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Aroma and Antioxidant Metabolites in Pleurotus Ostreatus Mushroom Detected By GC/MS Analysis, and Antioxidant Bioactivty Confirmed by DPPH Free Radical Scavenging Assay

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Abstract

Comparative studying of aroma metabolitesby GC/MS analysisrecorded 134metabolites are detected and classified in all tested *P. ostreatus* ethanolic extract samples includes substrate, fruiting bodies and spent. Antioxidant metabolites "ascorbic acid recorded that 0.3 g/100g in fruiting bodies and 0.2g/100g in spent" and "phenols 0.25µg/100g in fruiting bodies and 0.53µg/100g in spent". The antioxidant bioactivity is confirmed by using DPPH radical scavenging assay and extract 5 mg/ml recorded the best concentrations give the 93% reducing power of fruiting bodies and 85% in spent samples.

Key Words: *Pleurotus ostreatus; comparative studying; GC/MS; flavoring; Spent; Antioxidant; Ascorbic acid; Phenols and DPPH free radical scavenging assay*

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Introduction

Natural flavoring agents and fragrance are obtained from higher plants and animal and it used as food additives in many industries such as food, beverage, feed, cosmetics, detergents and pharmacology. Production of natural flavoring and faced many problems such as producing seasonally, time consuming, produced in minor quantities, extracted by very difficult and expensive methods. Through the past 60 years ago the production of natural flavoring and fragrance by microbial fermentation are prefer red due to increasing interest and worldwide demand because their production have many advantages such as very large amounts, through few days, easily extracted, on the in-expensive substrates under controlled environmental and nutritional factors. Science civilizations mushrooms are used as popular foods and food additives, it has many volatile compounds which responsible for their, good taste, odor, aroma and flavoring characters. Natural aroma agentsin cludeesters, aldehydes, and methyl ketones, fatty acids and lactones, alcohols, and phenolic compounds (Cag˘larırmak, 2007; De-Silva., *et al*. 2013; Eman & Farghaly, 2014; Gupta., *et al*. 2015; Nagy., *et al*. 2017).

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Pleurotus ostreatus has many advantages such as popular healthy foods, no starchy carbohydrates, low "calories, fats, and Na" but also it has highest nutritional value includes high level of water 80 to 90%, protein 40%, essential amino acids (arginine, alanine, glutamine, and glutamic acid); fatty acids (oleic, linoleic, linolenic and palmitic acids); glucans, mannitol and trehalose, vitamin B,C,D,K, thiamine, riboflavin, folic acid and niacin, minerals (Ca, P, Fe, K, Mn, Cu, Zn, Mg and Se. It also 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 (Khatua., *et al*. 2013; Eman & Fargally, 2014). Good quality of P. ostreatus depends upon many parameters includes color, flavor, taste, and texture. More than 200 different volatile organic compounds which determine the flavor of edible mushrooms have been identified.Mushrooms production around the world estimated that more than 10 million metric ton of edible and medicinal mushrooms were produced in 2004. Pleurotus, a close second rank and constitutes about 27% of the world's output (Royse, 2014).

After harvesting the fruiting bodies of mushroom residue includes mycelium and residue of the consumed digested substrate by derivative enzymes called spent. Spent has a bad impact and causing many environmental problems but it considered food wastes wealth that should be recycled. Spent has a high nutritional and medicinal value. The spent mushroom isutilized in many ways of life and has numerous biotechnological applications such as a re-cycling substrate for production of other mushrooms and production many industrial microbial bioactive metabolites. Spent is widely used as a soil conditioner, organic fertilizer, substrate for production of mushrooms, feeding animals and fish, pest management, carrier material for preparation of bio-inoculants; production of biogas, bioremediations of pollutants and dyes for purification of air (from H_2S), water (from radioactive elements and heavy metals), soils (from polycyclic aromatic hydrocarbons and pentachlorophenol) and substrates contaminated with pesticides (Bonatti., *et al*. 2004; Gern., *et al*. 2010; Phan & Sabaratnam, 2012; Purnomo & Mori., *et al*. 2013; Koutrotsios., *et al*. 2014).

