Bioactive Compounds of Fresh and Dried *Pleurotus ostreatus* Mushroom

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Abstract: Chemical composition of the fresh and dried mushroom *Pleurotus ostreatus* and their bioactive secondary metabolic products were studied. The ethanolic extracts of the *P. ostreatus* cultivated on rice straw supplemented by wheat bran were studied by Gas Chromatography/Mass Spectrometry (GC/MS) analysis. A total of hundred and seven metabolites were detected in tested samples. These include 56 metabolites only detected in fresh sample, 37 metabolites only detected in dry sample, and 14 metabolites were detected in the both samples. The detected metabolites could be classified into nine chemical groups including 2 metabolites related to acids, 5 alcohols, 27 alkane, 3 amides, 27 esters, 8 fatty acids, 4 terpenoids, 29 heterocyclic and 2 phenols. The bioactivity of the metabolic products could be classified: anticholesterol, anticancer and essential fatty acids which support human health. On comparison between fresh and dried *P. ostreatus* samples, we found that the high number of metabolites was recorded in the fresh extract. Fifty five aroma compounds were recorded and including (27 esters, 9 ketones, 7 thiols, 5 alcholos, 4 terpenoids, 2 phenols and 1 aldehyde). The selenium content in *P. ostreatus* samples was measured by using ACAL –APR -51-00 test methods and showed that the fresh sample has 58.24 mg/kg but the dry sample has 100.31 mg/kg.

Keywords: Mushroom, *Pleurotus ostreatus*, Chemical profile, Bioactive metabolites.

1. INTRODUCTION

Pleurotus ostreatus (Jacq. ex Fr.) [1], Paul Stamets mycologist who uses the tree oyster mushroom name. *Pleurotus ostreatus* was first cultivated in Germany during the first World War and is now grown commercially around the world for food [2], by now it is widely used all over the world [3]. It is a widely edible wild and cultivated mushroom. It has anise odor due to the presence of benzaldehyde which smells more like almonds. Therefore, it used as a food with good flavor, and take as ingredients in many other food industries [4].

Pleurotus ostreatus have highest nutritional value depend up on the presence of high level of essential amino acids (arginine, alanine, glutamine, and glutamic acid), carbohydrates (no starch, but found the glucans, mannitol and trehalose), water content (from 80 to 90%), protein (40%), vitamin B,C,D,K, thiamine, riboflavin, folic acid and niacin [3,5], minerals (Ca, P, Fe, K, Mn, Cu, Zn, Mg and Se [6]. In addition, it include also essentials unsaturated fatty acids including oleic, linoleic, α-linolenic and palmitic acids [7,8].

In addition, *P. ostreatus* has low calories (each 100 g have 28 k/Cal) and sodium. Therefore, this mushroom has high medicinal value and used for many years in folk medicine. It has numerous bioactive metabolites used as the largest untapped sources of powerful and new pharmaceutical products. It has gastronomic, nutraceutical properties [9,10]. It is preventing many diseases such as anti- (hypertension, cholesterol, atherosclerosis, cancer, viral and thrombotic) and immunomodulating agents [11]. The presence of folic acid which is used in the treatment of anemia. Sodium to potassium ratio (Na:K), is suitable for people with hypertension, diabetes, and obesity. Anticholesterol and heart disease metabolites including essential fatty acids and statins [4]. Polysaccharides, terpenoids, fatty acids, amino acid, steroids, and phenols were act as anticancer agents [12,13]. Glycoprotein's or polysaccharide–peptides such as lectins act as anticancer and immune enhancer according to [13-16]. *Pleurotus ostreatus* has 8 to 10 % fiber and used for reducing the body weight and hypertensive. The high level of K induced lowering of elevated blood pressure and reduces the risk of stroke. About 20 to 40 % of the daily value of Cu that has cardio-protective properties. Enhancement of health and fitness, can be classified into the category of dietary supplements/ mushroom nutriceuticals [9]. Patel *et al.,* [17] reported that the *P. ostreatus* have numerous active metabolites which used in prevention and treatment numerous human diseases, these metabolites act as anti-(ageing, cholesterol, hyper-glycemic, hypotensive, inflammatory, immunodefici-ency, microbial, mutagenic, neoplastic, oxidant and tumor), it also act as hepatoprotective and immuno-modulatory agents. Antioxidant and antimicrobial activities found when the presence of AAPH (2,20-azobis-2-amidino-propane dihydrochloride), β-carotene and DPPH (1,1-diphenyl-2-picrylhidrazyl). Antioxidant

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metabolites includes phenolic compounds specially flavonoid and organic acids. Antimicrobial agents includes glycosphingolipid, sugar alcohols (mannitol), lipopolysaccharides, thioacetamide proteins specially hemolysin and ubiquitin. Phenols including "benzoic acid, *trans*-3,4-dihydro-3,4,8-trihydroxynapthalen-1(2*H*)-one, 4-hydroxybenzal dehyde, indolo-3-carboxylic acid, DHN-melanin, L-phenylalanine, *trans*-cinnamic acid, β-hydroxy-phenyl-propionic acid and flavonoids (catechin, quercetin, chrysin*)* found in the *P. ostreatus* [9,17]. Also Oyetayo and Ariyo [18] reported that the phenolic compounds in wild *P. ostreatus* has antioxidant and antimicrobial activity. Edible *P. ostreatus* have been part of human diet for centuries and known to be medically active in several therapies, such as antitumour, antibacterial, antiviral, haematological and immunomodulating treatments. The therapeutic effect had been linked to the presence of bioactive compounds in mushrooms. Some of these bioactives include glycolipids, aromatic phenols, fatty acid derivatives, polyacetylamine, polyketides (lovastatin), sesquiterpenoids, polysaccharides (glucan, pleuran and pyran) and many other substances [17, 18].

