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CHROMATOGRAPHIC-MASS SPECTRUM ANALYSIS OF BIOLOGICALLY ACTIVE SUBSTANCES SEPARATED FROM THE HEXANE EXTRACT OF FUNGUS FUSARIUM OXYSPORUM SCHLECHT BELONGING TO THE CATEGORY FUSARIUM

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Abstract

In recent years, the type of microfungi that have a pathogenic effect on many micronutrients plants has been on the rise. But these fungi themselves also have the ability to synthesize biologically active metabolites. In our research, we aimed to investigate the fungal metabolites of the constellation Fusarium. Hexane extract of cultivated biomass of *Fusarium oxysporum Schlecht* fungus at 6–7 days were analyzed by the GC–MS method and a wide variety of substances were revealed. In this study, secondary metabolites of hexane extract obtained from the cultured biomass of Fusarium oxysporum Schlecht fungus for 6–7 days were analyzed. Gas chromatography–mass spectrometry (GC–MS) revealed a number of bioactive compounds, including 4-(1,1,3,3-tetramethylbutyl)phenol, methyl sis-3-[p-anisido]acrylate, formic acid, 1-(4,7-didro-2-methyl-7-oxopyrazolo[1,5-a]pyrimidine-5-yl)-, methyl ether, galactose, 4,6-O-nonidene, and 2,6-di-gesaddecanoate ether of L-(+)-ascorbic acid. These substances belong to the class of phenolic derivatives, acrylate ethers, nitrogen-sparing heterocyclic systems, carbohydrate complexes and ascorbate ethers of lipid nature and confirm the metabolic diversity of the fungus. The identified metabolites are distinguished by their antioxidant, antimicrobial, and cytoprotective properties, which indicates their practical value for fields of pharmaceuticals and biotechnology. The results obtained indicate that the species Fusarium oxysporum is structurally diverse and a source of biologically active natural compounds. In the future, it is promising to study these substances through isolation and mechanism studies on deeper bioactivity.

Keywords: *Fusarium oxysporum Schlecht, hexane extract, secondary metabolites, GC–MS, bioactive compounds, phenolic derivatives, ascorbate ethers.*

Log in

In recent years, biologically active metabolites of plant-pathogenic fungi have aroused special interest in science and practice. In particular, fatty acids, phenolic compounds, and various volatile organic compounds synthesized by strains belonging to the order Schlecht Fusarium oxysporum have been found to have broad-spectrum biological interactions (Raffaele et al., 2009; Liu et al., 2008). These microorganisms are studied not only as disease-causative pathogens in plants, but also as a source of secondary metabolites (Druzhinina et al., 2011). Many studies have shown that the substances isolated from the biomass or extract of Fusarium oxysporum Schlecht have antifungal, antibacterial, and cytotoxic properties (Pan et al., 2013; Stracquadanio et al., 2020). For example, fatty acids and sterol derivatives from hexane extracts may regulate plant–pathogen interactions in the soil microbiota (Abdel-Naime et al., 2019; Ding et al., 2019). Among such metabolites, cytotoxic and antimicrobial active substances have also been noted (Davis et al., 1997; Ghavam et al., 2021). Fatty acids, including palmitic, linoleic, and palmitoleic acids, have been recorded in many micromycetes, and they play important roles in natural defense mechanisms against plant diseases (Guo et al., 2010; Perez-Vich et al., 2016; Zhao et al., 2019). Among the many volatile metabolites identified through GC–MS analyses, benzene derivatives, terpenoids, and phenolic isomers are of particular importance (Reghmit et al., 2024; Azami et al., 2024; Nitish and Kumar, 2017). Such metabolites have been extensively studied in many studies on the genera Trichoderma and Fusarium (Ivan et al., 2023; Zeiad et al., 2023). There are prospects for application of the isolated secondary metabolites from extracts of F. oxysporum in pharmaceutical, agricultural, and biotechnology fields. Some of them inhibit the growth of pathogenic microorganisms and stimulate plant growth (Singh et al., 2021; Gajera et al., 2020). Also, substances identified in different extracts are being proposed as novel biomarkers (Khairillah et al., 2021; Jurakulova and Kamolov, 2021). Therefore, the aim of this study is to determine the composition of bioactive substances isolated from the hexane extract of Fusarium oxysporum and to elucidate their biological significance (Nomozova et al., 2025; Kamolov et al., 2021; Van Leeuwen et al., 2008). Substances identified through GC–MS. The application of antitumor, antioxidant, antimicrobial, and growth-stimulating properties of these fungal metabolites by researchers in the fields of pharmaceuticals, biotechnology, and agriculture is a promising direction. Also, studies by (Mishra et al., 2022) and Rahman and Singh (2023) have found that secondary metabolites synthesized by certain strains belonging to the genus Fusarium increase beneficial microorganism activity in plant root zones while inhibiting the growth of plant pathogens. El-Hassan et al., 2024) have highlighted the potential of these metabolites as bioconcentrates in the development of environmentally friendly fertilizers and pesticides. Therefore, the main goal of the present investigation is to determine the composition of bioactive substances isolated from hexane extract of Fusarium oxysporum, to analyze their biological activity and to elucidate the practical value of the obtained results. Research in this direction provides an important scientific basis for the development of environmentally friendly biotechnological solutions for the control of plant pathogens.