Free radical oxygen is very harmful and toxic to the living cells. Harman proposed relation of free radicals in the aging process. All living systems have protective natural metabolites and also have the repairing mechanism against oxygen free radical damage. The human disease occurs when the antioxidant defense system becomes unbalanced, deterioration of physiological functions and the essential molecules (DNA, RNA, Proteins, and lipids) become damaged according to the oxidative stress. As a result many humans occur diseases including atherosclerosis, cardiovascular diseases, several kinds of cancer, cirrhosis, diabetes, lung diseases, neurological disorders (Alzheimer`s disease, mild cognitive impairment, Creutzfeldt-Jacob disease, meningoencephalitis), Parkinson`s disease, senile and drug-induced deafness. Man tries to find the synthetic source of dietary supplements containing antioxidants such as butylated derivatives through consuming time they are suspected to be carcinogenic and to cause liver damage (Valko., *et al*. 2007; Khatua., *et al*. 2013).

Living cells have many antioxidant mechanisms for defenses the harmful effect of free radicals such as enzymatic defenses "superoxide dismutase, glutathione peroxidases, catalase etc.." and non-enzymatic defenses such as ascorbic acid "vitamin C", α-tocopherol "vitamin E", tocotrienols, vitamins "B; sterols specially "carotenoids"; glutathione; phenols "cinnamic acid, gallic acid, caffeic acid, vanillin, catechin, gentisic and tannic acid" and flavonoids "quercetin and rutin"; variegatic acid, 2 p-terphenyls, polysaccharide specially glucans and glucoprotein and also trace elements selenium and Zinc. These substances are responsible for antimicrobial, anti-inflammatory and anticancer (Percival, 1998; Mathew., *et al*. 2011; Reis., *et al*. 2011; Rashidi & Yang 2016).

This investigation has been accomplished to study the comparative studying of aroma and antioxidant value of local edible *P. ostreatus* mushroom (fruiting bodies and spent) GC/MS analysis. Determine the total phenols and ascorbic acid percentage and also antioxidant activity was confirmed by scavenging DPPH radical's analysis.

Materials and Methods

Collection of *P. ostreatus* samples

Pleurotus ostreatus substrate, fruiting bodies, and spent samples were obtained from Agricultural Mushroom Centre, Fac. Agric., and Assiut University, Egypt. Spores of *P. ostreatus* were obtained from the Egyptian National Center for Agricultural Ihuth. Spores were cultivated on rice straw substrate supplemented by 2-5% of each of wheat brain and agricultural gosibeium "$CaCO_3$"(Eman & Farghaly, 2014).

Preparation of the *P. ostreatus* samples ethanolic extracts

One hundred gram from each substrate, fruiting bodies and spent samples were homogenized individually for 10 min. in a high-speed blender at 16.000 rpm with 500 ml 98% ethanol and shacked for 24 hours on a shaker. The extraction procedure was repeated three times. Ethanolic extract was filtered, concentrated to dryness and used for the investigations (Eman 2012).

GC/MS analysis and determination the *P. ostreatus* flavoring value

Determination of *P. ostreatus* flavoringvalue of the ethanolic extracts of *P. ostreatus* samples by Gas Chromatography/Mass Spectrometry (GC/MS) Analysis.

Flavoring value of the substrate and spent of P. ostreatusethanolic extracts samples were detected by analysis using the methods (Blois 1958; Eman, 2012). GC/MS Analytic conditions include Apparatus: GC/MS 6890 N/5975B (Agilent Technologies, Palo Alto, CA, USA), Column: DB-5ms, GC-Conditions. Oven program: 40°C for 2min, then 10°C/min to 150°C for 3min, then 10°C/min to 220°C for 6 min, then 15°C/min to 280°C for 28 min. Run Time 61 min and 2 min (Post Run) 260°C. Flow Program: 0.5 mL/min for 10.9 min, then 1 mL/min per min to 1 mL/min for 30min.This was achieved by using Agilent GC/MS, at the analytical Chemistry Unit, ACAL, Chemistry Department, Faculty of Science, Assiut University, and Assiut, Egypt.

Determination of antioxidant metabolites in *P. ostreatus* ethanolic extracts

Determination of ascorbic acid (vitamin C)

Ascorbic acid in *P. ostreatus* amples were determined spectrophotometer at 760 nm by Folin–Ciocalteu's reagent according to Jagota & Dani (1982).