Pleurotus membranes and cell walls are riches by selenium [19], also incorporation into proteins reveals a great potential to improve the nutritional value of the *P. ostreatus* [20]. The recommended dose for an adult is 55 mg/day selenium which act as antioxidant, reduces the risk of prostate cancer and also act as immune enhancer [19-25].

This investigation was designed to study the chemical compositions of the *P. ostreatus* strain AUCU cultured on rice straw with wheat bran substrate by GC/MS analysis. Recorded their bioactive metabolites, show common and IUPAC names, their bioactivity and classified into different groups such as anticancer, anticholesterols, healthy essentials and flavoring agents.

2. MATERIALS AND METHODS

2.1. Collection of *P. ostreatus* Samples

Fresh and air dried (through one year at room temperature) samples of *P. ostreatus* strain cultivated on rice straw substrate supplemented by 2-5% from the each wheat bran and $CaCO_3$ were obtained from Assiut University Agricultural Mushroom Centre, Faculty of Agriculture, Assiut, Egypt (the original spores were obtained from the Egyptian National Center for Agricultural, Cairo, Egypt). Samples were processes within a maximum of 1-2 days after harvesting.

2.2. Extraction of the Samples

Fresh and air dried samples of the whole mushroom were divided into 100g for ethanolic extraction by homogenized for 10 min. in a high-speed blender at 16.000 rpm with 500 ml 95 % ethanol (by volume), 50 mL/500 ml flask. The mixture was filtered through Whatman filter paper no. 2 and dried by Na_2SO_4 anhydrous. The extraction procedure was repeated three times. The ethanolic extracts were combined, washed, filtered and concentrated to near dryness [16].

2.3. Detection of Chemical Compositions of *P. ostreatus* Ethanolic Extracts by (GC/MS)

The chemical compositions of the ethanolic extracts of two *P. ostreatus* (fresh and dry) samples were detected by GC/MS analysis [16,18,26-28].

2.4. Determination of the Selenium by Galvanometric Methods

Galvanometric method was used to determine the selenium in fresh and dried *P. ostreatus* samples [10,16,29].

Analysis of the ethanolic extract was performed using Agilent GC/MS, Model: 6890 N/5975B (Agilent Technologies, Palo Alto, CA, USA) and selenium determination determined at the analytical Chemistry Unit, ACAL, Chemistry Department, Faculty of Science, Assiut University, Assiut, Egypt.

3. RESULTS AND DISCUSSION

Results show that one hundred and seven metabolites were detected by GC/MS analysis in fresh and dry *P. ostreatus*. Fifty six metabolites were only detected in fresh sample. However 37 metabolites were detected in dry sample and 14 metabolites were found in both samples. The detected metabolites can be classified into nine chemical groups including 2 metabolites related to acids, 5 alcohols, 27 alkane, 3 amides, 27 esters, 8 fatty acids, 4 terpenoids, 29 heterocyclic and 2 phenols (Tables **1-3** and Figures **1-4**).

3.1. Acidic Metabolites

Two acidic metabolites including benzene,1-chloro-4-methoxy- (0.8 mass fractions) and benzene acetic acid (0.3 mass fractions). Benzene acetic acid act as active plant hormone, possessing a honey-like odor in low concentrations and used in some perfumes, also it used as precursor of penicillin G production. It is also employed to treat type II hyperammonemia to help

Table 1: Total Number, Mass Fractions of the Detected Metabolites (IUP Name and Different Chemical Groups) Determinates by GC/MS Analysis in Fresh and Dry *P. ostreatus*)

