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Chemical Composition ethanol and methanol extract of *Lycoperdon Pyriforme*

Rabab Majead Abed
Department of Biology, College of Education for Pure Sciences, University of Diyala, Iraq
Email: Mohammed.dawod.salman@uodiyala

Mohammed Dawod Salman
Department of Biology, College of Education for Pure Sciences, University of Diyala, Iraq
Email: rabab.majead81@gmail.com

***Abstract*---**For detect active compounds in gastrocap of *Lycoperedon periform* fungus that collected from some orchards of Diyala Governorate - Khalis district, which was characterized by the fact that the fruiting body was of the type Gastrocap almost spherical in shape, with a height of 6-8 cm, a width of about 10-13 cm, and a stem length of approximately 3 cm with a strong Woody, and the fruiting body of the fungus at the beginning of its growth is cylindrical in shape, then becomes pear-shaped, and at maturity it becomes spherical with a pointed base. The fruiting body is completely devoid of gills and in the form of a mass of Basidiospores, which is somewhat smooth and contains an oily drop of greenish-yellow color, which when placed in water gives a yellow color. The active compounds were detected in two types of alcoholic extracts of *Lycoperedon periform*, which were ethanolic extract and methanolic extract. Gas chromatography-mass spectrometry (GC-MS) was used for this purpose. The results showed that the total number of active compounds that were Its determination in both the ethanolic and methanolic extract was close, as the number of active compounds in the ethanolic extract was 41 compared to the number of active compounds in the methanolic extract, which was 43 active compounds. The results also showed that the concentration of the active compounds changed their concentration by changing the solvent used. The results showed that the compounds that were identified in both the methanol and ethanolic extract their concentration differed according to the solvent, as there were 13 active compounds in the methanolic extract that had higher concentrations compared to the ethanolic extract, and there are 8 active compounds in the ethanolic extract whose concentration is higher than the methanol and through the concentrations of the compounds It was

found that the methanolic extract was highly efficient in extracting the active compounds.

***Keywords*---*Lycoperedon periform*,** ethanolic extract, gastrocap, active compounds.

Introduction

The fungus *Lycoperdon pyriforme*, whose species is known as the puffball, is characterized by the fruiting body of the fungus being pear-shaped, and it is one of the fungi found in many parts of the world. The fungus is most common in the fall, and it is common under coniferous trees and wood (Vizzini et al., 2017). The fruiting body is 1.5 to 4.5 cm high and 2 to 4.5 cm in diameter. Often pear-shaped but may also be nearly spherical When the fruiting body is in its early development, the fruiting body is covered with small white spines that usually fall off before maturity Small developing pores may appear on top, while the base of the fruiting body is small and appears to be pinched Color ranges from White to yellowish brown with dark shades appearing with age, although the base of the fruiting body remains white (Steve and Trudell, 2009), the central pores rupture at late maturity to allow wind and rain to spread basidiospores. Thick, rope-like fungi (Kuo, 2004; Lincoff, 1981), the mass of basidiospores inside the fruiting body is white when it is young, but it becomes greenish-yellow color later tends to dark greenish-brown with age The spores measure from 3 to 4.5 µm and is round and smooth (Davis et al., 2012). The fruiting body of *L. pyriforme* is a good source of protein, carbohydrates and fats (Colak et al., 2009), and fatty acids such as linoleic acid, which constitutes 37% of the total fatty acids, oleic acid 24% and palmitic acid (14.5%) (Szummy et al., 2010) The surfaces of basidiospores of *L. pyriforme* contain many microscopic spines and can cause severe lung irritation when inhaled, which is known as lycoperidone disease (Dulger, 2005). It has been reported that this condition affects dogs that play or run where the bodies of the fungus are located Barros et al. 2009) Similar species *Lycoperdon excipuliforme* and *L. marginatum* are two of several puffball-like species. Several other species of jelly balls can be confused with *L. perlatum* found in the Pacific Northwest region of the United States, covered with granular spots, but these granules adhere to the surface more strongly than those of *L. perlatum* (Lamotte et al., 1978) The body lacks The fruiting fungus in *L. pyriforme* has prominent spines on the surface and the fungus grows on rotting wood - although it does grow on buried wood.