Determination of total phenols

Total phenols in *P. ostreatus* samples were determined spectrophotometer at 725 nm by Folin–Ciocalteu's reagent according to (Kovalvi & Nassuth 1995; Da az., *et al*. 2012).

Antioxidant bioactivity Confirmation by using DPPH radical scavenging assay.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay was performed by following the standard method of Blois (1958) with several modifications. Potential of extracts obtained from P. ostreatus fruiting bodies and spent samples were assessed by using an ethanolic solution of the "stable" free radical, DPPH. DPPH solution (0.2 mM) was prepared by dissolving DPPH powder in 70% ethanol, sealed in aluminum foil and kept in a Fridge. Two ml of this solution were added to 2 ml of the solution of all extracts in the ethanol-water mixture at different concentrations (0.5, 1, 2.5 & 5 mg/ml). The final concentration of DPPH solution in the assay mixture was 0.1 mM. The mixture was incubated in dark for 30 min. Absorbance was measured spectrophoto metrically at 517 nm (Spectra Max Plus 384, United States). Quercetin was used as a positive control and the sample solution without DPPH was used as a blank. The radical scavenging activity was measured as a decrease in absorbance of DPPH. Inhibition of DPPH free radicals was calculated by using the following formula: Inhibition of DPPH radical (%) = (A control–A sample/A control) × 100.

Where: A control = Absorbance of the control solution (Containing all reagents except the test extract), A sample = Absorbance of the test extract.

Statistical analysis

All experiments were carried out in triplicates. Data obtained were analyzed by one way ANOVA by Duncan's multiple range tests (SPSS 18 version). Differences were considered significant at $p \leq 0.05$.

Results and Discussion

Pleurotus ostreatusethanolic extract samples of substrate, basidium and spent were analyzed by GC/MS method and recorded 134 aroma metabolites (Table 1-4) and (Figure 1-4) and (figure 1, 2).

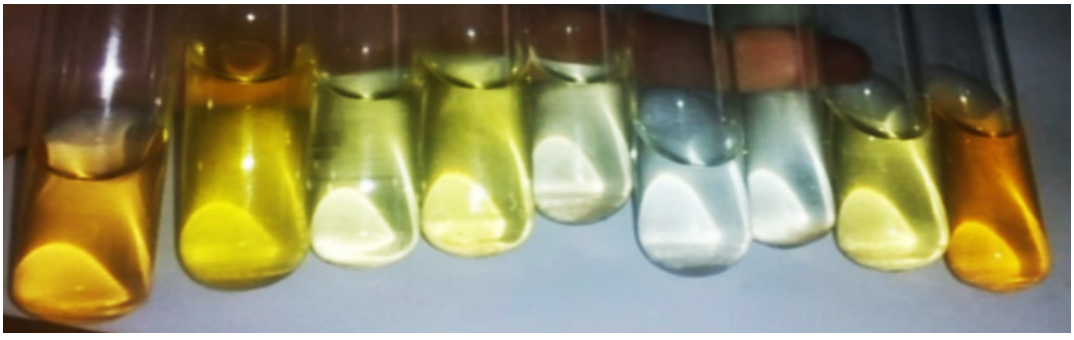


Figure 1: *Yeast extracts with NaOH reagent for detection the flavonoids by various degrees of yellow colors.*

