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Original Article

Profiling of volatile organic compounds from *Irpex laceratus*: An endophytic fungus isolated from *Glaucium fimbrilligerum*

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ABSTRACT

This study explored the volatile organic compounds (VOCs) of *Irpex laceratus*, a novel endophytic fungus isolated from *Glaucium fimbrilligerum* Boiss. Metabolites were extracted with ethyl acetate, diluted in n-hexane and n-butanol solvents, and identified by Gas Chromatography-Mass Spectrometry (GC-MS) using a non-polar column and NIST23 library matching (≥70%). The n-hexane extraction identified 47 distinct compounds, with simple hydrocarbons (40.42%) and organo-oxygen compounds (29.78%) being the most abundant classes. Other detected groups included organonitrogen (10.63%), organoiodine (6.38%), organosilicon (4.25%), and minor contributions from organochlorine, organosulfur, organobromine, and organofluorine compounds (each 2.12%). Most metabolites exhibited molecular weights between 200–400 g/mol, representing 44.67% of the total. In contrast, the n-butanol extract revealed 36 distinct compounds, with a different chemical distribution: simple hydrocarbons (33.33%), organo-oxygen compounds (27.77%), organosilicon (16.16%), organonitrogen and organochlorine (each 11.11%), and small proportions of organoarsenic, organosulfur, and organofluorine compounds (each 2.77%). The dominant molecular weight range for n-butanol metabolites was 200–300 g/mol (44.44%), followed by 300–400 g/mol (30.55%). The present study offers a comprehensive chemical profiling of *I. laceratus*, serving as a foundational contribution toward the systematic characterization of its VOC diversity. The identification of a broad spectrum of structurally and functionally diverse organic compounds underscores the metabolic complexity of the species and highlights its potential for discovering novel bioactive compounds.

KEYWORDS

Chemical ecology, Hydrocarbon, Myco-chemicals, Plant-microbe interaction, Secondary Metabolites.

INTRODUCTION

The genus *Glaucium* Mill. (*Papaveraceae*) comprises 28 species, 3 subspecies, and 3 varieties of annual, biennial, or perennial herbaceous plants distributed mainly in Europe, Central, and Southwest Asia (Kalis 1979, Kadereit 1993, Xu and Deng 2017). These plants are known for their diverse alkaloid content, particularly isoquinolines, which exhibit various pharmacological activities (Akaberi et al. 2021). *Glaucium fimbrilligerum* Boiss., an annual or biennial herb, is native to regions including Afghanistan, Iran, and parts of Central Asia (Máthé and Hassan 2015). In Iran, it is traditionally used to treat a wide range of

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ailments, such as pulmonary diseases, and serves as a laxative, sedative, anti-diabetic, and antidermatitic agent (Hossaini and Abarsaji 2009). The plant's aerial parts are used as narcotics or hypnotics, typically in the form of infusions or decoctions, while its seeds are employed as a laxative (Máthé and Hassan 2015). Although studies suggest that endophytic fungi often collaborate with plants to produce medicinal compounds, the microbial associations of *G. fimbrilligerum* remain largely unexplored due to its limited geographical distribution (Jia et al. 2016). Research on the isolation and identification of endophytic fungi within the *Papaveraceae* family is scarce, with prior studies focusing primarily on species such as *Argemone mexicana* (Singh et al. 2021), *Chelidonium majus* (Huang et al. 2019), *Macleaya* spp. (Jia et al. 2011), and *Hylomecon japonica* (Li et al. 2017), despite the family's significant diversity, encompassing around 42 genera and 775 species (Kalis 1979, Kadereit 1993, Xu and Deng 2017).

Notably, within *Papaveraceae*, all identified endophytes have been ascomycetes, with no reports of basidiomycetes (Jia et al. 2011, Li et al. 2017, Huang et al. 2019, Singh et al. 2021). Recently, the study by Tajik Gharibi et al. (2024) identified 32 root endophytic fungal species from *G. fimbrilligerum*, *G. contortuplicatum*, *C. majus*, *Papaver macrostomum*, *P. chelidoniifolium*, *P. pavoninum*, and *P. rhoeas* in Iran, with 96.87% belonging to *Ascomycota*. *Irpex laceratus* was the only Basidiomycete isolated from *G. fimbrilligerum*. *I. laceratus* (previously known as *Ceriporia lacerata* and *Emmia lacerata*) is a basidiomycete commonly found on decaying wood, where it causes white rot, playing a key role in forest ecosystems (Soleimani 1976, Totmaj et al. 2021, Fusco et al. 2022). Recent studies have revealed diverse ecological behaviors of *I. laceratus*, including its endophytic associations with plant species such as *Cleistocalyx operculatus*, *Phlegmariurus phlegmaria*, *Oryza sativa*, and *Piper nigrum* (Wang et al. 2013, Zhang et al. 2015, Sreeja et al. 2016, Lapuz et al. 2018), while pathogenicity has been reported in tomatoes (Adedire et al. 2022). *I. laceratus* is also regarded as a potential source of antifungal, antibacterial, and bioactive compounds (Furtado et al. 2019, Feng et al. 2021, Fusco et al. 2022). In northern Iran, this species has been frequently observed in forests, and unpublished local research suggests its presence as an endophyte in wild plants from the Alborz Mountains (Hatamzadeh et al. 2020, Hatamzadeh et al. 2023). This study investigated the volatile organic compounds (VOCs) produced by *I. laceratus* (G41 and G.f), an isolate identified by Tajik Gharibi et al. (2024) from *G. fimbrilligerum* in Iran, using Gas Chromatography-Mass Spectrometry (GC-MS). The purpose of this study is to provide a comprehensive chemical profiling of *I. laceratus* to systematically characterize its diverse VOCs and reveal its potential for novel bioactive compound discovery.