Due to the lack of studies on the fungus *Lycoperdon periform* and its therapeutic uses, the current study aimed to:

- Collect the fungus *Lycoperdon periform* from its natural place
- Detection of the active compounds of the fungus *Lycoperdon periform*

Materials and Methods

Lycoperedon periform mushroom collection

The mushrooms used in the experiment under study were collected from the orchards of Diyala Governorate - Khalis District - Mansourieh District - Sherwin village during November of the year 2021. varicella and basidiospores (Pacioni,

1981 Bates; et al., 2009). The samples were washed with distilled water to remove suspended dust, then dried, and after drying, they were ground by an electric mill and kept after turning into powder in glass bottles away from light until use.

Figure (1) *Lycoperedon periform* mushroom in natural habitat

Preparation of the ethanolic extract of the fungus *Lycoperedon periform*

The method used was followed by (Hu et al., 2009), where mushroom powder was extracted at a rate of 25 g of powder per 100 ml of ethyl alcohol at a concentration of 95% in a 1 liter glass beaker and its nozzle was closed with a rubber stopper and covered with aluminum foil to prevent evaporation. Then the beaker was placed on the magnetic stirrer -hot plat for 24 hours for the purpose of stirring the mixture and ensuring proper extraction. The mixture was filtered using medical gauze and then by Whatman 1 filters paper. After that, the alcohol was disposed of using a rotary evaporator for disposal, then the filtrate was placed in glass Petri dishes, then the dishes were placed in the incubator at 35 °C. For 24 hours for the purpose of getting rid of the alcohol, the process was repeated several times for the purpose of obtaining a sufficient amount of the extract and it was kept in the freezer until it was used.

Preparation of the methanolic extract of *Lycoperedon periform*

The method used was followed by (Hu et al., 2009), where mushroom powder was extracted at a rate of 25 g of powder per 100 ml of methanol at a concentration of 95% in a 1 liter glass beaker and its nozzle was closed with a rubber stopper and covered with aluminum foil to prevent evaporation, then the beaker was placed on the magnetic stirrer stirrer-hot plat for 24 hours in order to stir the mixture and ensure proper extraction. The mixture was filtered using medical gauze, then by Whatman1 filter paper. Then the alcohol containing the extract was dried using a rotary evaporator for the purpose of getting rid of the alcohol. Then the filtrate was placed in glass Petri dishes, then the dishes were placed In the incubator at

35 °C for 24 hours for the purpose of getting rid of the alcohol, the process was repeated several times for the purpose of obtaining a sufficient amount of the extract and it was preserved and kept in the freezer until it was used.

Detection of active compounds in the crude extract of *Lycoperedon periform*

The active compounds of *Lycoperedon periform* were detected using a gas chromatography-mass spectrometry (GC-MS) device in Basra Governorate - South Oil Company, which included extracting compounds where 1 ml of alcoholic fungal extract was taken and injected into a glass tube. The capacity is 1 ml and then 1 micron of the alcoholic extract was taken and inserted into the device, where the temperatures are then raised incrementally, starting from 40 degrees Celsius until it reaches 360 degrees Celsius, and then the device begins to separate the active compounds according to the boiling point, molecular weight and area. The effective compounds by comparing them with the library in the device. Table (1) shows the working conditions of the device.