Research methodology

1. Fungal cultivation and biomass preparation

The strain of Fusarium oxysporum Schlecht was isolated from the soil and stored under sterile conditions. For experiment, fungus was incubated in a potato dextrose agar (PDA) environment at a temperature of 24.5–28 °C for 6–7 days. Subsequently, 250 ml of Potato Dextrose Broth (PDB) feed medium was poured into 500 ml Erlenmeyer tubes and a suspension of 107–108 spores/ml concentration was inoculated into it. Fungal biomass was isolated from the cultured liquid cultured for 6–7 days in the shaker incubator (180 rpm). The resulting biomass was washed with distilled water, dried at high 38 °C temperature and used in the following extraction process.

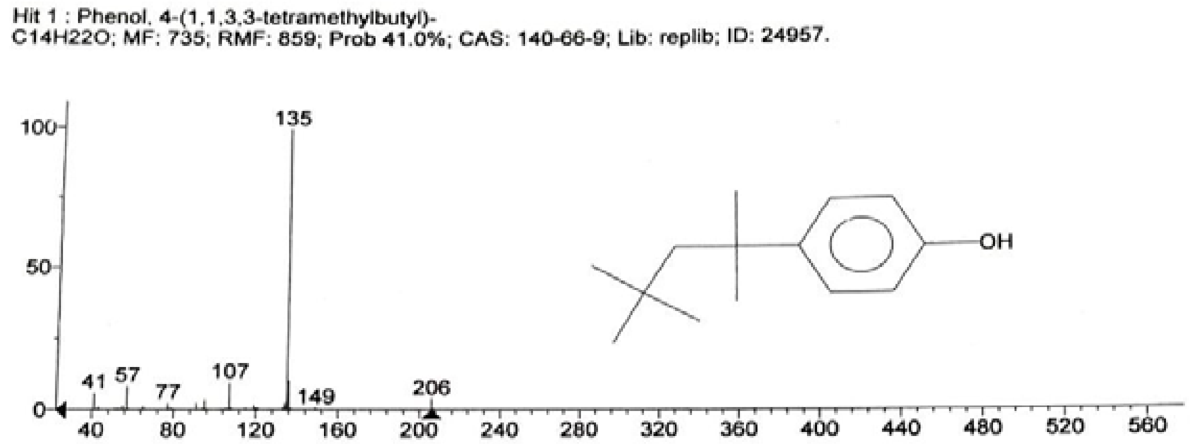
2. Extract using hexane

To extract bioactive substances from the dried biomass, an extraction apparatus was used. 50 g of dried litter powder were extracted for 8–10 hours with 500 mL hexane. The resulting crude extract was concentrated under vacuum using a rotary evaporator (Büchi R-210) at a temperature of 40 °C. The result was a dark yellow extract. The extract, comprising of a total complex of secondary metabolites, was given to chromatographic mass spectrum for determination of biologically active compounds contained in the formed yellow extract.