	Fresh	Dry	IUP name of the detected metabolites and their different chemical groups
Acids	0.08	-	benzene,1-chloro-4-methoxy-
	0.03	-	benzeneacetic acid
Alkanes	0.69	-	tetrapentacontane,1,54-dibromo-
	0.49	-	heptacosane
	0.37	0.01	hexadecane
	0.35	-	hexatriacontane
	0.25	0.02	dodecane
	0.25	-	docosane
	0.22	-	Pentadecane
	0.19	-	3-ethyltetracosane
	0.19	-	tridecane
	0.18	-	9-octylheptadecane
	0.14	-	triacontane
	0.13	-	tetratriacontane
	0.12	0.01	nonadecane
	0.10	0.12	7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione (alkenone)
	0.09	-	7-hexyl- docosane
	0.09	0.02	eicosane
	0.08	-	heptadecane
	0.08	0.08	tetracosane
	0.08	0.04	octadecane
	0.08	0.02	undecane
	0.07	-	octacosane
	0.24	-	2,2'-bi-1,3-dioxolane,4,4,4',4',5,5,5',5'-octamethyl-
	-	0.21	2,2-dideutero-heptadecanal
	0.14	-	ethane,1,1-dichloro-2,2-difluoro-;
	0.12	-	ethane,2,2-dichloro-1,1,1-trifluoro-;
	0.10	-	15thane, chlorotrifluoro-
	-	0.01	2-pentanone,5-(acetyloxy)-
Alcohols	0.16	-	9-phenanthrenol,4b,5,6,7,8,8a,9,10-octahydro-1,3,4-trimethoxy-4b,8,8-trimethyl-2-(1-methyl ethyl)
	0.13	-	(2*Z*,13*E*)-2,13-octadecadien-1-ol
	-	1.50	3,4,6,7-tetramethylidenebicyclo[3.2.1]octan-2-exo-ol
	0.01	-	cyclohexanol,1*R*-4-acetamido-2,3-*cis*-epoxy
	0.02	-	1,3-benzenediol,5-pentyl
Amides	-	1.21	*N*-1,*N*-1-dimethyl-*N*-2-*N*-pentylformamidine
	0.02	-	*N*-methylmaleamic acid
	0.32	-	1-naphthalenecarboxamide, *N*-(3-chlorophenyl)

(Table 1). Continued.

Fatty acids	-	29.19	9,12-octadecadienoic (*Z, Z*) [*Cis*-linoleic acid]
	-	0.05	9-octadecenoic (*Z*) [Oleic]
	-	6.21	9-octadecenamide, (*Z*) [*cis*-linoleic amide]
	-	6.98	hexadecanoic [palmitic acid]
	-	1.36	pentadecanoic [pentadecanoic acid]
	-	0.21	dodecanoic [lauric acid]
	-	0.32	tetradecanoic [myristic acid]
	-	0.04	pelargonic [nonanoic acid]
Esters	5.98	18.53	linoleic acid ethylester
	5.04	2.59	9,12-octadecadienoic acid (*Z,Z*)-,2-hydroxy-1-(hydroxymethyl) ethyl ester
	3.32	3.34	hexadecanoic acid, ethyl ester
	1.68	-	didecyl phthalate
	1.53	-	2-ethylhexyl methacrylate
	1.41	-	di-hexylphthalate
	0.99	-	ethyl oleate
	0.41	-	1,2-benzenedicarboxylic acid,butyl-8-methyl nonyl ester
	0.25	0.39	pentadecanoic acid,ethyl ester
	0.23	-	1,2-benzenedicarboxylic acid, ditridecyl ester
	0.18	-	1-heneicosyl formate
	0.10	-	l-proline,*N*-ethoxycarbonyl-,butyl ester
	0.10	-	sulfurous acid, hexyl octyl ester
	0.05	-	sulfurous acid, butyl dodecyl ester
	0.05	-	6-tetradecanesulfonic acid, butyl ester
	0.03	-	cyclohexanecarboxylic acid,2,2-dimethyl propyl ester
	-	1.16	proopyl-trans-4-[trans-4-(trans-4- propylcyclohexyl)cyclohexyl]cyclo hexane-carboxylate;
	-	0.53	butyl citrate
	-	0.39	hexadecanoic acid,2-hydroxy-1-(hydroxyl methyl) ethyl ester
	-	0.25	2,4,6-tribromophenyl decanoate
	-	0.17	butanoic acid, ethyl ester
	-	0.11	2-ethylhexyl methacrylate
	-	0.10	5.β-cholan-24-oic acid,3.α.-hydroxy-,methyl ester,trifluoro acetate
	-	0.06	1,2-benzenedicarboxylic acid,bis(2-methyl propyl) ester
	-	0.06	1,2-benzenedicarboxylic acid,dibutylester
	-	0.06	methyl-*N*-hydroxybenzenecarboximidoate
	-	0.05	methyl-2,8-dimethylundecanoate
Terpenoids	47.21	10.04	ergosta-5,7,22-trien-3-ol, (3*β*,22*E*)
	1.48	-	ergost-5,8(14)-dien-3-ol
	0.88	0.99	(22*E*)-ergosta-5,7,9(11),22-tetraen-3*β*-ol
	-	0.02	*D*-xenialactol

(Table 1). Continued.