Table (1) Separation conditions for GC-MS combined gas chromatography-mass spectrometer for ethanolic and methanol alcoholic extracts of *Lycoperedon periform*

100m	Primary column temperature
280m	Final column temperature
10m/min	temperature rise rate
200m	Ionization detector temperature
10cm/min	Carrier helium gas flow rate
25mm x 0.20 mm	Shaft dimensions: length x inner diameter
%25 phenyl and 5% dimethyl polysiloxane	Column components
1 ml	injection volume
Ion source EL	Detector type

Results

***Lycoperdon periform* fungus**

The fruiting body of the Gastrocap of the fungus *Lycoperdon periform* is almost spherical in shape, with a height of 6-8 cm and a width of about 10-13 cm. The length of the stem is approximately 3 cm, which is woody in texture. Tapered base the fruiting body is completely devoid of gills and in the form of a mass of Basidiospores, which is somewhat smooth and contains inside it an oily drop of greenish-yellow color, which, when placed in water, gives a yellow color, as shown in Figure (2), which shows the mature fruiting body of the fungus, as well as Basidioid spores under the microscope.

Figure (2) the picture on (A) shows the mature fruiting body of *Lycoperdon periform*, Picture (B) shows the smooth basidoid spores of *Lycoperdon periform* under the light microscope at magnification x 40.

Active compounds of the ethanolic and methanolic extract of *Lycoperdon periform*

The results of the detection of the active compounds of the methanol and ethanolic extract of the fungus *Lycoperdon periform* by Gas Chromatography-mass spectrometry (GC-MS) device show the identification of a number of active compounds, as the results shown in Table (2) and Figure (3) show that 41 active compounds have been identified In the ethanolic extract of the fungus *L. periform*, while Table (3) and Figure (4) show that the methanolic extract had the highest number of active compounds recorded in it and their number was 43 active compounds. Table (4) also shows the compounds that were identified in each of the methanol and ethanolic extracts and their concentration, as it shows that there are 13 active compounds in the methanolic extract that had higher

concentrations compared to the ethanolic extract, and there are 8 active compounds in the ethanolic extract whose concentration is higher than the methanol and through The concentrations of the compounds showed that the methanolic extract was highly efficient in extracting the active compounds.

Table (2) Determination of chemical compounds in the ethanolic extract of *Lycoperdon periform* by GC-MS technique

%	RT	Molecular formula	compound	No
0.427938	7.501	C9H12	Tricyclo[3.2.1.0(2,4)]octane, 8-methylene-	1
0.743748	10,522	C10H22	Octane, 3,5-dimethyl-	2
0.227473	13.437	C6H14Si	Silane, ethenylethyldimethyl-	3
0.661476	14,195	C7H14O	1-Methyl-1-ethoxycyclobutane	4
0.273443	14,415	C6H6O3	5-Hydroxymethylfurfural	5
0.509753	14,687	C5H10O3	1,2,3-Cyclopentanetriol	6
0.295004	15,481	C22H43NO4	l-Isoleucine, N-methoxycarbonyl-, tetradecyl ester	7
0.245943	15,729	C8H13NO3	N-Pentyloxazolidin-2,4-dione	8
0.801834	16,275	C10H9ClO3	Propanoic acid, 3-chloro-, 4-formylphenyl ester	9
0.245749	17.176	C7H16OSi	Cyclobutanol, TMS derivative	10
0.313954	17.338	C6H10O5	.beta.-D-Glucopyranose, 1,6-anhydro-	11
0.495218	17.932	C9H10O3	Benzeneacetic acid, 4-hydroxy-, methyl ester	12
5.999,629	17.971	C6H10O5	.beta.-D-Glucopyranose, 1,6-anhydro-	13
0.938827	18.132	C11H14O3	Isobutyl 4-hydroxybenzoate	14
3.931296	18,508	C6H12O5	.beta.-l-Arabinopyranoside, methyl	15
0.809255	18,664	C8H8O3	Benzeneacetic acid, 4-hydroxy-	16
0.590732	19.31	C7H10Cl2O3	Dichloroacetic acid, 2-tetrahydrofurylmethyl ester	17
11.93208	19.402	C5H8O3	Butanoic acid, 2-oxo-, methyl ester	18
11.40057	19.462	C6H2	Hexa-1,3,5-triyne	19
13.89103	19.643	C6H12O5	.beta.-l-Arabinopyranoside, methyl	20
1.885723	19.692	C9H22O2Si	1-Pyrrol[tert-butyl(dimethyl)silyl]oxymorphopropan-2-ol	21
0.255815	20.414	C14H12O2	Ethanone, 1-(4-hydroxyphenyl)-2-phenyl-	22
1.718331	22,262	C16H22O4	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	23
0.377686	.22,672	C15H22N2O3	Tolycaine	24
0.293727	22,772	C17H34O2	Hexadecanoic acid, methyl ester	25
7.536375	23.104	C16H32O2	n-Hexadecanoic acid	26
0.404702	23.173	C12H17NO	Diethyltoluamide	27
0.506494	23.2	C16H22O4	Dibutyl phthalate	28
21.04232	23,765	C6H14O6	D-Mannitol	29
0.310272	24.41	C19H34O2	9,12-Octadecadienoic acid, methyl ester	30
0.497827	24,458	C19H36O2	13-Octadecenoic acid, methyl ester	31
1.937922	24.746	C18H32O2	9,12-Octadecadienoic acid (Z,Z)-	32
3.152253	24,789	C18H34O2	Oleic Acid	33
0.266375	24.974	C18H36O2	Octadecanoic acid	34
0.914075	28.25	C24H38O4	Phthalic acid, di(2-propylpentyl) ester	35
0.265378	29.3	C20H37ClO2	2-Chloroethyl oleate	36