MATERIALS AND METHODS

Fungal preparation

Irpex laceratus was obtained from Gorgan University of Agricultural Sciences and Natural Resources. This isolate was identified in previous studies at Gorgan University of Agricultural Sciences and Natural Resources, and the Internal Transcribed Spacer (ITS) and the Large Subunit (LSU) ribosomal DNA genomic regions of the endophytic fungus has been deposited in the National Center for Biotechnology Information (NCBI) under accession numbers MH880256 and MK447560, respectively (Tajik Gharibi et al. (2024). The fungus was cultured on Potato-Dextrose-Agar (PDA) medium (Merck 110130) with a pH adjusted to 7 ± 0.2 and incubated under dark conditions at 25 ± 2 °C for three days. For liquid culture, 5-mm mycelial discs, excised from the edge of a 3-day-old fungal colony on PDA, were inoculated into 250 mL Erlenmeyer flasks containing 100 mL of Potato-Dextrose-Broth (PDB) medium (HiMedia M403), also adjusted to pH 7 ± 0.2. The flasks were incubated in darkness at 25 ± 2 °C on an orbital shaker set to 120 rpm for 14 days (Akbari Oghaz et al. 2022).

Metabolite extraction

Extraction of VOCs followed the method of Siddiquee et al. (2012) with slight modifications. After incubation, the fungal culture was filtered through a 400-mesh filter to separate the mycelium and spores from the broth. An equal volume of ethyl acetate (Merck, HPLC grade) was added to the filtrate, and the mixture was allowed to stand for 12 hours to ensure complete fungal cell death. The ethyl acetate phase was carefully separated from the aqueous phase using a Buchner vacuum filtration funnel. The resulting extract was concentrated by evaporation at 60°C using a rotary evaporator (Heidolph Hei-VAP Advantage) set at 60 rpm. After evaporation, the extract was resuspended in 100 mL of n-hexane (Merck, HPLC grade), and n-butanol (Merck, HPLC grade) for further analysis. The prepared extracts were either subjected immediately to GC-MS analysis or stored at -20°C in a laboratory-grade freezer (Thermo Fisher Scientific) until analysis.

Metabolite identification

A Shimadzu GC (Japan) equipped with a QP5050A Mass Selective Detector (MSD) was employed to analyze the VOCs. The GC-MS system operated at an electron ionization energy of 70 eV. Chromatographic separation was performed on a non-polar capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness, DB-5MS or equivalent). The oven temperature program was as follows: an initial temperature of 60 °C held for 1 min, followed by a ramp of 10 °C/min to 300 °C, with a final hold at 300 °C for 5 min. The injector was maintained at 300 °C in splitless mode, and the detector temperature was set at 320 °C. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The MS detector was operated with an ion source temperature of 200 °C and a scan range of 35–450 m/z, with a scan rate of 0.50 scans per second.

A solvent delay of 3 minutes was applied to prevent solvent interference. Metabolites were identified by comparing mass spectra against the National Institute of Standards and Technology (NIST23) library version 23, with a match probability of ≥70%. (Siddiquee et al. 2012). A positive control consisting of a caffeine standard (Sigma-Aldrich) was included to validate the GC-MS method, while a negative control utilizing sterile media without fungal inoculation was established to account for any background noise or contamination. Each treatment, including both controls, was subjected to three separate injections to allow for replication and enhance statistical robustness. For semi-quantitative analysis, n-hexadecane (Sigma-Aldrich, purity ≥99%) was used as an internal standard and added to each extract at a final concentration of 10 µg/mL prior to GC-MS injection.