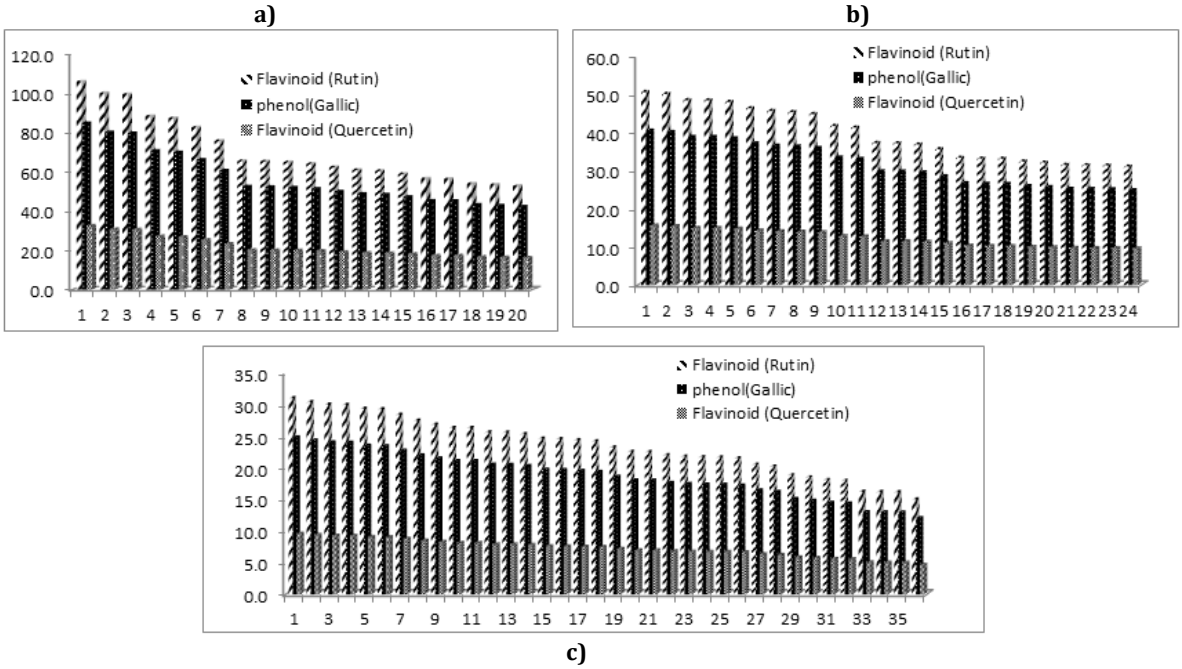


Figure 2: *High 20, moderate 24 and low 36 tested yeast methanolic extracts produce total flavonoids with rutin and quercetin as standard materials, total phenol with gallic acid µg/g DW. a) High producers 20 methanolic yeast extracts; moderate producers 24 methanolic yeast extracts and c. Low producers 36 methanolic yeast extracts*

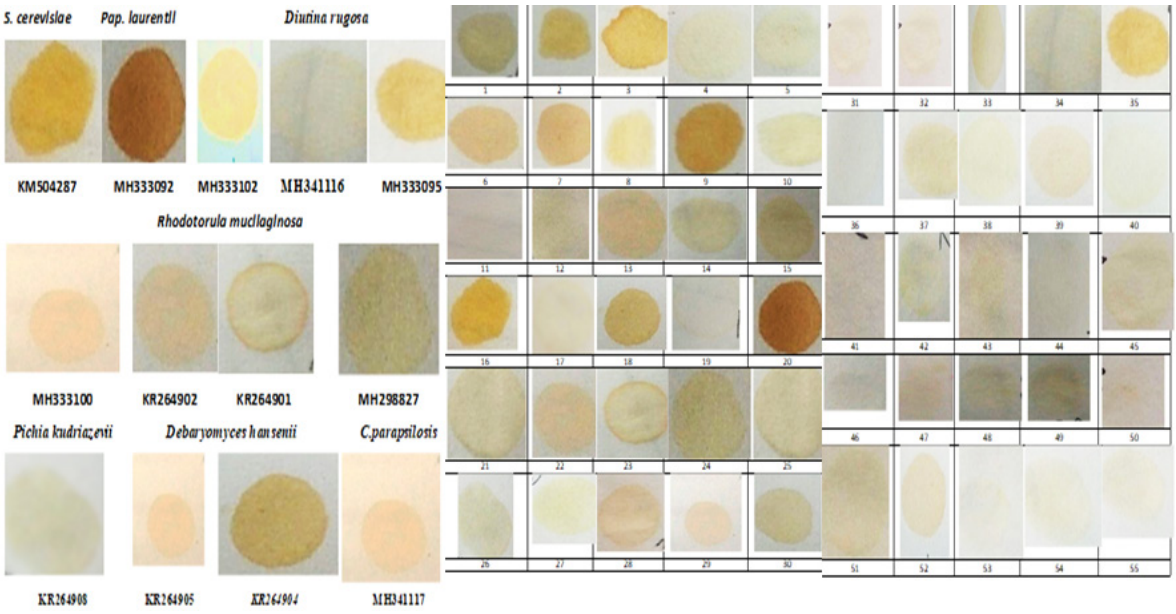


Figure 3: *Screening of production of flavonoids by 80 yeast methanolic extracts by using ammonia vapor on the each methanolic extract spot on paper chromatography (PC) of 80 yeast isolates (yellow color degree are ranged between finitely yellow, deep yellow and brown.*