Heterocyclic	-	1.7	3-(2-indolyl)isocoumarin
	-	0.05	*N,N*-dimethyl-2H-pyran-2-iminium chloride
	0.01	-	2-methylpiperidine
	0.23	-	propionaldehyde, ethylhydrazone
	0.26	-	3-pyridinecarboxamide
	0.46	-	*N*-[3-methylaminopropyl]aziridine
	0.02	-	3-pyridinecarboxylic acid
	-	0.22	pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)
	0.33	-	2-ethoxycarbonyl-5-oxopyrrolidine
	0.07	-	4-amino-2,6-dihydroxypyrimidine
	-	0.04	1H-pyrazole,3-ethyl-4,5-dihydro
	-	0.10	5-methyloxazole
	0.06	-	4,6-dimethoxy-3-phenylindole
	-	0.03	9-acetyl-3-methoxy-1,2-dimethyl-9H-carbazole;
	0.14	-	1,3-propanediamine, *N,N*-dimethyl
	0.02	-	oxo-1-(1-pyrrolidinyl)-6-octadecanone
	0.33	-	2,5-pyrrolidinedione
	0.50	-	7-benzylidene-6-(biphenyl-2-yl)-6,7-dihydropyrrolo[3,4-b]pyridin-5-one
	0.06	-	2-amino-1-methyl-1H-imidazole-4,5-dione
	0.34	-	2(3H)-furanone, dihydro-3-hydroxy-4,4-dimethyl-
	0.20	-	3,9-dimetyoxy-1,11-dimethylbenzo(1,2-B:5,4-B')bisbenzofuran-6,12-dione
	0.22	-	nepenthone B
	-	0.13	7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione
	-	0.16	3,5,5-trimethyl-2-hydroxy-1-cyclohexanone-2-ene
	-	0.70	3-butyl-thiophene-1,1-dioxide
	0.01	-	2-naphthalenecarboxamide, *N*-5H-1,3,2,4-dithia(3-*SIV*)diazol-5-ylidene
	-	0.32	[1*S*-(1α.,5.α)-6.α-methyl-6.*β*-(4-methyl-3-penten-1-yl)-3-azabicyclo[3.1.0]hexan-3-one
	0.03	-	4-oxazolidinone,3-ethyl-2-thioxo
	-	0.05	disulfide, di-tert-dodecyl
Phenols	-	0.71	2,5-di-tert-butylphenol
	0.11	-	phenol,2,4-bis(1,1-dimethylethyl)

Total detected metabolites = 107, In the both sample = 14, In fresh sample only= 56, In dry sample=37.

reduce the amounts of ammonia in a patient's bloodstream by forming phenylacetyl-CoA, which then reacts with nitrogen-rich glutamine to form phenylacetyl glutamine which secreted by the patient's body.

3.2. Alcoholic Metabolites

From fresh samples, five metabolites were classified as an alcohols recorded in fresh sample and ranged between (0.16-0.0) mass fraction, except (3,4,6,7-tetramethylidenebicyclo [3.2.1]octan-2-exo-ol) was the only alcohol recorded in dry sample. Alcohols act as flavoring agents, antifreeze, antiseptics, fuels, preservative, solvents, antioxidant and antimicrobial [16].

3.3. Alkanes Metabolites

Twenty seven alkanes were recorded including 25 alkanes in the fresh sample and fluctuated between

Table 2: The Number of the Different Detected Metabolites Determinates by GC/MS Analysis in Fresh and Dry *P. ostreatus* (Summarized from Table 1)

Metabolites	Heterocyclic		Alkane		Ester		Fatty Acid	Alcohols	
Kind of the Sample	Fresh	Dry	Fresh	Dry	Fresh	Dry	Dry	Fresh	Dry
No. in fresh & dry	18	11	21	8	16	15	8	4	1
Fluctuated between	0.01-0.5	0.5-1.7	0.08 - 0.69	0.1- 0.08	5.98-0.0.03	18.53-0.1	29.19-0.04	0.16-0.01	1.5
Total number	29		27		27		8	5	
Metabolites	**Terpenoids**		**Amids**		**Acids**		**Phenols**		
Kind of the Sample	**Fresh**	**Dry**	**Fresh**	**Dry**	**Fresh**		**Fresh**	**Dry**	
No. in fresh & dry	3	3	2	1	2		1	1	
Fluctuated between	47.21-0.88	10.04-0.99	0.32 -0.02	1.21	0.03-0.08		0.11	0.71	
Total number	4		3		2		2		

Table 3: The Number of the Different Aroma Compounds Detected by GC/MS Analysis in Fresh and Dry *P. ostreatus* (Summarized from Table 1)

Chemical Groups	Esters	Ketones	Thiols	Alcohols	Terpenoids	Phenol	Aldehydes
Total No. of Compounds	27	9	7	5	4	2	1