RESULTS

VOCs from n-hexane solvent

The GC-MS analysis, utilizing n-hexane as the solvent, identified 47 distinct organic compounds (Supplementary Fig. S1; Table 1). Among these, the most abundant metabolite was Oxime-, methoxy-phenyl- (18231922), while the least abundant was Oxalic acid, cyclobutyl pentadecyl ester (204998) (Table 1). Sebacic acid, di(2-ethylbutyl) ester exhibited the longest retention time at 43.91 minutes, while p-Xylene had the shortest retention time at 6.13 minutes (Table 1). The compound with the highest %Area was Oxime-, methoxy-phenyl- at 22.24%, and the lowest was Oxalic acid, cyclobutyl pentadecyl ester at 0.25% (Table 1). Compounds with molecular weights between 200 and 300 g/mol accounted for approximately 38.29% of the identified *I. laceratus* metabolites in n-hexane, while compounds with molecular weights between 100 and 200 g/mol and 300 and 400 g/mol comprised 14.89% and 29.78%, respectively (Table 1). Only 10.63% of the metabolites had molecular weights between 400 and 500 g/mol (Table 1). The highest molecular weight was observed in the organobromine compound 1,54-Dibromotetrapentacontane at 917.2 g/mol, followed by Tetratetracontane and the organofluorine compound Octacosyl trifluoroacetate, with molecular weights of 619.2 g/mol and 506.8 g/mol, respectively (Table 1). Simple hydrocarbons represented the largest class of compounds, accounting for 40.42% of the metabolites, followed by organo-oxygen compounds at 29.78% (Table 1). The remaining metabolites were classified as organonitrogen compounds (10.63%), organosilicon compounds (4.25%), organoiodine compounds (6.38%), organochlorine compounds (2.12%), organosulfur compounds (2.12%), organobromine compounds (2.12%), and organofluorine compounds (2.12%) (Table 1).

VOCs from n-butanol solvent

The GC-MS analysis, using n-butanol as the solvent, identified 36 distinct organic compounds (Supplementary Fig. S2; Table 2). Six compounds-Cyclotrisiloxane, hexamethyl, Tridecane, Hexadecane, Heneicosane, Heptacosane, 1-chloro, and Bis(2-ethylhexyl) phthalate-were detected in both n-hexane and n-butanol extractions (Tables 1 and 2). Similar to the results obtained using n-hexane, the compound with the highest relative abundance in n-butanol was Oxime-, methoxy-phenyl-, with a relative abundance of 2,386,397 (Table 2). In contrast, Heptacosane, 1-chloro had the lowest abundance, registering at 57,372 (Table 2). The metabolite with the longest retention time was 1,2-Bis(trimethylsilyl)benzene at 43.92 minutes, whereas Dodecahydropyrido[1,2-b]isoquinolin-6-one exhibited the shortest retention time at 5.2 minutes (Table 2). The highest %Area was again associated with Oxime-, methoxy-phenyl- (18.59%), and the lowest %Area with Arsenous acid, tris(trimethylsilyl) ester (0.29%) (Table 2). Unlike the n-hexane solvent analysis, no organo-iodine or organo-bromine compounds were identified in the n-butanol solvent (Tables 1 and 2). However, organo-arsenic compounds, such as Arsenous acid, tris(trimethylsilyl) ester, were detected in n-butanol but not in n-hexane (Table 2).

The metabolite profile of n-butanol was distinct from that of n-hexane (Tables 1 and 2). Simple hydrocarbons comprised 33.33% of the identified *I. laceratus* metabolites in n-butanol, while organo-oxygen compounds accounted for 27.77% (Table 2). The remaining identified compounds included organonitrogen (11.11%), organosilicon (16.16%), organoarsenic (2.77%), organochlorine (11.11%), organosulfur (2.77%), and organofluorine (2.77%) compounds (Table 2). The highest molecular weight was attributed to the organofluorine compound Octatriacontyl pentafluoropropionate, with a molecular weight of 697 g/mol (Table 2). This was followed by 17-Pentatriacontene, Heptacosane, 1-chloro, and 2-Methyloctacosane, with molecular weights of 490.9, 415.17, and 408.8 g/mol, respectively (Table 2). Compounds with molecular weights ranging from 100 to 200 g/mol constituted 13.88% of the identified metabolites, while those between 200 and 300 g/mol accounted for 44.44% (Table 2). Additionally, compounds with molecular weights between 300 and 400 g/mol made up 30.55%, and those between 400 and 500 g/mol comprised 8.33% of the total identified organic compounds in the n-butanol extraction (Table 2).

DISCUSSION

Possible bioactive potential of *Irpex laceratus*

As the results showed, organosilicon compounds included approximately 10.52% of all identified *I. laceratus* metabolites. Organosilicon metabolites are typically absent in biological systems, with carbon-silicon bonds formed only through microbial enzymes (Houk et al. 1979). Organosilicon compounds are widely used in pharmaceuticals, antifoams, adhesives, and agricultural adjuvants (Hiyama and Shirakawa 2002).