1.715114	31.42	C35H46O2	Anthraergostatetraenol benzoate	37
0.487033	31.634	C28H40	Anthraergostapentene	38
0.29951	31.982	C24H36O2Si2	4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative	39
0.99059	33.578	C28H46O	Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)-	40
0.40752	38.376	C29H57NO4	L-Leucine, N-methyl-N-(2-ethylhexyloxycarbonyl)-, tridecyl ester	41

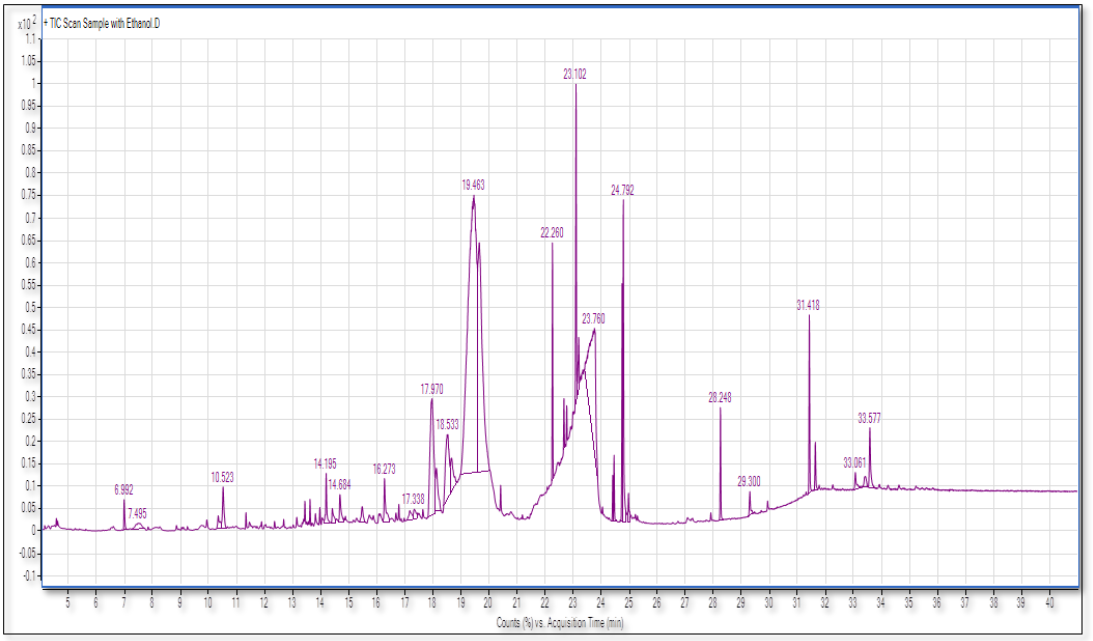

Figure (3) Curves of chemical compounds in the ethanolic extract of *Lycoperdon periform*

