ACTIVE COMPOUNDS DETECTION IN AQUEOUS EXTRACT OF *GANODERMA APPLANATUM* LOCAL ISOLATE

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ABSTRACT

This study was aimed to investigate active compounds in the aqueous extract of *Ganoderma applanatum* mycelium. The aqueous extract of fungus mycelium was prepared and some active compounds were detected, including phenols, flavonoids, tannins, alkaloids, glycosides and resins, all of which gave a positive detection. The effective compounds were diagnosed by GC-Mass Chromatography technique, was revealed that there are a number of compounds that can produce mycelium with ability to inhibit microorganisms, inhibit free radicals and prevent oxidation, as well as their effect in reducing cancerous tumors. As for the effective groups, they were diagnosed by FTIR technique.

Key words: GC-Mass Chromatography, phenols, flavonoids, tannins, FTIR.

الكشف عن المركبات الفعالة في المستخلص المائي للعزلة المحلية *Ganoderma applanatum*

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المستخلص

هدفت الدراسة الى التعرف على المركبات الفعالة في المستخلص المائي للغزل الفطري *Ganoderma applanatum*. تم تحضير المستخلص المائي للغزل الفطري وشخصت فيه بعض المركبات الفعالة والتي شملت، الفينولات، الفلافونويدات، التانينات، القلويدات، الكلايكوسيدات والراتنجات، والتي اعطت جميعها كشفاً موجباً. حددت المركبات الفعالة بتقنية GC–Mass. لوحظ وجود عدد من المركبات التي تمنح الغزل الفطري القدرة على تثبيط الأحياء المجهرية، وتثبيط الجذور الحرة وتمنع الأكسدة، فضلاً عن تأثيرها في اختزال الأورام السرطانية. اما المجاميع الفعالة فقد تم تشخيصها بتقنية FTIR.

الكلمات المفتاحية: GC–Mass Chromatography، الفينولات، الفلافونويدات، التانينات،FTIR .

Received:16/8/2021, Accepted:12/1/2022

INTRODUCTION

Ganoderma fungus was found in many countries of the world, as it is considered one of the great economic resources. In Mekong Province, which has an area of 2.5 million square kilometers and part of Yunnan Province of China, there is a spread of at least 650 types of fungi, and the large proportion of these fungi is Ganoderma in addition to other fungi could be cause diseases to plants (6). In some Iraqi governorates, the fungi appear in grasses, fields and forests form a large presence that is worth searching for. Fruiting bodies belonging to the genus Ganoderma have been discovered. *G. lucidum* species in Tikrit governorate during the inspection tours by other researchers (2,3) and the discovery of other types of Ganoderma in Nineveh governorate that were identified by molecular diagnosis.The Ganoderma mushroom belongs to the Ganoderma family, which is characterized by the most prominent decomposers of herbs as well as being non-toxic to humans. Basidium fungi are a wide source of biologically active elements that are therapeutically useful. There are more than 700 types of Basidium fungi that have been discovered to have a great and effective therapeutic effect. It has been proven by studies at the present time that many types of Ganoderma are used in treatment and that they have great efficacy and medicinal properties, as well as protect kidney and liver and act against cancer, hyperlipidemia, and hypertensive disorders (4,7,13, 15,16). Ganoderma is a wide source of natural products with biomedical applications and high pharmacological efficacy, and one of these products that have been available in the markets in several countries of the world is Ganodermic acid, and one of its advantages is that it can fight aging and provide vitality and glasses for the skin. Also, 119 organic compounds were isolated from the bodies of mushrooms. Mycelium is a basic acid, including lucidenic acid, in addition to proteins that contain amino acids, which are mainly rich in lysin and leucine (1,9). Since its emergence, mass spectrometry (MS) is increasingly perceived as an essential tool in nearly all phases of drug discovery and development (8).

MATERIALS AND METHODS

The local isolate *Ganoderma applanatum* from Mosul University was cultured in natural medium of wheat bran and the mycelium was collected as published in previous research by authors.

Extraction of the active compounds from the fungus mycelium: The method of Yao, et al.(19) was followed in preparing the aqueous extracts by mixing 20 g of the local fungus *Ganoderma applanatum* mycelium with 400 ml of distilled water in a 1000-liter beaker, then leaving the mixture in a vibrating water bath at a temperature of 40°C for 24 hours.The mixture was filtered by using Whattman No.1filter paper and keep the filtrate in a refrigerator at 4°C.

Qualitative detection of the active compounds in the aqueous extract of mycelium: Detection of alkaloids: one ml of the filtrate was placed in a test tube with reagents:

A. Dargandrof reagent: An orange precipitate appeared indicating the presence of alkaloids.

B. Wachner reagent: the appearance of a yellow precipitate indicates the presence of alkaloids.

C. Mayer's reagent: the appearance of a white precipitate indicates the presence of alkaloids.

Tannins: the solution was boiled, filtered and left to cool. Then the filtrate was divided into two parts:The first part: A 1% ferric chloride solution was added to it, where the appearance of a bluish-green color indicates the presence of tannins. The second part: A 1% lead acetate solution was added to it, which indicates the appearance of a white, jelly-like precipitate on the presence of citrates (17).