Results and discussion

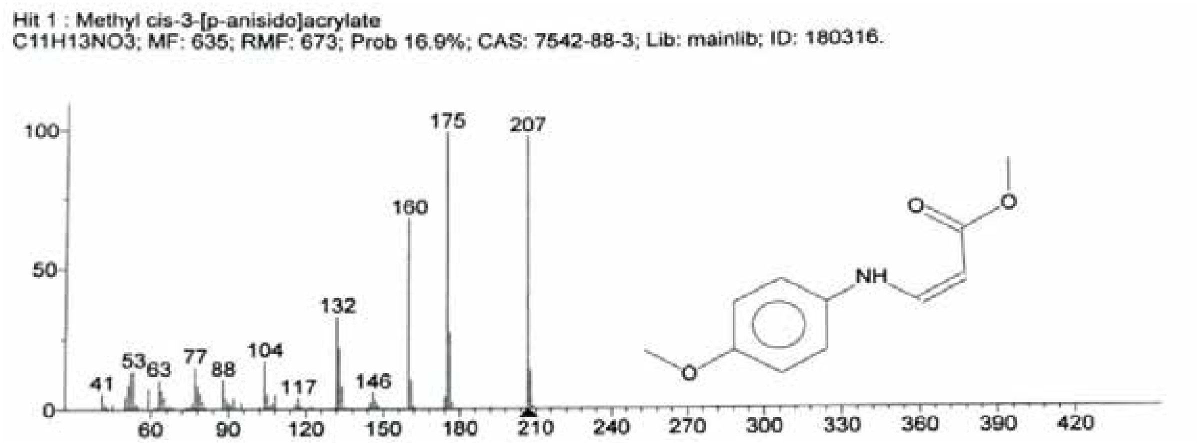
Hexane extract from the grown biomass of Fusarium oxysporum Schlecht fungus for 6–7 days was analyzed using gas chromatography–mass spectrometry (GC–MS). According to the results of the analysis, several bioactive components were revealed in the extract, indicating that they contained such substances as 4-(1,1,3,3-tetramethylbutyl) phenol, methyl sis-3-[p-anisido]acrylate, formic acid, 1-(4,7-didro-2-methyl-7-oxopyrazolo[1,5-a]pyrimidine-5-yl)-, methyl ether, galactose, 4,6-O-nonidene, and I-(+)-ascorbin acid, 2,6-dihexadecanoate.

Figure 1. *Spectrum of phenol 4-(1,1,3,3-tetramethylbutyl) substance obtained by GC–MS method*



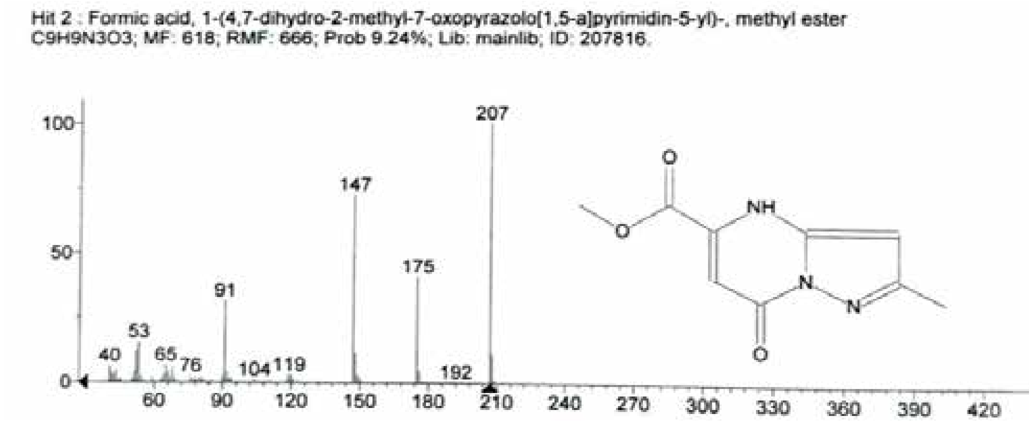
The chemical structure and bioactive properties of the identified compounds indicate their synthesis through various metabolic pathways. 4-(1,1,3,3-tetramethylbutyl)phenol is a substance of phenolic nature, which has a strong antioxidant and antimicrobial effect was previously noted in fungi of the genus Aspergillus and Penicillium. Its presence indicates the presence of protective mechanisms in the fungus of F. oxysporum that inhibit lipid peroxidation processes.

