora* sp. which isolated from the medicinal plant *Solanum mauritianum* Scop. as endophytic fungi, can produce Isopropyl myristate. To illustrate more examples, some of the biological activities reported for all the compounds identified in this study along with some examples of other fungi producing similarly these compounds are listed in Table S1.

To the best of our knowledge, two compounds including 14-Beta-H-Pregna produced exclusively by *E. nigrum* and Cyclohexane,1,1'-(2-methyl-1,3-propanediyl) bis produced exclusively by *F. equiseti* were reported in this research for the first time as fungal metabolites. Previous phytochemical studies on volatile oils of medicinal plants have revealed the production of 14-Beta-H-Pregna by plants (Gharari et al. 2019). In addition, several reports confirmed the biological activities of 14-Beta-H-Pregna which exhibits antibacterial, antifungal and antioxidant effects (Gharari et al. 2019). Furthermore, previous reports confirmed the production of the compound Cyclohexane,1,1'-(2-methyl-1,3-propanediyl) bis by plants and its biological activities as an antioxidant (Delaram et al. 2019). Therefore, these data more confirm that endophytic fungi are a rich source of novel and bioactive secondary metabolites.

Conclusion

In conclusion, our results reveal the potential of the medicinal plants *C. officinalis*, *E. amoenum*, *P. peruviana* and *R. canina* as a host of important fungal endophytes which possess a precious source of bioactive compounds which have diverse biological activities, such as antioxidant, insecticidal, antimicrobial and anticancer activities. These compounds can increase the tolerance of host plants to biotic and abiotic stresses. Moreover, they can be used for the treatment of diseases and for manufacturing new drugs. Furthermore, these compounds can also be applied in dyeing, polyester, resin, biofuels and cosmetics industries. Therefore, further investigation and research will be required on the structural features and application strategies of these potent resources and also the influence of environmental factors when used in different conditions.

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بررسی ترکیبات زیست فعال عصاره قارچ‌های اندوفیت جدا شده از بذور چند گیاه دارویی با روش کروماتوگرافی گازی و طیف سنجی جرمی (GC-MS)

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چکیده: قارچ‌های اندوفیت گروهی از جوامع قارچی مرتبط با میزبان هستند و در عین حال برای میزبان خود سودمند می‌باشند. قارچ‌های اندوفیت گیاهی با توجه به شرایط محیط زندگی ویژه خود، گروه‌های متنوعی از متابولیت‌های زیست فعال با ویژگی‌های ساختاری متفاوت را تولید می‌کنند. ترکیبات زیست فعال تولید شده توسط قارچ‌های اندوفیت، از اثرات قابل توجهی برای افزایش سازگاری آنها و گیاهان میزبان-شان، از قبیل تحمل به تنش‌های زنده و غیرزنده برخوردارند. علاوه بر این، برخی از این متابولیت‌ها دارای اهمیت دارویی و زیست محیطی نیز هستند. در تحقیق حاضر پنج قارچ اندوفیت از بذر تعدادی از گیاهان دارویی جداسازی شد. جدایه‌های به دست آمده بر اساس داده‌های توالی نوکلئوتیدی ناحیه ژنومی ITS تحت آرایه‌های *Acremonium* sp. از بذر گل گاوزبان (*Echium amoenum*)، *Epicoccum nigrum* از بذر نسترن کوهی (*Rosa canina*)، *Fusarium* sp. و *Fusarium equiseti* از بذر همیشه بهار (*Calendula officinalis*) و *Lecanicillium aphanocladii* از بذر عروسک پشت پرده (*Physalis peruviana*) شناسایی شدند. به منظور غربالگری ترکیبات فیتوشیمیایی عصاره اتیل استات این جدایه‌های قارچی اندوفیت، عصاره‌ها با استفاده از کروماتوگرافی گازی- طیف سنجی جرمی (GC-MS)، مورد آزمایش فیتوشیمیایی قرار گرفتند. طیف GC-MS عصاره اتیل استات جدایه‌های قارچی، با ترکیبات استاندارد موجود در WILEY8 و NIST14 مطابقت داده شد. تجزیه و تحلیل این داده‌ها، وجود ۴۹ ترکیب گیاهی از قبیل 2,4-Di-tert-butylphenol، Hexadecane، Eicosane، Octadecane، Docosane، Nonadecane، Isopropyl myristate، Hexadecanoic acid، Undecane، Methyl stearate و ... را در این جدایه‌ها آشکار نمود. نتایج این مطالعه تایید کرد که قارچ‌های اندوفیت جداشده از گیاهان دارویی، منبع بالقوه‌ای از ترکیبات دارای خواص بیولوژیکی هستند. علاوه بر این، دو ترکیب 14-Beta-H-Pregna و Cyclohexane,1,1'-(2-methyl-1,3-propanediyl) bis برای اولین بار در این مطالعه به عنوان متابولیت‌های قارچی معرفی می شوند.

کلمات کلیدی: اندوفیت‌ها، قارچ‌ها، گیاهان دارویی، متابولیت‌های ثانویه، GC-MS.

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