Detection of glycosides: the solution was filtered and Fehling reagent was added to it. The appearance of the dark red color is evidence of the presence of glycosides.

Detection of resins: the solution was boiled at 100°C for one minute, then the solution was filtered and 100 ml of hydrochloric acid at a concentration of 4% was added. The appearance of turbidity is evidence of the presence of Resin materials.

Detection of flavonoids: The method of Sofowara (14) was followed in the detection of

flavonoids, the appearance of yellow color indicates the presence of flavones in the test sample.

Diagnostics of active compounds using gas-liquid chromatography (GC-Mass spectrometer): The active compounds diagnostic device GC-Mass was used to estimate and detect the active compounds in the fungus mycelium under study by using the instrument Gas chromatograph: Agelint (7820A) VSA G Mass spectrometer

Diagnosing the active groups of mycelium using the FTIR device: The active groups of the local fungus *G. appplantnim* were diagnosed by using a spectrometer FTIR. The examination model represented by dried and ground mycelium was prepared for the purpose of diagnosing these groups. The device depends in the measurement on the carbon atoms absorption of radiation, which is represented by giving results recorded in the form of peaks within specific frequencies, which indicate the type of bonds and effective groups present in the mycelium.

RESULTS AND DISCUSSION

Preparation of the aqueous extract of the local mushroom mycelium *G. applanatum*: The aqueous extract was obtained from the mycelium of the fungus *G. applanatum* prepared according to the method mentioned above, and to identify its components, subsequent tests were conducted.

Qualitative detection of some active compounds: The laboratory results showed that the local fungus *G. applanatum* contained a number of active compounds, using some chemical reagents. Table (1) shows a group of active compounds and methods for their detection, noting the presence of compounds such as alkaloids, glycosides, resins and tannins. These compounds make these fungi have acquired the importance of nature and nutrition in some of their species and thus considered them to be biologically active organisms and used them in more than life uses at the health level and other fields (5). The researcher (11) found that the aqueous extract of the fungus *G. applanatum* contained Saponins, Tannins, Phenols and Terpenoids and was free of alkaloids and steroids. The difference may be due to the environment from which the fungus was isolated, as well as the fungal strain.

Table1. Qualitative detection of some active compounds in aqueous extract of *G. applanatum* mycelium

Effective group	Detection method	detection guide	Detection result
Alkaloids	Wachner detector	Yellow precipitate	+
Alkaloids	Dargendorf detector	Orange precipitate	+
Alkaloids	Meyer's detector	White residuum	+
Glycosides	Fehling detector	dark red color	+
resins	hydrochloric acid 4%	Turbidity	+
tannins	ferric chloride	bluish green color	+

Identification of active compounds by GC-Mass technology for the local mycelium of the fungus *G. applanatum*:

An analysis of the aqueous extract of the local *G. applanatum* mycelium was carried out with a GC-Mass device to identify the active compounds. These compounds were differ in the amount of their presence in the molecule, as in Table (2) and Figure (1), which represents the results of spectral analysis from the peak number, retention time in minutes and area %, with the diagnosis of the compound according to the device reading. It is noted that the compounds likely to be present in the peak 11 are (ethanone, 2-imino-1,2-diphenyl, semicarbazone), which constitute the highest percentage in the mycelium of the local fungus *G. applanatum*, followed by the peak 13 containing (11-Octadecenoic acid methyl ester, cis-vaccenic acid).The 12th peak was characterized by the presence of Oleic acid and Ascorbic acid, and the fourth peak was characterized by the presence of Carvone, one of the terpenoid compounds, and all these compounds can give mycelium the ability to inhibit microorganisms, inhibit free radicals and prevent oxidation, as well as their effect in reducing cancerous tumors (20). Some

researchers conducted an analysis of *G. lacidum* mycelium using GC-Mass and showed the presence of alcohols, aldehydes, acids, phenols, amino acids and amino compounds (18). As (12) explained, when he estimated the compounds present in the mycelium of *G. lacidum* using GC-Mass, it contained saturated and unsaturated fatty acids in addition to alkanes. The local mushroom *G. applanatum*'s content of these compounds is of great importance in the possibility of exploiting it because it contains effective compounds that are biologically important and the possibility of exploiting them in the preservation and safety of some food products as well as pharmaceutical uses because it contains high levels of phenols, flavonoids, tannins, fatty acids, amino acids and other important compounds.