Figure 2. *Spectrum of methylcis-3-[p-anisido]acrylate obtained by GC–MS method*



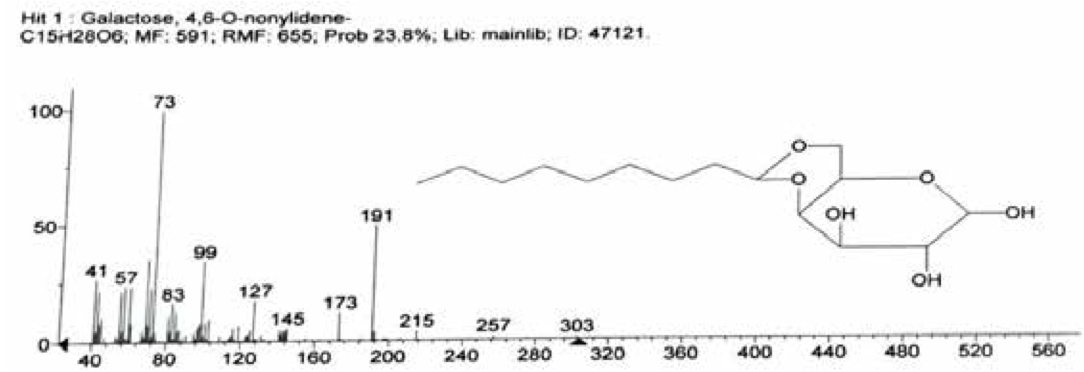
Meanwhile, methyl sis-3-[p-anisido]acrylate belongs to the class of acrylate ethers and is characterized by anti-inflammatory and antibacterial activity. The presence of this compound indicates the presence of a complex biosynthetic pathway of phenolic and etheric components in the genus Fusarium.

Figure 3. *Spectrum of formic acid, 1-(4,7-dihydro-2-methyl-7-oxopyrazolo[1,5-a] pyrimidin-5-yl)-methyl ester obtained by GC–MS method*



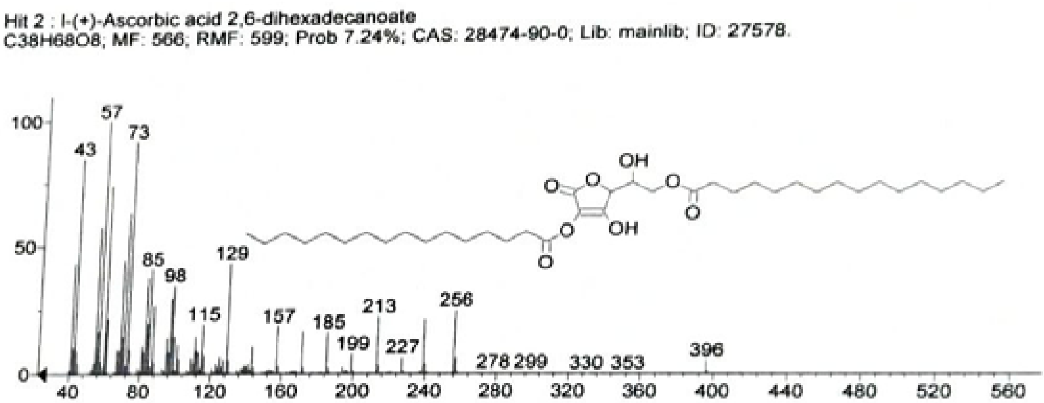
It is also noteworthy as a substance with a nitrogenous heterocyclic system, 1-(4,7-didro-2-methyl-7-oxopyrazolo[1,5-a] pyrimidine-5-yl)-, methyl ether, which is a derivative of formic acid. Compounds in this class have been reported in most literature as having antitumor, antifungal, and cytotoxic activity. Their presence suggests that the species F. oxysporum has the ability to form pyrozolo and pyrimidine rings during the process of secondary metabolite biosynthesis.