Table (3) Determination of chemical compounds in the methanolic extract of *Lycoperdon periform* by GC-MS technique

%	RT	Molecular formula	compound	No
1.688031	6.897	C5H4O2	3- Furaldehyde	1
0.24894	7.485	C5H7LiO2	2,4-Pentanedione, ion(1-), lithium	2
0.287016	9.911	C6H6O2	2-Furancarboxaldehyde, 5-methyl-	3
2.621938	10.532	C12H25F	Dodecane, 1-fluoro-	4
0.686588	12,641	C6H6O3	Levoglucosenone	5
0.453404	13.417	C6H14Si	Silane, ethenylethyldimethyl-	6
0.93079	14.144	C6H8O4	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	7
1.369836	14,181	C7H14O	1-Methyl-1-ethoxycyclobutane	8
0.644813	14,408	C6H6O3	5-Hydroxymethylfurfural	9

0.666368	14,684	C7H14O5	.alpha.-L-Galactopyranoside, methyl 6-deoxy-	10
0.255901	15.265	C6H10O4	Dianhydromannitol	11
0.874262	15.479	C7H12OS	3(2H)-Thiophenone, dihydro-5-(1-methylethyl)-	12
0.750737	15.721	C7H14O2	Butanoic acid, 2-methyl-, ethyl ester	13
0.528559	16.278	C10H9ClO3	Propanoic acid, 3-chloro-, 4-formylphenyl ester	14
0.885435	17.332	C6H10O5	.beta.-D-Glucopyranose, 1,6-anhydro-	15
0.24427	17.935	C9H10O3	Benzeneacetic acid, 4-hydroxy-, methyl ester	16
0.849532	18,019	C13H28OSi	3-Dimethyl(octyl)silyloxy-1-propene	17
21.84303	18,063	C6H10O5	.beta.-D-Glucopyranose, 1,6-anhydro-	18
0.259062	18.126	C11H14O3	Isobutyl 4-hydroxybenzoate	19
1.110749	18,481	C6H12O5	1,4-Anhydro-d-galactitol	20
2.297106	18,684	C5H12O5	Ribitol	21
4.034517	19.23	C7H18Si	Silane, butyltrimethyl-	22
3.77914	19,257	C12H25F	Dodecane, 1-fluoro-	23
0.304704	21.526	C14H30O	Heptane, 1,1'-oxybis-	24
4.532082	22,263	C16H22O4	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	25
0.710848	22,772	C17H34O2	Hexadecanoic acid, methyl ester	26
2.099364	23.101	C16H32O2	n-Hexadecanoic acid	27
0.646232	23.2	C16H22O4	Dibutyl phthalate	28
1.449527	24.411	C19H34O2	9,12-Octadecadienoic acid, methyl ester	29
1.038123	24,459	C19H36O2	13-Octadecenoic acid, methyl ester	30
1.220074	24,743	C17H30	1,8,11-Heptadecatriene, (Z,Z)-	31
2.656155	24.786	C18H34O2	Oleic Acid	32
0.471818	26,748	C18H35NO	9-Octadecenamide, (Z)-	33
13.21087	28.253	C24H38O4	Phthalic acid, di(2-propylpentyl) ester	34
13.09501	31.424	C35H46O2	Anthraergostatetraenol benzoate	35
2.311223	31.636	C28H40	Anthraergostapentene	36
0.300705	32.084	C24H36O2Si2	4-Methyl-2,4-bis(p-hydroxyphenyl)pent-1-ene, 2TMS derivative	37
0.33045	33.433	C28H44O	Ergosta-5,8,22-trien-3-ol, (3.beta.,22E)-	38
6.834657	33.579	C28H46O	7,22-Ergostadenol	39
0.219686	33.916	C22H13F10N	Benzeneethanamine, N,N-bis[(pentafluorophenyl)methyl]-	40
0.308456	34.616	C29H50O	.gamma.-Sitosterol	41
0.491503	35.222	C28H42O	Anthraergostatetraenol	42
0.485902	35.822	C21H32F7NO3	l-Proline, n-heptafluorobutyryl-, dodecyl ester	43

