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ISOLATION OF METABOLITES FROM TRICHODERMA ASPERELLUM FUNGUS AND STUDY OF THEIR PROPERTIES

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Abstract

Today, the issues of restoring human health, meeting the demand for food, or maintaining soil fertility in a moderate state are among the issues of a wide range of issues. Therefore, in order to solve these problems, today more and more natural resources are being eliminated. Therefore, we also aimed to isolate the secondary metabolites from the fungus Trichderma asperellum, which contains metabolites of different properties in itself, and to study their properties. During our treatment, the extract sum obtained from the fungus of Trichoderma asperellum was fractionated in different solvent systems (hexane, hexane chloroform, chloroform, chloroform methanol, methanol) and its secondary metabolites were extracted. In this study, we thought it necessary to introduce some chromatographic analyses of the fractionated secondary metabolites in the chloroform methanol 19:1 system and the properties of some of them.

Keywords: *Trichoderma asperellum, extract, secondary metabolites, chromatographic analyses*

Introduction

Identification of beneficial plants, organisms and microorganisms and the study of their beneficial properties is a wide network around the world. Our research team also carries out practical work on the identification of microorganisms, including micro- and macrofungi and the isolation and determination of metabolites they produce in order to study their beneficial and harmful properties. In this regard, in order to make our research more correct and complete, we have identified several sources on the isolation of metabolites of fungi of the constellation Trichoderma, the study of their biological properties. Volatile organic compounds produced by the trichoderma atrovirid fungus have been found to be related to plants, that is, these metabolites are associated with mycoparasitic interactions with host fungi. Three of them – described as agents of an antifungal nature – were highly secreted by the Δhda1 mutant, while 3-octanone was found in smaller amounts in the head cavity. The metabolites produced by T. atroviride were also significantly enhanced in the growth and development of B. cinerea

Δhda1 (Verena Speckbacher et al., 2024). In addition, 25 different MVOCs were found when Trichoderma atroviride was applied in biocontrol cultures (spectral compatibility factor of at least 90% and LTPRI ± 2% maximum relative deviation from the literature values). These metabolites were known to be VOCs of the classes of alkanes, alcohols, ketones, furans, pyrones (mainly bioactive 6-pentyl-alpha-pyrone), mono- and sesquiterpenes, while the production of 13 Trichoderma spp was not previously identified and 11 volatile substances were additionally validated using valid standards (Norbert Stoppacher et al., 2010). In another study, the effect of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) synthesized by the T. harzianum IM 0961 strain on lipidome and selected extracellular substances was studied, and it was found that the herbicide 2,4-D affects the lipid moiety in the mycelium and that the herbicide has lipophilic properties (Julia Mironenka et al., 2020). Available data reveal the important importance of secondary metabolites of T. harzianum, which may reduce side effects in antioxidant and anti-inflammatory treatments. Therefore, T. harzianum has retained a potent antioxidant property and effectively clears ROS by lowering T-reg markers. (Alblihed et al., 2023). In order to study the peptidaibiotic substances synthesized by fungi of the constellation Trichoderma chromatographic and spectroscopic methods were used. All of these peptidaibiotic groups have been found to exhibit biological activity including antibacterial, antifungal, antiviral effects, as well as cytotoxic effects such as immunosuppressive and neuroleptic properties (Adigo Setargie et al., 2024). Ethyl acetate extract of TH-TIND02 Gas Chromatography – Mass spectrometry analyses revealed 21 major and small volatile organic compounds counted as a multiplicity component: acetamide, 2, 2, 2-trifluoro-N, N-bis trimethyl-Icylyl-C (94.74%), along with the isolation produced hydrolytic enzymes chitinase, cellulose, β-1, 3 glucanase and protease as a component (Abhay K et al., 2024). Trichoderma releases various volatile compounds, including alcohols, aldehydes, ketones, ethylene, hydrogen cyanide, and monoterpenes, as well as non-volatile compounds, including peptaibols, and diketopiperazine-like glycotoxins and gliovirin, which show antibiotic activity. (Amrita Saxena et al., 2025). In addition, when root samples of the plant were inoccated with Trichoderma asperellum, sometime after inoculation with Ganoderma. boninense, fungus-releasing metabolites were detected and detected in the GC–MS method (Muniroh Ms et al., 2025). Of the several isolates of another Trichoderma, the following most abundant substances were nabbed: 6-Pentil-2H-pyran-2-A, 2,3,5,5,8a-pentamyl-6,7,8,8a-tetrahydro-5H-chrome-8-ol Toluene, 2,4, Ditert-butyl phenol, 1,5, Dimethyl-6-methylene spiro (2,4) heptanes and 2,4, Ditert-butylphenol, 1,5, Dimethyl-1-methylenpyro (2,4) heptanes and N, N-dimethyl-1-(4-methylphenyl)ethanol, Benzenethanol, 1,5-dimethyl-6-methylenpyro(2.4) and 6-pentil-2H-pyran-2-one, Anethanol and 1-hydroxy-2,4-di.tert lequinyl Benzene vs Benzene (Srinivasa Nagappa Chowluru et al., 2017). In another study with T. hamatum, it was extracted in ethyl acetate and several secondary metabolites were obtained and purified and studied by HRESIMS, NMR, UV, IQ, circular dichroism, and Mosher deposition method. In this case, 1–7 structure compounds not previously known, 8 known are obtained. Metabolites 1, 2, and 9 are rare, compounds 3–8 are cyclonean sesquiterpenes, 6–5–4–7 are harzian diterpenes composed of tetracyclic carbon skeletons, and compound 5 is known to be cycloneranesesquiterpenes, which is the first to replace -OOH (Li Huang et al., 2024). Metabolites of Trichoderma strains found in the sea have also been studied. Molecular diversity of Trichoderma metabolites, especially the abundance of metabolites that conserve the skeletons of cycloneran, bisabolan, harzian, sorbitsillinoid, and peptaibol, has been identified. Among the metabolites, 235 members are known to have several bioactiveites such as microalgal, antifungal, cytotoxic, zooplankton-toxic, antibacterial, anti-inflammatory, anti-inflammatory (Yin-Ping Song et al., 2024). Ethyl acetate extraction sum of TRI07 isolate When GC–MS was examined, spathulenol, triacetin, and aspartame were checked to be the major substances, and were observed to be 28,90, 14,03, and 12,97%, respectively. The analysis of TRI07-VOC with the solid-phase microextraction technique