Table 2. the results of the analysis of the mycelium of the fungus *G. applanatum* with the GC-Mass device

PK	RT	Area	Library/ID	Ref
1	3.884	1.61	Acetamide, 2-(2-hydroxyethoxy)- Adenosine, 4'-de(hydroxyethoxyl)-4'-(N-ethy1aminoformyl)- Silver acetate	9056 225242 35237
2	4.759	0.78	sec-Butyl nitrite 1,4-Butanedimine Carbamic acid, acetyl-, ethyl ester	4544 1969 13842
3	5.430	0.73	Butanedioic acid, 2,3-bis (acety1oxy)-, (R-(R*,R*)- Malic Acid Propionic acid, 2-chloro-, isopropyl ester	89393 14971 24140
4	10.562	6.36	(-)-Carvone D- Carvone D- Carvone	23676 23643 23675
5	15.880	2.10	Acetamide, N- (3-oxo-4-isoxazolidinyl)-, R- 2-Propenoic acid , 2- (acetylamino)- B, 9, 9, 10, 10, 11-Hexafluoro-4, 4-dimethy1-3, 5-dioxatetracyclo [5.4.1.0(2,6) .0(B,11)] dodecane	20526 12825 145819
6	16.840	1.12	Acetamide, N- (3-oxo-4-isoxazolidiny1)-, (R)- 3-Piperidino1, 1,4-dimethyl-, cis- Carbamic acid, (3,4,4-trimethyl-1,2-dioxetan-3-y1) methy1 ester	20526 12983 42049
7	18.632	1.83	Glycy1-d1-alanine Ethanol, 2-bromo- 3-Piperidinol, 1,4-dimethyl-, cis-	21745 10243 12983
8	19.210	1.68	2-Amino-8-[3-d-ribofuranosyl|imidazo[1,2-a]-s-triazin-4-one Glycyl-d1-alanine z-1,9-Hexadecadiene	129649 21745 79559
9	20.136	0.88	4-Fluoro-1methy1-5carboxylic acid, ethy1 (ester) 2-Trifluoroacetoxytridecane Oleic Acid	40101 140818 129337
10	20.985	0.75	3-Trimethylsiloxypalitic acid, trimethylsily1 ester Nonadecanoic acid, trimethylsilyl ester Octadecanoic acid, 12-oxo, trimethylsilyl ester	217846 197044 196782
11	22.472	36.06	Ethanone, 2-imino-1,2-diphenyl-, semicarbazone Diphenylacetylene 2-Cyclopropen-1-one, 2,3-dipheny1-	115444 44148 66195
12	23.373	9.16	Oleic Acid 1-(+) –Ascorbic acid 2,6- dihexadecanoate cis-Vaccenic acid	129337 242221 129347
13	23.933	19.92	11-Octadecenoic acid methyl ester 9- Octadecenoic acid, methyl ester, (E)- cis-Vaccenic acid	141291 141310 129339
14	24.757	17.01	cis-Vaccenic acid cis-11-Eicosenoic acid cis-10-Nonadecenoic acid	129339 153110 141266

Identification of active groups in *G. applanatum* mycelium using FTIR technique: The diagnosis of active compounds in the fungus mycelium under

study by FTIR technique revealed the effective groups responsible for the vitality of the compound, as Table (3) and Figure (2) show clear absorption bands at a frequency of 3390.56 (cm) for the mycelium, which is due to the presence of hydroxyl alcohol (OH) groups, which It refers to phenols in a high concentration, followed by the CH packs. The model gave another frequency, which is 1635.64, which belongs to the C=O group, as well as the convergent frequencies 1575.84 to 1523.78, as they belong to the C=C groups in addition to the Amine group. It was shown by (10) when performing a diagnosis of the active groups in the aqueous extract of *G. applanatum*, the absorption bands ranged between 619.9 belonging to C=O groups up to 3156.78, which belong to the hydroxyl groups.

Table 3. Absorption Packs of Dried Mycelium *G. applanatum* with FTIR Technology

Absorption frequency of the active group	the active group	Group name
3390.86	OH	Phenols
2926.01	CH	Aromatic
1635.64	C=O	Amide
1575.84	C=C	Amine
1541.12	C=C	Amine
1523.76	C=C	Amine
1411.89	C-O/C-H	Amine
1377.17	C-O	Amine
1249.87	C-O-C	Alkyl Ketone
1074.35	C-O	Alkyl Amine
1041.56	C-O	Alkyl Amine
773.46	C=O	Alkyl halide
609.51	C=O	Alkyl halide
524.64	C=O	Alkyl halide
435.91	C=O	Alkyl halide

CONCLUSION

In conclusion, the detection of active components were ethanone, 2-imino-1,2-diphenyl, semicarbazone, which constitute the highest percentage in the mycelium of the local fungus *G. applanatum*, followed by octadecenoic acid methyl ester, cis-vaccenic acid and oleic acid and Ascorbic acid, these compounds give mycelium the ability to inhibit microorganisms, prevent oxidation, as well as their effect in reducing cancerous tumors

ACKNOWLEDGMENTS

All the experiments of this study were done in the laboratories of Jabir Ibn Hayyan Medical University/Iraq. ORCID ID:https//orcid.org/0000-0001-5883-0494
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