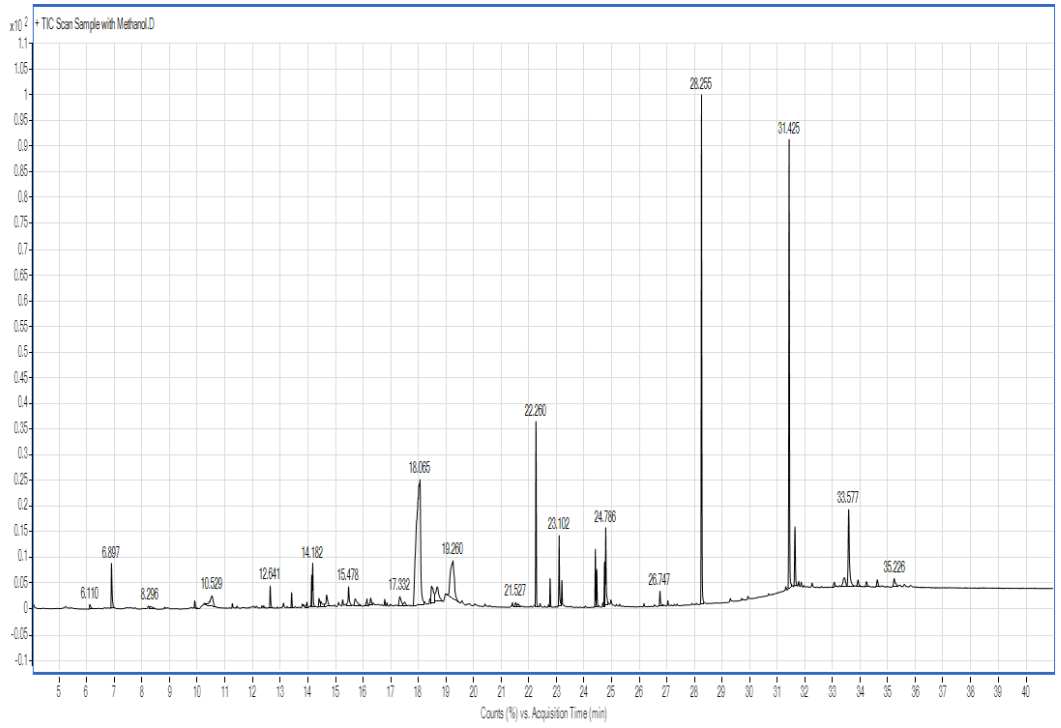

Figure (4) Curves of chemical compounds in the methanolic extract of *Lycoperdon periform*

Table (4) The co-active compounds identified in the ethanolic and methanolic extract of *Lycoperdon periform* by GC-MS technique

methanol %	ethanol %	compound	No
0.453404	0.227473	Silane, ethenylethyldimethyl-	1
1.369836	0.661476	1-Methyl-1-ethoxycyclobutane	2
0.644813	0.273443	5-Hydroxymethylfurfural	3
0.528559	0.801834	Propanoic acid, 3-chloro-, 4-formylphenyl ester	4
0.885435	0.313954	.beta.-D-Glucopyranose, 1,6-anhydro-	5
0.24427	0.495218	Benzeneacetic acid, 4-hydroxy-, methyl ester	6
21.84303	5.999,629	.beta.-D-Glucopyranose, 1,6-anhydro-	7
0.259062	0.938827	Isobutyl 4-hydroxybenzoate	8
0.24427	0.809255	Benzeneacetic acid, 4-hydroxy-	9
0.750737	11.93208	Butanoic acid, 2-oxo-, methyl ester	10
4.532082	1.718331	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	11
0.710848	0.293727	Hexadecanoic acid, methyl ester	12
2.099364	7.536375	n-Hexadecanoic acid	13
0.646232	0.506494	Dibutyl phthalate	14