revealed that the most common compounds include ethanol, hydroperoxide, 1-methylhexyl, and 1-octen-3-one. When TRI07 interacts with Alt3, 34 compounds with key components including 1-octen-3-one, ethanol, and hexanedioic acid, bis(2-ethylhexyl) ester were identified. (Philip B et al., 2024). The filamentous fungus Trichoderma reesei is a multi-producer of plant cell wall-disrupting enzymes that are regulated in response to various environmental signals for optimal adaptation, but also produce a wide range of secondary metabolites (Schalamun M et al., 2023). To investigate the antiviral properties of secondary metabolite compounds derived from four Trichoderma spp. culture filtrates. GC–MS analysis. 24 substances with a relative amount of more than 10% of secondary metabolites have been reported (Rizk M. N. et al., 2024). In addition, identification of volatile metabolites isolated from Trichoderma strains and gas chromatography-mass spectrometry (GC–MS) assays revealed 98 volatile compounds with antifungal activity through GC–MS analysis: phenethyl alcohol, benzene derivatives, D-limonene, octadecanoic acid methyl ester, toluene, hexadecanoic acid, several phenolic isomers, and important volatile compounds with antifungal activity such as eicosamine and eicosamine (Abdenaceur R. et al., 2024). Our previous studies have provided information on the isolation of secondary metabolites from T. asperellum fungus and GC–MS analysis and their significance (Nomozova M. Z et al., 2023).

Method

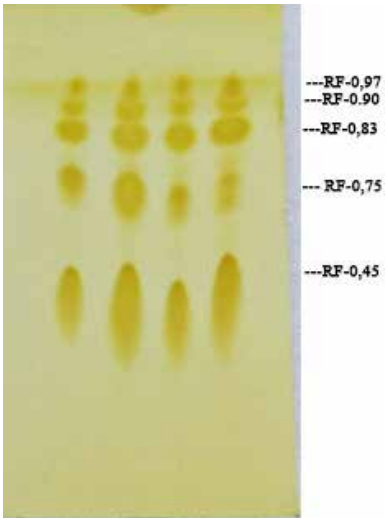
Preparation of biomass

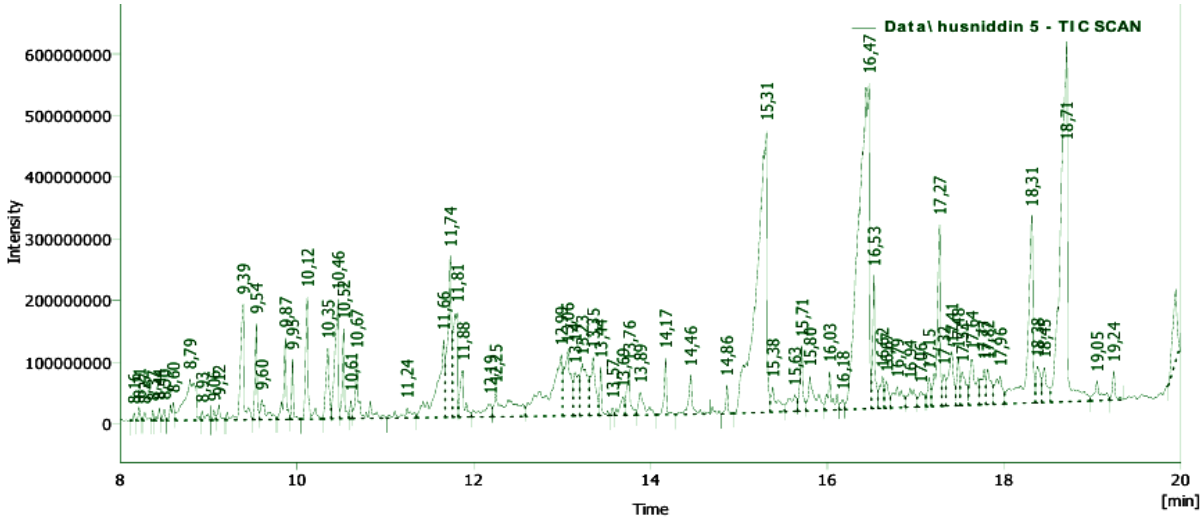
The strain of T. asperellum was primited for 2 weeks under mandible feed conditions, followed by isolation using filitation paper (Whatman #1.5). The collected biomass was dried at a temperature (+ 45 °C). In the next step, the biomass was ground until it became a powder. The resulting mass ethyl alcohol was mixed in a 1:2 state and put on a pendulum shaker at 155 µl/min for 20 h. The alcohol mixture was then subjected to filtration process and the obtained filtrate was driven in a rotary drive at 40 µL/min at 78.5 °C. This process was repeated 7–8 times. The total extract mass was collected and dried, so that the finished mass was 15gr.