In this study, only two organo-iodine compounds were identified among the metabolites of *I. laceratus*, despite over 3,000 such compounds known, many of which have hormonal activity or are used as pesticides (Grelier et al. 2018). Additionally, 6.4% of identified metabolites were organochlorine compounds, previously linked to pesticide use (Blus 2002). The study also found organo-sulfur compounds, known for their antimicrobial properties and potential therapeutic benefits, particularly in cancer chemoprevention (Moriarty et al. 2007, Sagdic and Tornuk 2012). Other antimicrobial organo-bromine, organo-fluorine, and organo-arsenic compounds were also identified (Gribble 2000, Chen et al. 2020, Ogawa et al. 2020).

Consistent with our findings, Burhamzah et al. (2020) found that the endophytic isolate *I. laceratus* DF1 exhibited significant antibacterial activity against several pathogens at a 10% extract concentration. Subsequently, Zhang et al. (2022) reported that VOCs from *Ceriporia lacerata* HG2011 effectively controlled

Table 1. Details of secondary metabolites identified from *Irpex laceratus* using n-hexane solvent.

Pick No.	Metabolite	Chemical formula	Molecular weight (g/mol)	Abundance	Time (min)	%Area	%Match against NIST*	CAS** registry No. reference
1	p-Xylene	C_8H_{10}	106.16	1052402	6.130	1.28	90	106-42-3
2	Oxime-, methoxy-phenyl-	$C_8H_9NO_2$	151.16	18231922	8.024	22.24	78	222-86-6
3	Acridine, 9-methyl	$C_{14}H_{11}N$	193.24	4932120	9.554	6.02	77	611-64-3
4	Octamethylcyclotetrasiloxane	$C_8H_{24}O_4Si_4$	296.61	907853	10.758	1.11	86	556-67-2
5	Cyclotrisiloxane, hexamethyl	$C_6H_{18}O_3Si$	222.46	3415344	13.747	4.17	86	541-05-9
6	1H-Trindene, 2,3,4,5,6,7,8,9-octahydro-1,1,4,4,9,9-hexamethyl-	$C_6H_{10}N_4$	138.17	541189	14.701	0.66	77	55682-87-6
7	Tridecane	$C_{13}H_{28}$	184.37	2651495	17.042	3.23	83	629-50-5
8	Hexadecane	$C_{16}H_{34}$	226.41	5986877	22.526	7.30	90	544-76-3
9	Tridecane, 3-methyl	$C_{14}H_{30}$	198.38	591849	24.108	0.72	90	6418-41-3
10	Decane, 2,3,5-trimethyl	$C_{13}H_{28}$	184.36	2755468	25.011	3.36	90	62238-11-3
11	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32	593210	25.406	0.72	97	96-76-4
12	Cyclopentane, 1-butyl-2-propyl	$C_{12}H_{24}$	168.32	599990	25.831	0.73	77	62199-50-2
13	2-Methylpentacosane	$C_{26}H_{54}$	366.7	833871	25.992	1.02	72	629-59-4
14	Hexadecyl octyl ether	$C_{24}H_{50}O$	354.65	1127725	26.241	1.38	78	376-72-8
15	Methoxyacetic acid, 2-tridecyl ester	$C_{16}H_{32}O_3$	272.42	644469	26.506	0.79	91	282-04-8
16	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	296.23	611143	26.682	0.75	86	73105-67-6