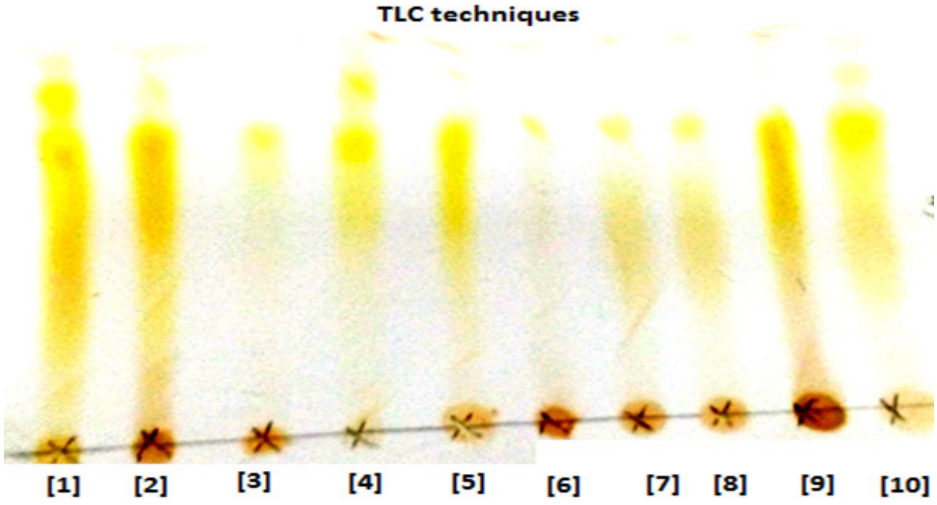


Figure 4: *Flavonoids detected by TLC the highest selected results recorded by ammonium test and Spectrophotometric methods.*

1. Saccharomyces cerevisia KM504287
2. Diutina rugosa MH333102 (AUMC13568)
3. Unidentified yeast isolates 1
4. Rhodotorula mucilaginosa MH298827
5. Rhodotorula mucilaginosa MH29882
6. Rhodotorula mucilaginosa KR264901
7. Pichia kudriazevii14
8. Diutina rugosa MH333095
9. Unidentified yeast isolates 2
10. PapiliotremalaurentiiMH333092 (AUMC13571)