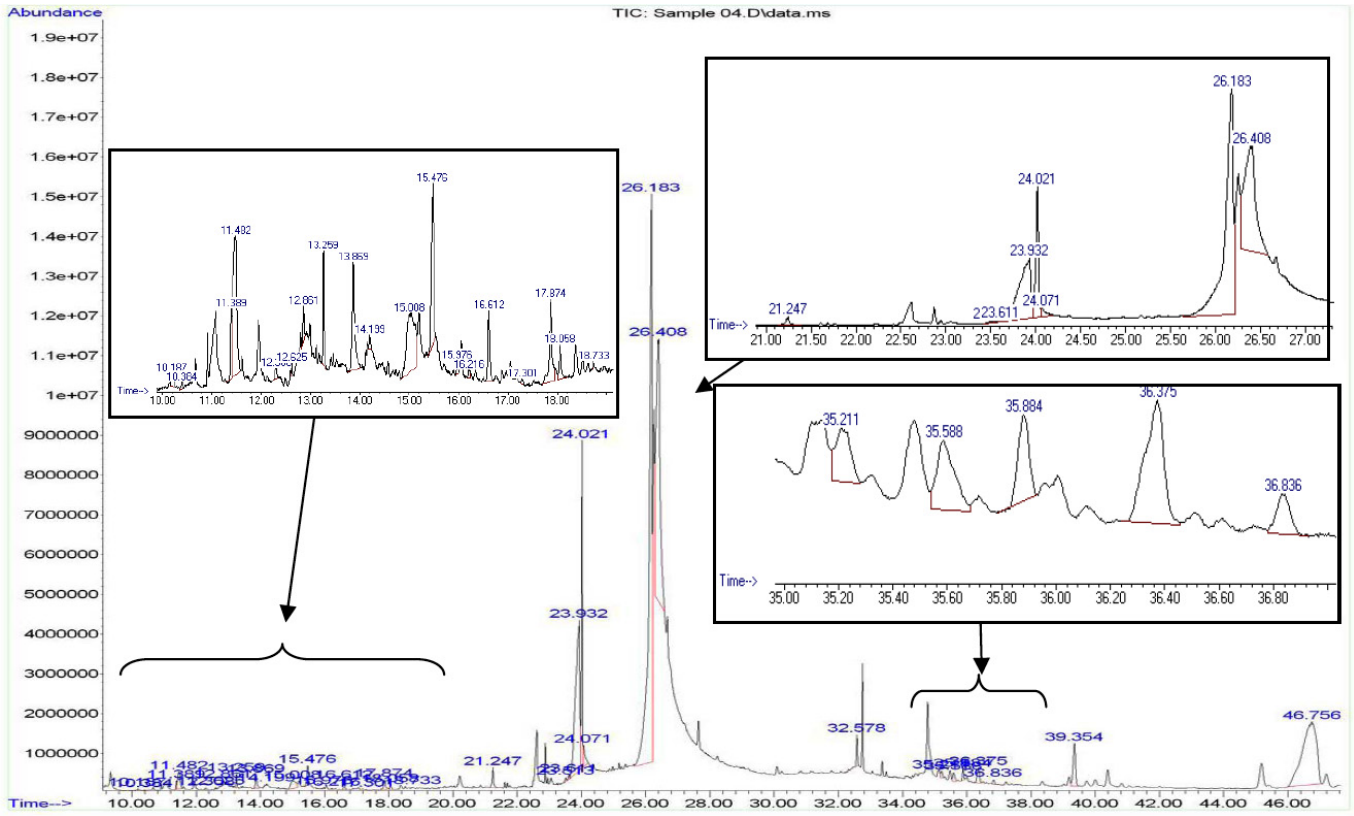

Figure 1: GC/MS analysis of different detected metabolites in dry *P. ostreatus* sample.