17	Nonadecane	$C_{19}H_{40}$	268.51	8002588	27.362	9.76	94	629-92-5
18	Undecane, 2,3-dimethyl	$C_{13}H_{28}$	184.36	612722	29.582	0.75	91	17312-77-5
19	Hexadecane, 2,6,10,14-tetramethyl	$C_{20}H_{42}$	282.54	566549	29.707	0.69	90	638-36-8
20	10-Methylnonadecane	$C_{20}H_{42}$	282.5	439177	30.682	0.54	86	56862-62-5
21	Heneicosane	$C_{21}H_{44}$	296.6	621170	30.734	0.76	86	629-94-7
22	Heptacosane, 1-chloro	$C_{27}H_{55}Cl$	415.17	343243	30.932	0.42	80	62016-79-9
23	Nitric acid, nonyl ester	$C_9H_{19}NO_3$	189.25	999029	31.035	1.22	87	20633-13-0
24	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	296.23	685815	31.092	0.84	86	73105-67-6
25	Heptadecane, 8-methyl	$C_{18}H_{38}$	254.49	4251308	31.699	5.19	93	13287-23-5
26	Oxalic acid, allyl octadecyl ester	$C_{23}H_{42}O_4$	382.6	1383331	31.860	1.69	72	309-24-5
27	Phthalic acid, 2,2-dimethylpent-3-yl dodecyl ester	$C_{27}H_{44}O_4$	432.63	481757	33.131	0.59	78	415-53-7
28	2-Nonadecanone	$C_{19}H_{38}O$	282.5	487831	33.770	0.60	78	629-66-3
29	Phthalic acid, butyl hex-3-yl ester	$C_{19}H_{28}O_4$	320.4	3538627	34.973	4.32	86	356-95-5
30	Octacosane	$C_{28}H_{58}$	394.8	772827	35.088	0.94	72	630-02-4
31	Eicosane	$C_{20}H_{42}$	282.5	2189015	35.606	2.67	97	112-95-8
32	n-Tetracosanol-1	$C_{24}H_{50}O$	354.65	643130	35.824	0.78	78	506-51-4
33	Isobutyl tetradecyl ether	$C_{18}H_{38}O$	270.5	437585	35.881	0.53	83	406-32-7
34	Eicosane, 1-iodo	$C_{20}H_{41}I$	408.4	434683	37.656	0.53	88	406-31-8
35	Sulfurous acid, butyl tetradecyl ester	$C_{18}H_{38}O_3S$	334.6	540727	38.076	0.66	72	309-18-1
36	Hentriacontane	$C_{31}H_{64}$	436.8	691095	38.180	0.84	87	630-04-6
37	1,54-Dibromotetrapentacontane	$C_{54}H_{108}Br_2$	917.2	582088	38.351	0.71	80	156-09-4
38	Methoxyacetic acid, 2-tetradecyl ester	$C_{17}H_{34}O_3$	286.4	1324038	38.414	1.62	91	282-04-8
39	Dodecane, 6-cyclohexyl	$C_{18}H_{36}$	252.47	1026773	38.631	1.25	83	13151-86-5
40	Octacosyl trifluoroacetate	$C_{30}H_{57}F_3O_2$	506.8	392873	38.906	0.48	72	351-74-9
41	Eicosane, 10-methyl	$C_{21}H_{44}$	296.57	559931	40.494	0.68	91	630-01-3
42	Eicosyl nonyl ether	$C_{29}H_{60}O$	424.8	435976	40.728	0.53	74	406-37-8
43	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.6	2146730	41.859	2.62	91	117-81-7
44	Tetratetracontane	$C_{44}H_{90}$	619.2	336055	42.222	0.41	87	7098-22-8
45	Oxalic acid, cyclobutyl pentadecyl ester	$C_{21}H_{38}O_4$	354.5	204998	42.466	0.25	73	309-70-5
46	9-Octadecenamide	$C_{18}H_{35}NO$	281.5	466762	43.706	0.57	77	301-02-0
47	Sebacic acid, di(2-ethylbutyl) ester	$C_{22}H_{42}O_4$	370.6	898806	43.919	1.10	78	356-11-1