Figure 4. *Spectrum of Galactose, 4,6-O-nonydene obtained by GC–MS method*



Galactose, 4,6-O-nonidene, is a carbohydrate complex substance that performs protective functions in connection with glycosides and polysaccharides. Compounds of this type play an important role in maintaining the stability of the fungal cell wall, as well as in regulating metabolic balance.

Figure 5. *Spectrum of L-(+)-Ascorbic acid, 2,6-dihexadecanoate, obtained by GC–MS method*



The 2,6-di-hesadekanoate ether of L-(+)-ascorbic acid is an ascorbate derivative of lipid nature and is characterized by its strong antioxidant and membrane-protecting activity. Such compounds confirm that it was formed as a natural defense system against oxidative stress in microfungi such as F. oxysporum. This substance is the esterified form of ascorbic acid with fatty acids, which actively functions in the lipophilic environment in the cell membrane.

Table 1. *Extracts from the hexane extract of the grown biomass of Fusarium oxysporum Schlecht fungus in 6–7 days*

No	Substance Name	Chemical formula	Molecular mass (g/mol)	RT (min)
1.	4-(1,1,3,3-tetramethylbutyl)-phenol	$C_{14}H_{22}O$	206.32	11.635
2.	Methylcis-3-[p-anisido]acrylate	$C_{11}H_{13}NO_3$	205,9	13,928
3.	Formic acid, 1-(4,7-dihydro-2-methyl-7-oxopyrazolo[1,5-α]pyrimidine-5-yl)-, methyl ester	$C_9H_9N_3O_3$	206,1	13,951
4.	Galactose, 4,6-O-nonydene	$C_{15}H_{28}O_6$	302,5	14,090
5.	L-(+)-Ascorbin acid, 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	648,2	14,114

In Table 1, the increase in the RT value shows a delay in the release time as the mass and nonpolarity of the molecules increase. Therefore, among the substances in the table, phenols and ethers are eliminated more quickly, and shaker and ascorbin derivatives are more slower. Overall, the identified metabolites indicate that the fungus F. oxysporum has high biochemical adaptability and the potential to form a second-second metabolite. The diversity of the composition of these substances opens promising areas for their pharmacological application. In particular, phenolic and ascorbate derivatives can be used in the development of antimicrobial, antioxidant and antitumor drugs, and acrylate and pyrosolo-pyrimidine derivatives in the study of bioactive synthesis pathways. The results indicate that Fusarium oxysporum fungus is a potential biotechnological facility capable of synthesizing complex, biologically active metabolites. Therefore, future research should be directed towards identifying the bioactivity, toxicological properties and pharmaceutical value of these compounds.

Conclusion

The detection of bioactive metabolites belonging to the phenolic, indolic, phosphonate, and ester class in the hexane extract of Fusarium oxysporum Schlecht fungus showed that these microorganisms have a complex secondary metabolism system. The obtained results confirm the practical value of these compounds as antimicrobial, antioxidant and environmentally stable bioregulators. The results of the research expand the possibility of using fungi of the genus Fusarium as a source of bioactive substances in the pharmaceutical and biotechnology fields.

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