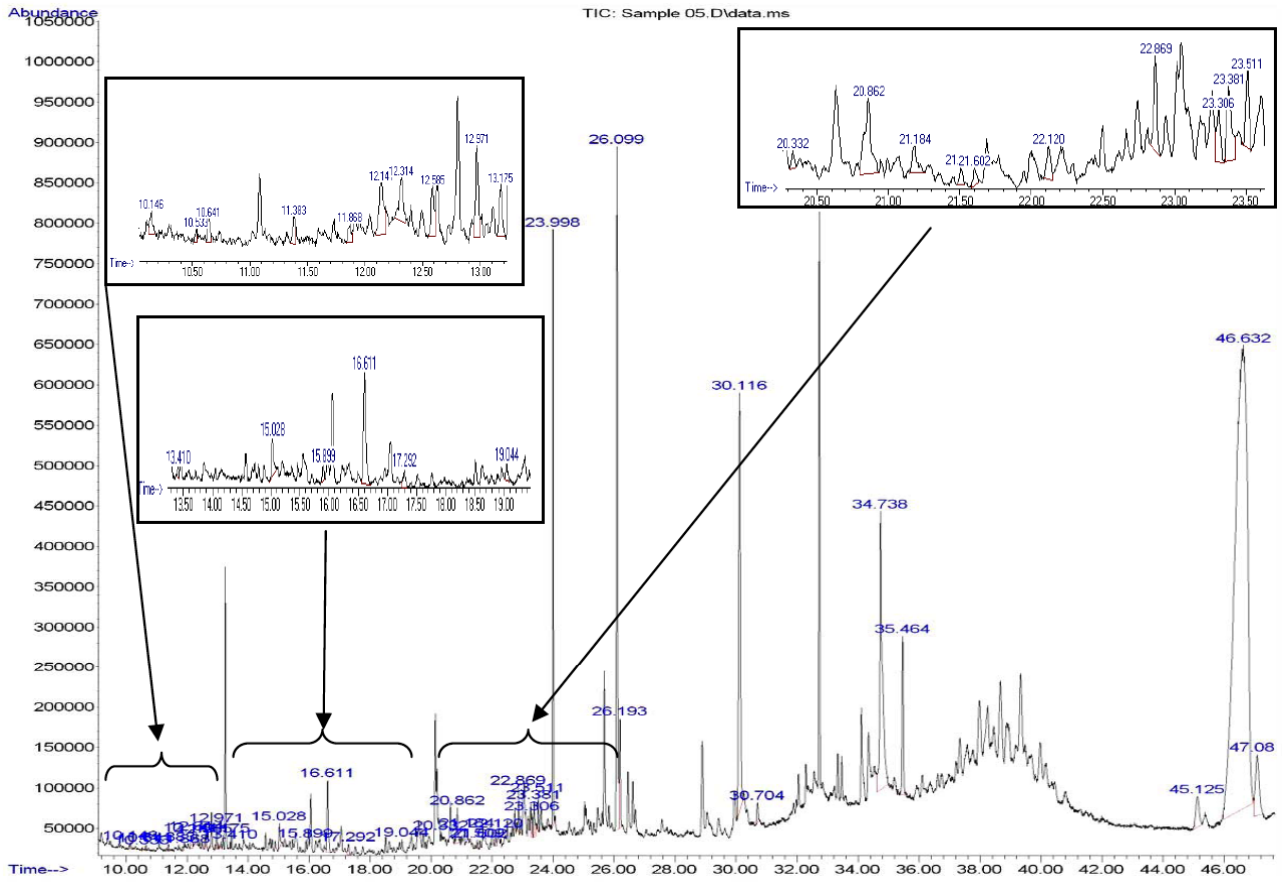

Figure 2: GC/MS analysis of different detected metabolites in fresh *P. ostreatus* sample.

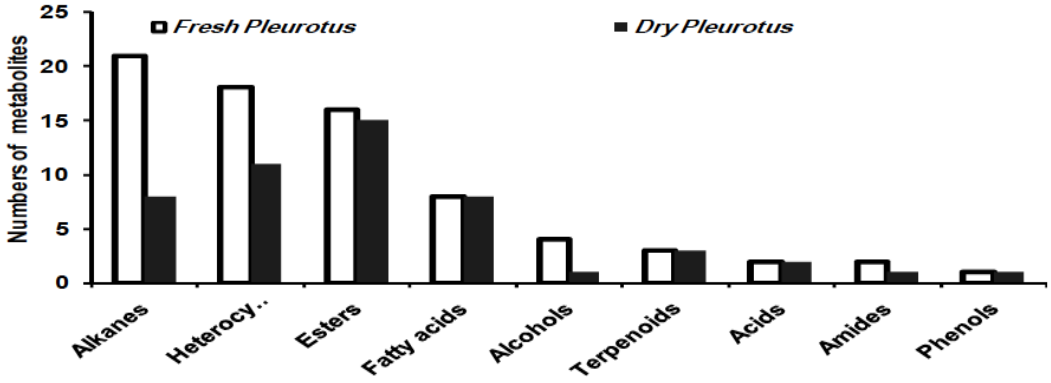

Figure 3: Numbers of different detected metabolites which determined by GC/MS analysis in both fresh and dry *P. ostreatus* samples.

0.69 - 0.07, but the dried sample recorded ten alkanes in low concentrations from 0.08 - 0.01. Eicosane (CAS) recorded 0.09 and 0.02 fraction in fresh and dried sample, respectively. Dried sample recorded 2,2-dideutero-heptadecanal at 0.21 fraction. Zawirska-Wojtasiak *et al*., [33] reported that three main eight-carbon atom volatiles (3-octanone, 3-octanol and 1-octen-3-ol) were detected in different *P. ostreatus* strains as aroma volatiles metabolites. Paltel *et al.,* [17] recorded the presence of eicosanoids in *P. ostreatus* samples.

3.4. Amides

Three amides were detected including two in fresh samples ranged between 0.02 -0.32 but *N*-1,*N*-1-dimethyl-*N*-2-*N*-pentylformamidine only was detected in dry sample at 1.21 mass fraction. Eman [16] and Paltel *et al.,* [17] proved that the phosphoric-triamid recorded

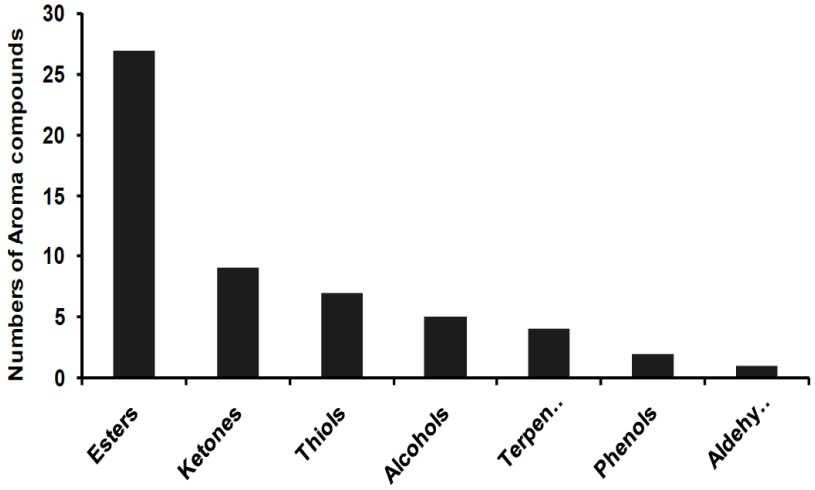

Figure 4: Numbers of aroma compounds which determined by GC/MS analysis in fresh and dried *P. ostreatus* samples.

in the *Agarics bisporus* and reported that this compounds act as antioxidant and anticancer agents.