*: National Institute of Standards and Technology. **: Chemical Abstracts Service

Table 2. Details of secondary metabolites identified from *Irpex laceratus* using n-butanol solvent.

Pick No.	Metabolite	Chemical formula	Molecular weight (g/mol)	Abundance	Time (min)	%Area	%Match against NIST*	CAS** registry No. reference
1	Dodecahydropyrido[1,2-b]isoquinolin-6-one	$C_{13}H_{21}NO$	207.31	205268	5.201	1.60	79	8873-36-5
2	N-nitroso-4H-1,2,4-triazole-3,5-diamine	$C_2H_4N_6O$	128.09	294664	5.227	2.30	87	396-10-8
3	Benzene, 1,3-dimethyl	C_8H_{10}	106.16	327953	5.875	2.55	86	108-38-3
4	Oxime-, methoxy-phenyl-	$C_{19}H_{15}NO_5$	337.33	2386397	7.484	18.59	83	222-86-6
5	Benzeneacetic acid, TBDMS derivative	$C_{14}H_{22}O_2Si$	250.40	443163	9.274	3.45	78	78323-99-6
6	Decane	$C_{10}H_{22}$	142.28	93936	10.535	0.73	79	124-18-5
7	Cyclotrisiloxane, hexamethyl	$C_6H_{18}O_3Si_3$	222.46	266298	13.607	2.07	83	541-05-9
8	Carbonic acid, undecyl vinyl ester	$C_{14}H_{26}O_3$	242.35	465004	17.015	3.62	78	382-54-9
9	Tridecane	$C_{13}H_{28}$	184.36	1166261	22.515	9.08	90	629-50-5
10	Heptadecane	$C_{17}H_{36}$	240.5	527598	25.006	4.11	86	629-78-7
11	Phenol, 2,5-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	206.32	93048	25.406	0.72	90	5875-45-6
12	Dodecane	$C_{12}H_{26}$	170.33	129611	25.987	1.01	72	112-40-3
13	Octatriacontyl pentafluoropropionate	$C_{41}H_{77}F_5O_2$	697.0	174835	26.231	1.36	77	351-89-1
14	Oxalic acid, isobutyl nonyl ester	$C_{15}H_{28}O_4$	272.38	98628	26.677	0.77	72	309-37-4
15	Hexadecane	$C_{16}H_{34}$	226.44	1666875	27.356	12.98	94	544-76-3
16	Sulfurous acid, butyl nonyl ester	$C_{13}H_{28}O_3S$	264.43	109924	29.577	0.86	72	309-17-6
17	2-Methyloctacosane	$C_{29}H_{60}$	408.8	116158	30.734	0.90	78	376-72-8
18	Diethylmalonic acid, monochloride, pentadecyl ester	$C_{22}H_{41}ClO_3$	389.0	134540	31.040	1.05	70	369-41-8
19	1-Octanol, 2-butyl	$C_{12}H_{26}O$	186.33	142160	31.092	1.11	78	3913-02-8
20	9-Methylheptadecane	$C_{18}H_{38}$	254.5	898142	31.694	7.00	90	26741-18-4
21	17-Pentatriacontene	$C_{35}H_{70}$	490.9	245522	31.860	1.91	80	6971-40-0
22	n-Pentadecanol	$C_{15}H_{32}O$	228.41	138861	33.775	1.08	74	629-66-3
23	Phthalic acid, 5-methylhex-2-yl butyl ester	$C_{19}H_{28}O_4$	320.4	562977	34.973	4.39	86	371-09-2
24	Carbonic acid, nonyl prop-1-en-2-yl ester	$C_{13}H_{24}O_3$	228.33	89389	35.088	0.70	73	382-53-9
25	Heneicosane	$C_{21}H_{44}$	296.6	422965	35.606	3.29	90	629-94-7
26	Trichloroacetic acid, pentadecyl ester	$C_{17}H_{31}Cl_3O_2$	373.78	90436	35.824	0.70	70	74339-53-0
27	Heneicosane, 11-(1-ethylpropyl)	$C_{26}H_{54}$	366.70	242380	38.413	1.89	86	55282-11-6
28	Dodecane, 5-cyclohexyl	$C_{18}H_{36}$	252.47	122697	38.631	0.96	73	13151-85-4
29	Trichloro(octadecyl)silane	$C_{18}H_{37}Cl_3Si$	387.93	131214	40.494	1.02	72	112-04-9
30	Icosan-1-ol	$C_{20}H_{42}O$	298.5	111821	40.722	0.87	73	629-96-9
31	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.6	424084	41.859	3.30	83	117-81-7
32	Heptacosane, 1-chloro	$C_{27}H_{55}Cl$	415.17	57372	42.227	0.45	72	62016-79-9
33	9-Octadecenamide	$C_{18}H_{35}NO$	281.5	235614	43.706	1.84	87	3322-62-1
34	Arsenous acid, tris(trimethylsilyl) ester	$C_9H_{27}AsO_3Si_3$	342.49	36994	43.758	0.29	79	55429-29-3
35	Bis(trimethylsilyl) diethyl silicate	$C_{10}H_{28}O_4Si_3$	296.58	82809	43.804	0.65	80	13183-70-5
36	1,2-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	222.47	101778	43.924	0.79	80	17151-09-6

*: National Institute of Standards and Technology. **: Chemical Abstracts Service.

Penicillium spp. and *P. italicum*, identifying organo-oxygen compounds, including n-pentadecanol and 2-nonadecanone. Our study also identified additional compounds with potential antifungal, antibacterial, and insecticidal properties. (Li et al. 2025) identified 770 VOCs from *I. lacteus* (a fungal species closely related to *I. laceratus*), narrowing them down to 26 key compounds from Using SPME-GC-MS, demonstrated strong antifungal activity against *Botrytis cinerea*, *Fusarium oxysporum*, and *Alternaria alternata*. These findings suggest that *I. laceratus* endophyte isolates may be a promising source of bioactive compounds for industrial, pharmaceutical, and agricultural applications, though further research is needed to explore their potential.

Chemical constituents of n-hexane extract

A comprehensive analysis of the chemical compounds extracted in n-hexane solvent, based on the literature review, reveals that 34% of the constituents exhibit diverse properties and applications across various domains. p-Xylene is a crucial chemical feedstock, primarily used in the production of polymers such as polyethylene terephthalate (PET) and terephthalic acid, and is also polymerized to produce parylene (Al-Mussawii et al. 2022). Oxime-methoxy-phenyl (MPO), an alkaloid from *Conocarpus lancifolius*, exhibits antibacterial properties against *Bacillus subtilis*, has low cytotoxicity, and is extracted with a methanol yield of 1.6% (Al-Mussawii et al. 2022). Acridine derivatives, historically used for antibacterial and antimalarial treatments, are gaining renewed interest for their effectiveness against drug-resistant bacteria, especially when combined with low-power light (Wainwright 2001). Octamethylcyclotetrasiloxane (D4), a cyclic siloxane commonly used in silicone production, has low toxicity but is classified as a substance of high concern due to its persistence and bioaccumulation (Franzen et al. 2017).