No.		Flavonoids µg/g DW		No.	Flavonoids µg/g DW		No.	Flavonoids µg/g DW		No.	Flavonoids µg/g DW	
		Rutin	Quercetin		Rutin	Quercetin		Rutin	Quercetin		Rutin	Quercetin
High	1	105.8 ± 4.3	32.6 ± 1.3	6	82.5 ± 0.9	25.4 ± 0.3	11	64.1 ± 0.5	19.7 ± 0.1	16	56.5 ± 8.5	17.4 ± 2.6
	2	100.0 ± 3.8	30.8 ± 1.2	7	75.7 ± 1.1	23.3 ± 0.3	12	62.4 ± 3.1	19.2 ± 1	17	56.4 ± 0.6	17.4 ± 0.2
	3	99.4 ± 1.4	30.6 ± 0.4	8	65.5 ± 1.1	20.2 ± 0.3	13	60.9 ± 4.2	18.8 ± 1.3	18	54.0 ± 1.3	16.6 ± 0.4
	4	88.2 ± 1.2	27.2 ± 0.4	9	65.3 ± 1.1	20.1 ± 0.3	14	60.5 ± 0.2	18.6 ± 0.1	19	53.5 ± 2.7	16.5 ± 0.8
	5	87.3 ± 0.8	26.9 ± 0.2	10	64.9 ± 0.6	20.0 ± 0.2	15	58.9 ± 1.1	18.1 ± 0.3	20	52.8 ± 0.6	16.3 ± 0.2
Moderate	1	50.5 ± 5	15.5 ± 1.6	7	45.6 ± 0.8	14.0 ± 0.2	13	37.1 ± 1.7	11.4 ± 0.5	19	32.3 ± 0.5	10.0 ± 0.2
	2	50.0 ± 0.8	15.4 ± 0.2	8	45.3 ± 0.4	13.9 ± 0.1	14	36.8 ± 0.5	11.3 ± 0.2	20	32.0 ± 1.1	9.9 ± 0.3
	3	48.4 ± 1.4	14.9 ± 0.4	9	44.8 ± 1.1	13.8 ± 0.3	15	35.5 ± 1.6	10.9 ± 0.5	21	31.4 ± 0.7	9.7 ± 0.2
	4	48.4 ± 0.4	14.9 ± 0.1	10	41.6 ± 1.2	12.8 ± 0.4	16	33.3 ± 0.8	10.3 ± 0.2	22	31.3 ± 0.5	9.6 ± 0.1
	5	47.9 ± 1	14.8 ± 0.3	11	41.2 ± 1	12.7 ± 0.3	17	33.1 ± 1.2	10.2 ± 0.4	23	31.3 ± 0.5	9.6 ± 0.2
	6	46.3 ± 0.4	14.2 ± 0.1	12	37.2 ± 3.4	11.4 ± 1.1	18	33.0 ± 0.7	10.2 ± 0.2	24	31.0 ± 1.8	9.5 ± 0.6
Low	1	30.9 ± 1.8	9.5 ± 0.5	10	26.2 ± 2.5	8.1 ± 0.8	19	24.4 ± 2	7.5 ± 0.6	28	20.1 ± 3	6.2 ± 0.9
	2	30.3 ± 0.9	9.3 ± 0.3	11	26.2 ± 1.5	8.1 ± 0.5	20	24.3 ± 0.9	7.5 ± 0.3	29	18.7 ± 10.3	5.8 ± 3.2
	3	29.9 ± 2.6	9.2 ± 0.8	12	25.5 ± 0.6	7.9 ± 0.2	21	24.1 ± 1	7.4 ± 0.3	30	18.4 ± 0.9	5.7 ± 0.3
	4	29.8 ± 0.9	9.2 ± 0.3	13	25.5 ± 0.8	7.8 ± 0.2	22	23.1 ± 0.1	7.1 ± 0	31	18.0 ± 1.9	5.5 ± 0.6
	5	29.3 ± 0.2	9.0 ± 0.1	14	25.2 ± 1.6	7.8 ± 0.5	23	22.4 ± 0.5	6.9 ± 0.2	32	17.8 ± 0.8	5.5 ± 0.3
	6	29.2 ± 1.5	9.0 ± 0.5	15	24.5 ± 5.3	7.5 ± 1.6	24	22.4 ± 0	6.9 ± 0	33	16.1 ± 23	5.0 ± 7.1
	7	28.3 ± 0.4	8.7 ± 0.1	16	25.5 ± 0.8	7.8 ± 0.2	25	21.5 ± 1.9	6.6 ± 0.6	34	16.1 ± 1.1	5.0 ± 0.4
	8	27.3 ± 2	8.4 ± 0.6	17	25.2 ± 1.6	7.8 ± 0.5	26	21.4 ± 15.1	6.6 ± 4.6	35	16.1 ± 1.2	4.9 ± 3.7
	9	26.7 ± 1.4	8.2 ± 0.4	18	24.5 ± 5.3	7.5 ± 1.6	27	20.4 ± 3.5	6.3 ± 1.1	36	14.9 ± 1	4.6 ± 3.7

Table 1: *Flavonoids screening of 80 yeast methanolic extracts detected by spectrophotometer at 362 nm.*

Summarized categories results of flavonoids recorded in table 1.

High = H, 20 yeast producers quercetin = ≥ 16 and rutin = ≥ 51.

Moderate = H, 24 yeast producers quercetin = 15.9- 9.5 and rutin = 50.9-31.

Low = H, 36 yeast producers quercetin = ≤ 9.5 and rutin = ≤ 30.9.