3.5. Esters

Twenty seven esters were recorded including 16 esters in the fresh sample and fluctuated between (0.03-5.98). However, dried sample recorded 15 esters reached to higher mass fractions (0.01–18.53) Tables **1** and **2**. The esters enhancement the different fruit fragrance such as methyl-butyrate (apple fragrance), methyl butanoate (pineapple), ethyl butyrate (orange), ethyl butanoate (pineapple), pentyl-butyrate (pear), pentyl-butanoate (apricot) are reported elsewhere [30-33].

3.5. Fatty Acids

Eight free fatty acids were detected only in dry sample, it were ranged between 0.04 - 29.19 mass fractions. The recorded fatty acids includes two ω unsaturated (*Cis*-linoleic 29.19, *Cis*-linoleic amide 6.21 and oleic 0.05) and five saturated fatty acids which includes (palmitic 6.98, pentadecanoic 1.36, myristic 0.32, lauric 0.21, nonanoic 0.04 mass fractions). The *P. ostreatus* is riches by fatty acids these results were recorded by many authors [12,13,16,17,34]. Palmitic acid represented 5-14, stearic 0.8-5.5, oleic 2.9-22; linoleic 5.7-65 mg/kg according to Barros *et al.* [35], palmitic and stearic acids and their unsaturated derivatives palmitoleic, oleic, linoleic and linolenic acids [36], saturated fatty acids specially linoleic, linolenic, oleic, myristic, palmitic, palmitoleic and heptadecenoic [37,38]. Fatty acid composition in the *P. ostreatus* includes linoleic acid concentrations are ranged from 13% to 59%. The other major fatty acids were, respectively, palmitic, oleic, stearic and arachidoic acids. Linolenic acid levels were low [11]. Four fatty acids and their derivatives including linoleic, oleic, stearic, palmitic and their corresponding methyl esters [9,17].

Table 4: Detected Selenium by Using Test Methods ACAL-APR-51-00

Samples	Value mg/kg
Fresh *Pleurotus*	58.236
Dry *Pleurotus*	100.306

3.6. Terpenoids

Four terpenoids related metabolites were detected including ergosta-5,7,22-trien-3-ol, (3.β.,22E) represented as (47.21 in fresh and 10.04 mass fraction in dry sample), (22E)-ergosta-5,7,9(11),22-tetraen-3.β.-ol (0.88 in fresh and 0.99 mass fraction in dry sample), ergost-5,8(14)-dien-3-ol; was reached to about 1.48 in fresh sample and *D*-xenialactol also detected in dry sample at 0.02 mass fraction. Ergothioneine, dimethylhydrazine, gosterol, N,N,Ntrishydrazine-carbonyl all these metabolites are act as antioxidant and anticancer agents [16,17]. Triterpenoids (sterols and steroids) have many bioactive actions including anti-(oxidant, cancer cholesterols, cardiovascular, and inflamatory), health support agents and immune enhancer [17,39]. Recently, El Enshasy and Hatti-Kaul [40] reported that the mushroom terpenoids act as immunomodulatory agents.

3.7. Heterocyclic Metabolites

It is worth to mention that twenty nine heterocyclic metabolites were recorded and including all the

(nitrogenous, sulphorus, oxygenated heterocyclic metabolites). Eleven heterocyclic were recorded in the dry sample and fluctuated between 0.03 - 1.7, but the fresh sample recorded 18 heterocyclic at low mass fractions 0.01 – 0.50.