Cyclotrisiloxane, hexamethyl (D3), a reactive organosilicon compound, is valuable for producing dimethylsilanols in organic chemistry (Jiang et al. 2010). Tridecane (n-tridecane), a combustible alkane, functions as a pheromone in insects like stink bugs and is also used in distillation (Krall et al. 1999). Hexadecane (cetane) plays a critical role in determining diesel fuel efficiency (cetane number of 100) and is used in drug delivery and radiolabelling due to its hydrophobic properties (Solyanikova and Golovleva 2019). 2,4-Di-tert-butylphenol (2,4-DTBP), a toxic component of essential oils, regulates biological processes in its producers (Zhao et al. 2020). 1-Iodo-2-methylundecane (1I2MU) acts as an estrogen-dependent chemosignal in female mice, influencing reproductive behaviors (Achiraman et al. 2010). The n-nonadecane is an ideal phase change material for thermal energy storage due to its high latent heat and favorable phase change temperatures (Li et al. 2010). Heneicosane serves as a pheromone in termites and mosquitoes, with concentration-dependent effects that allow it to attract or repel insects, offering potential for mosquito control (Seenivasagan et al. 2009).

Phthalic acid esters (PAEs), commonly used plasticizers, exhibit antimicrobial and allelopathic properties and are naturally detected in various plant and microbial sources (Huang et al. 2021). Octacosane, extracted from *Marantodes pumilum*, has antioxidant properties that support wound healing and collagen synthesis, while also functioning as an insect repellent (Rajkumar and Jebanesan 2004, Okechukwu 2020, Balachandran et al. 2023). Icosane (eicosane), used in paraffin wax and thermal energy storage, has been identified as a potential biomarker for Parkinson's disease (Lan et al. 2004). Hentriacontane, known for its antitumor and anti-inflammatory properties, exhibits cytotoxic effects on lymphoma cells and has antimicrobial effects when isolated from *Acanthospermum hispidum* (Sultana et al. 2024). Lastly, bis(2-ethylhexyl) phthalate (DEHP), a plasticizer in PVC products, is essential in industry but requires regulation due to its environmental and health impacts (Astuto et al. 2023). Each compound exhibits distinct chemical properties with applications ranging from industrial uses to ecological and pharmaceutical significance.

Exploring the chemical diversity of n-butanol solvent

Compounds extracted using n-butanol solvent, such as Bis(2-ethylhexyl) phthalate, Cyclotrisiloxane, hexamethyl, Heneicosane, Oxime-methoxy-phenyl, and Tridecane, were similar to those found in n-hexane. Additionally, several distinct compounds were identified, showcasing diverse properties and applications across industrial, pharmaceutical, and environmental sectors, highlighting the chemical versatility of n-butanol extracts. Benzene, 1,3-dimethyl (m-Xylene) is an aromatic hydrocarbon and one of the isomers of dimethylbenzene, commonly used in the production of isophthalic acid, which modifies the properties of polyethylene terephthalate, though its toxicity primarily manifests as narcotic effects upon exposure (Zhou et al. 2012). Decane, an alkane hydrocarbon present in gasoline and kerosene, serves as a nonpolar solvent but is of limited significance as a chemical feedstock compared to other alkanes (Van Buuren et al. 1993). Heptadecane, a straight-chain alkane, is widely used as a phase change material for thermal energy storage due to its favorable phase change temperature and high latent heat, making it ideal for smart textiles and thermal regulation (Can and Žigon 2022).

Dodecane functions primarily as a solvent, distillation chaser, and scintillator component, increasingly explored as a kerosene-based aviation fuel surrogate due to its molecular mass and hydrogen-to-carbon ratio (Lemmon and Huber 2004). Hexadecane, also known as cetane, is essential in measuring diesel fuel ignition quality and is used in chemotherapy drugs and medical imaging through radiolabelling due to its hydrophobic properties (Graner et al. 2003). Trichloro(octadecyl)silane forms self-assembled monolayers on hydroxyl-containing surfaces, enhancing stability in perovskite solar cells and creating stable monolayers for biosensors and photovoltaics (Iimura and Kato 1998, Zheng et al. 2021). Icosan-1-ol (Arachidyl alcohol), derived from peanut oil, is an emollient used in cosmetics, produced through the hydrogenation of arachidic or arachidonic acid, with its name originating from the peanut plant *Arachis* (Ventola et al. 2002). Collectively, these compounds demonstrate a diverse range of industrial and scientific applications.