Fractionation technology

For extraction of the extracted dry curd mass, colonical (columnar) chromatography was used to isolate the solids. The length of the glass column is 60 cm, and it is considered to have a diameter of 12 cm. Initially, the kalonka was washed in hexane substance, then 240 gr of 100/250 m size from silicagel L (Chemapol Prana-Czechoslovakia) was placed on this kalonka. The 15g biomass was thoroughly mixed with 14g silicagel and turned into paroshok and put into a column over the silicagel. Fractionation was applied sequentially according to the polarity of the solvents and each separated fraction was verified by TLCH analysis.

Figure 1. *Thin-layer and gaseous liquid chromatography of chloroform methanol 19:1 fraction*





Results

In this study, we cited several analyses and some of the metabolites obtained when the fractionation process was conducted in a ratio of 19:1 to chloroform methanol. During this step of fractionation, the obtained fractions were treated with thin-layer (chloroform, methanol 9:1 solvent system was used) and gaseous liquid chromatographic analysis was performed (Fig. 1).

The volatile substances detected by the obtained chromatographic examinations were found to be the following compounds when compared with the data in the MS data library (Table 1).

Table 1. *Chloroform: methanol 19:1 in the fraction of metabolites decomposed*

№	Metabolit name	Molecular formula	Moleculyar mass g/mol	Time to absorption
1	Trans-2-undecenoic acid	C11H20O2	184.28	11,879
2	Cyclopropaneoctanoic acid, 2-octyl-, methyl ester	C20H38O2	310.5	12,990
3	9-Tetradecenal, (Z)	C14H26O	210.36	13,061
4	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C24H38O4	390.55	13,226
5	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C24H38O4	390.55	13,352
6	2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)	C13H22O	194.3132	13,438
7	9,12-Octadecadienoyl chloride, (Z, Z)-	C18H31ClO	298.9	13,688
8	Tetradecanoic acid	C14H28O2	228.37	13,760
9	Pentadecanoic acid	C15H30O2	242.40	14,455
10	Hexadecanoic acid, methyl ester	C17H34O2	270.4507	14,864
11	n-Hexadecanoic acid	C16H32O2	256.4241	15,312
12	11-Octadecenoic acid, methyl ester	C19H36O2	296.4879	16,028
13	Oleic Acid	C18H34O2	282.4614	16,473
14	Octadecanoic acid	C18H36O2	284.4772	16,530
15	Hexadecanoic acid, ethyl ester	C18H36O2	284.4772	17,272

№	Metabolit name	Molecular formula	Moleculyar mass g/mol	Time to absorption
16	7,10,13-Eicosatrienoic acid, methyl ester	C21H36O2	320.5093	17,318
17	Linoleic acid ethyl ester	C20H36O2	308.4986	17,956
18	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	C21H40O4	356.53	18,311
19	Diisooctyl phthalate	C24H38O4	390.6	18,709
20	Oxiraneoctanoic acid, 3-octyl-, cis-	C18H34O3	298.4608	19,053
21	Retinoic acid, methyl ester	C20H28O2	300.4351	19,243

Discussion

During this further fractionation, several undissociated metabolites were detected: 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, 1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, 2-Butanone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl), retinoic acid, methyl ester. When the properties of retinoic acid were studied, it was found that vitamin A and its active metabolite all-trans-retinoic acid (ATRA) are a collection of substances that regulate several physiological functions in some organ systems, and normal immunity was considered important. Vitamin A derivatives are useful in the treatment of cancer, and ATRA is used in the differential treatment of acute promyelocytic leukemia (APL) (Łukasz Szymański et al., 2020). Vitamin A and retinoid derivatives are recognized as morphogens that govern body structure and skeletogenesis and, when excessive, cause profound defects (Alanna C. Green et al., 2016). At the same time, the decomposed linoleic acid (18:2ō6; cis, cis-9,12-octadecadienoic acid) was also known to be the most consumed PUFA in the human diet (Jay Whelan et al., 2013).

Conclusion

The results of the above study show that as a result of this stepwise fractionation, organic substances, unlike some other fractions, have decomposed and these substances are substances of specific biologically active properties.

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