Papaspyridi *et al.,* [9] reported on the appetite-suppressing properties of the alkaloids in the *P. ostreatus*. 3-(2-indolyl)isocoumarin, which protected the plants against grazing animals. Though the compound has a pleasant sweet odor, it has a bitter taste, and animals tend to avoid it. Coumarins have shown some evidence of many biological activities, but they are approved for few medical uses as pharmaceuticals. Coumarin activity includes anti- (HIV virus, tumor, hypertension, arrhythmia, inflammatory, osteoporosis and septic) and analgesic. It is also used in the treatment of asthma and lymphedema [17,41]. Pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro-3-(2-methylpropyl)-. Pyrrole is essential to the production of many different chemicals. *N*-methylpyrrole is a precursor to N-methylpyrrolecarboxylic acid, a building-block in pharmaceutical chemistry. Although there is a claim that pyrrole is used as an additive to cigarettes, it is typically listed as a constituent of tobacco smoke and not as an ingredient pyrrole. Also, 3-pyridinecarboxylic acid is used as a solvent and as a base. The same is true for certain derivatives: *N*-formylpiperidine is a polar aprotic solvent with better hydrocarbon solubility than other amide solvents, and 2,2,6,6-tetramethylpiperidine is a highly sterically hindered base, useful because of its low nucleophilicity and high solubility in organic solvents. Papaspyridi *et al.,* [41] were identified benzoic acid, trans 3,4-dihydro-3,4,8-trihydroxy napthalen-1(2H)-one, 4-hydroxybenzaldehyde, indolo-3-carboxylic acid and uracil. 3-formyl-pyrrole, 4-hydroxy-benzoic acid, uridine, nicotinic acid and nicotinamide have bioactivity. 3-formyl pyrrole is a member of pyrrole exhibiting anticancer, antibacterial and anti-inflammatory activities. In addition, 4-hydroxy-benzoic acid and its derivatives used in preparation of parabens which using in cosmetics and pharmaceutical industries, find important applications as dietary antioxidants, natural flavors and medicines. Moreover, uridine and its derivatives are viewed promising candidates as therapeutic agents for a variety of diseases. Sathyaprabha *et al.,* [42] recorded that the *Pleurotus species* contains pyridine-3-carboxamide, 4-dimethylamino-N-(2,4-difluorophenyl), piperidin-4-carboxylic acid, aspidofractinine-3-methanol, (2à, 3á5à)-indolizine, 2-(4-methylphenyl)-, imidazolidine, 1, 3-dinitro, phenol, 2-methyl-4-(1, 1, 3, 3-tetramethyl-butyl), aspidofractinine-3-methanol, (2à, 3á, 5à) and squalene [42-45].

3.8. Phenolic Compounds

Phenolic compounds were detected including phenol, 2,4-bis(1,1-dimethyl ethyl); in fresh samples in between 0.11 mass fraction but 2,5-di-tertbutylphenol only detected in dry sample at 0.71 mass fraction.

3.9. Aroma Compounds

Fifty five aroma compounds were recorded (27 esters, 9 ketones, 7 thiols, 5 alcholos, 4 terpenoids and 2 phenols and 1 aldehyde). Forty-two aroma compounds were recorded (4 alcohols, 2 benzaldehydes, 27 esters, 3 lactones, 1 phenol, 1 terpenoid, 3 thiol compounds and 1 acetate-3-mercapto butyric acid) by Moharram *et al*., [33]. Aroma compounds, such as volatile fatty acids or esters, lactones, aldehydes, alcohols and ketones which play a significant role in the production of food to improve its flavor. aroma compounds have high biological activity, low toxicity for using in folk medicine, food, perfume, cosmetic and pharmaceutical industries, as defoaming agents and to improve shelf-life and safety of minimally processed fruits [30-33]. The presence of thiols metabolites gives unpongee odor, undesirable taste lowering their food quality but increased the medicinal value. Many investigations recorded that the *P. ostreatus* is riches by many bioactive metabolites with pharmaceutical and nutraceutical interest [41-45].

3.10. Selenium Content

The selenium content was measured by using test methods ACAL-APR-51-00 and recorded (58.24 mg/kg for the fresh *P. ostreatus* sample and 100.31 mg/kg for the dry *P. ostreatus* sample). *Pleurotus ostreatus* was grown in coffee husks enriched with various concentrations of sodium selenite. The highest level of selenium absorption was obtained by adding 51 mg/kg of sodium selenite [6]. Consumption of one gm of dried *P. ostreatus* grown on substrate with 3.2 mg/kg of Se is enough to supply the amount of selenium recommended for adults, 55 µg/day. *Pleurotus ostreatus* is a very good Se accumulator, reaching 858 mg/kg when cultivated on substrate enriched with 102 mg/kg of Se. The capacity to accumulate Se was verified in *Agaricus bisporus* when the mushrooms were irrigated with water plus Se, as these mushrooms absorbed 52.8 mg/kg of Se [43]. selenium absorption rate by *P. ostreatus* were obtained when grown in coffee husks containing 12.8 mg/kg of Se. Selenium

content in *P. ostreatus* mushrooms grown in coffee husk without selenium enrichment ranged from 0.12 to 0.96 mg/kg; these levels could be considered low compared to other mushrooms found in natural conditions [24].

4. CONCLUSIONS AND FUTURE PROSPECTS

GC/MS analysis of the ethanolic extracts of the *P. ostreatus* samples, fresh sample recorded the highest numbers and highest mass fractions of the detected metabolites but the dry sample recorded the lowest numbers and amounts of the detected metabolites. Carboxylic acids and free fatty acids were recorded only in dry sample. Terpenoids followed by fatty acids and esters represented the highest metabolites detected in high mass fractions which are responsible about the bioactivity (which used in pharmacological and medicinal industries), aroma and flavoring of the mushroom (for give desirable fruit fragrances in food, perfume and cosmetic industries). The fresh mushroom sample is rich by aroma compounds more than the dry sample. As a vies veers the dry sample is rich by selenium more than the fresh mushroom. We recommended the using of the fresh mushroom as a source of the aroma compounds but the dry samples as a antioxidants.

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