CONCLUSSION

This study identified 75 distinct VOCs from an endophytic *I. laceratus* isolate using GC-MS analysis of n-hexane and n-butanol extracts. The detected compounds probably include bioactive substances, which may have potential applications in pharmaceuticals, agriculture, and industry. These metabolites appear to possess diverse functions, possibly including antimicrobial, pesticidal, energy storage, and polymer production roles. It is important to note that GC-MS primarily detects VOCs and semi-VOCs, so non-VOCs or thermally unstable metabolites present in the crude extracts might not have been captured. Our literature review suggests promising insights into the possible bioactive capabilities of these compounds, but confirming such activities would depend on future direct experimental studies. Despite current limitations, *I. laceratus* could be a valuable source for bioactive metabolites, warranting further research to optimize extraction and explore practical applications.

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AUTHOR CONTRIBUTION

All authors contributed to the conception and design of the study. Yasaman Tajik Gharibi contributed to performing all tests and writing the original draft. Kamran Rahnama contributed to conceptualization, supervision, validation, review, and editing original draft. Amir Zolfaghary contributed to software and data analysis. Khodayar Hemmati contributed to the methodology, review, and editing original draft. Afsaneh Graan contributed to optimizing tests, visualization, and investigation; All authors have read and approved the final draft of the manuscript.

DATA AVAILABILITY

The raw data that support the findings of this study are available from the corresponding author, upon reasonable request.

DECLARATION

The author declares that there is no statement of interest.

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ETHICS APPROVAL

Not applicable.

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نمایه‌سازی ترکیبات آلی فرّار *Irpex laceratus*: یک قارچ اندوفیت جداسازی‌شده از گیاه *Glaucium fimbrilligerum*

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چکیده

این مطالعه به بررسی ترکیبات آلی فرّار (VOCs) قارچ *Irpex laceratus*، یک قارچ اندوفیت جدید جداسازی‌شده از گیاه *Glaucium fimbrilligerum* پرداخته است. متابولیت‌ها با استفاده از استات اتیل استخراج شده، در حلال‌های ان-هگزان و ان-بوتانول رقیق‌سازی گردیدند و با بهره‌گیری از دستگاه کروماتوگرافی گازی-طیف‌سنجی جرمی (MS-GC) با ستون غیرقطبی و تطابق با کتابخانه NIST نسخه ۲۳ (≤۷۰٪) شناسایی شدند. در استخراج با ان-هگزان، ۴۷ ترکیب متمایز شناسایی شد که هیدروکربن‌های ساده (۴۰/۴۲٪) و ترکیبات آلی اکسیژنه (۲۹/۷۸٪) بیشترین فراوانی را داشتند. سایر گروه‌های شناسایی‌شده شامل ترکیبات آلی نیتروژنه (۱۰/۶۳٪)، آلی یددار (۶/۳۸٪)، آلی سیلیکونی (۴/۲۵٪) و مقادیر اندکی از ترکیبات آلی کلردار، گوگرددار، برم‌دار و فلوئوردار (هرکدام ۲/۱۲٪) بودند. بیشتر متابولیت‌ها دارای وزن مولکولی بین ۲۰۰ تا ۴۰۰ g/mol بودند که ۴۴/۶۷٪ از کل ترکیبات را شامل می‌شد. در مقابل، استخراج با ان-بوتانول ۳۶ ترکیب متمایز را نشان داد که توزیع شیمیایی متفاوتی داشتند: هیدروکربن‌های ساده (۳۳/۳۳٪)، ترکیبات آلی اکسیژنه (۲۷/۷۷٪)، آلی سیلیکونی (۱۶/۱۶٪)، آلی نیتروژنه و کلردار (هرکدام ۱۱/۱۱٪)، و مقادیر اندکی از ترکیبات آلی آرسنیک‌دار، گوگرددار و فلوئوردار (هرکدام ۲/۷۷٪). بیشترین دامنه وزنی مولکولی در متابولیت‌های ان-بوتانول بین ۲۰۰ تا ۳۰۰ g/mol (۴۴/۴۴٪) و پس از آن ۳۰۰ تا ۴۰۰ g/mol (۳۰/۵۵٪) بود. مطالعه حاضر، نمایه‌سازی شیمیایی جامعی از قارچ اندوفیت *I. laceratus* ارائه می‌دهد و به عنوان یک مشارکت پایه‌ای در جهت شناسایی نظام‌مند تنوع VOCهای آن به شمار می‌رود. شناسایی طیف گسترده‌ای از ترکیبات آلی با ساختار و عملکرد متنوع، پیچیدگی متابولیکی این گونه را نشان داده و پتانسیل آن در کشف ترکیبات زیست‌فعال جدید را برجسته می‌سازد.

کلمات کلیدی: اکولوژی شیمیایی، تعامل گیاه-میکروب، ترکیبات شیمیایی قارچها ،میکوکمیکال، هیدروکربن.

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