1.449527	0.310272	9,12-Octadecadienoic acid, methyl ester	15
1.038123	0.497827	13-Octadecenoic acid, methyl ester	16
2.656155	3.152253	Oleic Acid	17
13.21087	0.914075	Phthalic acid, di(2-propylpentyl) ester	18
13.09501	1.715114	Anthraergostatetraenol benzoate	19
2.311223	0.487033	Anthraergostapentene	20
0.33045	0.99059	Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)-	21
1.688031	----	-3Furaldehyde	22
0.24894	----	-2,4Pentanedione, ion(1-), lithium	23
0.287016	----	2-Furancarboxaldehyde, 5-methyl-	24
2.621938	----	Dodecane, 1-fluoro-	25
0.686588	----	Levoglucosenone	26
0.93079	----	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	27
0.666368	----	.alpha.-L-Galactopyranoside, methyl 6-deoxy-	28
0.255901	----	Dianhydromannitol	29
0.874262	----	3(2H)-Thiophenone, dihydro-5-(1-methylethyl)-	30
0.849532	----	3-Dimethyl(octyl)silyloxy-1-propene	31
1.110749	----	1,4-Anhydro-d-galactitol	32
2.297106	----	Ribitol	33
3.77914	----	Dodecane, 1-fluoro-	34
0.304704	----	Heptane, 1,1'-oxybis-	35
1.220074	----	1,8,11-Heptadecatriene, (Z,Z)-	36
6.834657	----	7,22-Ergostadenol	37
0.219686	----	Benzeneethanamine, N,N-bis[(pentafluorophenyl)methyl]-	38
0.308456	----	.gamma.-Sitosterol	39
0.491503	----	Anthraergostatetraenol	40
0.485902	----	l-Proline, n-heptafluorobutyryl-, dodecyl ester	41
----	0.427938	Tricyclo[3.2.1.0(2,4)]octane, 8-methylene-,	42
----	0.743748	Octane, 3,5-dimethyl-	43
----	0.509753	1,2,3-Cyclopentanetriol	44
----	0.295004	l-Isoleucine, N-methoxycarbonyl-, tetradecyl ester	45
----	0.245943	N-Pentyloxazolidin-2,4-dione	46
----	11.40057	Hexa-1,3,5-triyne	47
----	13.89103	.beta.-l-Arabinopyranoside, methyl	48
----	1.885723	1-Pyrrol[tert-butyl(dimethyl)silyl]oxymorphopropan-2-ol	49
----	0.255815	Ethanone, 1-(4-hydroxyphenyl)-2-phenyl-	50
----	0.377686	Tolycaine	51
----	21.04232	D-Mannitol	52

----	0.266375	Octadecanoic acid	53
----	0.265378	2-Chloroethyl oleate	54
----	0.40752	L-Leucine, N-methyl-N-(2-ethylhexyloxycarbonyl)-, tridecyl ester	55

The above results confirm that the type of solvent used has a role in the type, number and concentration of the chemical compounds extracted. Several studies have recommended the use of methanol as a solvent as the optimal solvent to obtain a high percentage of effective compounds, including the study of (Kim and Lee, 2017; Truong et al., 2019). The study (Borges et al., 2020) also indicated that the use of ethanol as a solvent outperformed acetone and water in extracting effective compounds and in the nature of the extracted materials, which were characterized by having different therapeutic and pharmacological properties. The study of Morales-Olán et al., 2020 also showed that many phenolic compounds can be extracted using ethanol or methanol as a solvent with different concentrations. The study (Bharathiraja and Chandran, 2020) also indicated that the methanol extract has the ability to produce the largest number of effective compounds from the mushroom extract, followed by the ethanolic extract. The current study agrees with the study (Ali Akbar et al., 2020) of hexane extract of the fungus *Lycoperdon pyriforme* in producing effective compounds because it contains 7.22 Ergostadenol and 5-Ergosterol.

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