Strain Name	GB	AUMC	PC Spot color on with ammonia	TLC	Spectro.		HPLC µg /L			Antibacterial Bioactivity
					Rutin	Quercetin.	Gallic	Rutin	Quercetin.	
1. D. rugosa	MH333102	13568	faintly orange	+++	106	33	150	1	1	4H + 2L
2. R. mucilaginosa	MH298828	13565	yellow green	++	87	27	-	-	-	6H
3. Pa. laurentii	MH333092	13569	brown	++	100	31	-	-	-	3H + 2M + L
4. R. mucilaginosa	MH298827	13567	yellow green	++	76	23	2600	12	50	4H + 2M
5. R. mucilaginosa	MH333100	13564	faintly orange	-	99	31	18800	1	1	6L
6. R. mucilaginosa	KR264902	_	faintly yellow	-	88	27	23500	11	1	L + 5VL
7. R. mucilaginosa	MH333091	_	faintly yellow orange	-	83	25	100	50	2	H + 1M + 4L

8. D. rugosa	MH341116	13566	faintly yellow green	-	53	16	-	-	-	6H
9. D. rugosa	MH333095	13571	Yellow	+	46	14	-	-	-	3H + 3M
10. De. hansenii	KR264905	_	faintly orange	+	48	15	-	-	-	3H + 3M
11. S. cerevisiae	KM504287	_	deep yellow	+++	65	20	-	-	-	3H + 3M
12. R. mucilaginosa	MH341115	13570	faintly orange	-	24	7	-	-	-	H + 2M + 3L
13. S. cerevisiae	-	GHM	faintly yellow	-	48	15	-	-	-	H + 2M + L
14. S. cerevisiae	-	EC1118	faintly yellow	-	59	18	-	-	-	M + 5L
15. C. parapsilosis	MH341117	13563	faintly orange	-	37	11	-	-	-	M + 5L
16. De. hansenii	KR264904	_	faintly orange	-	45	14	-	-	-	H+M+4L
17. R. mucilaginosa	KR264901	_	faintly yellow orange	+	59	18	-	-	-	M+5L
18. P. kudriazevii	KR264908	_	faintly yellow green	+	33	10	-	-	-	L+3VL+2-Ve
19. Unidentified1	_	_	faintly orange	+	65	20	-	-	-	6L
20. Unidentified2	_	_	faintly yellow	++	36	11	-	-	-	6L

Table 2: *Screening of phenols and flavonoids by many analytical methods (ammonia test, spectrophotometer, TLC & HPLC). Relationship between flavonoids concentration and antibacterial bioactivity of 20 tested yeast strains and isolates methanolic extract.*

UV & florescent of different flavonoids classes (Mabry., *et al*. 1970)	In this investigation	
Tested yeast strains Color recorded		
Deep purple when spry with ammonia gives		
1] Yellow, yellow-green or brown a) Flavones with 5 OH and 4-OH or 3-OH substituted flavonols with 5-OH and 4-OH Some 5-OH flavanones and 4-OH chalcones lacking B-ring hydroxyl groups	Papiliotrema laurentii MH333092	Brown
	S. cerevisiae KM504287	deep yellow
	D. rugosa MH333102	Yellow
	S. cerevisiae GHM & EC1118	faintly yellow
	R. mucilaginosa KR264902	faintly yellow
2] Little or no color change		
Flavones or flavonols with 5-OH but with the 4-OH absent or substituted a) Isoflavones dihydroflavonols & flavanones +5-OH but without a free 2- or 4-OH b) Chalcones with 2- or 6-OH	The remain tested yeast	
3] Light blue, Red or orange		
Some 5-OH flavanones, Chalcones with a free 2- and /or 4-OH		
4] Fluorescent yellow-green or fluorescent blue-green	R. mucilaginosa KR264901	faintly yellow-green
a) Flavones and flavanones lacking a free 5-OH b) Flavonols lacking a free 5-OH but with the 3-OH substituted	Pichia kudriazeviiKR264908	faintly yellow-green
	R. mucilaginosa MH298828 &298827	yellow-green
	D. rugosa MH341116	faintly yellow-green

1] Little or no color change Isoflavones lacking a free 5-OH 2] Bright fluorescent light blue Light blue		
Isoflavones lacking a free 5-OH		
Fluorescent light blue Invisible		
Isoflavones lacking a free 5-OH		
Little or no color change Yellow or orange		
Flavonols with a free 3-OH and with or without a free 5-OH		
Orange or red no color Aurones with a free 4-OH and some- 2- or 4-OH chalcones	Diutina rugosa MH333102	faintly orange
	R.mucilaginosa MH333091, 333100 &341115	faintly yellow-orange
	C. parapsilosis MH341117	faintly orange
	De. hansenii KR264904 &264905	faintly orange
Yellow, yellow green, blue-green or green		
a) Aurones lacking a free 4-OH and flavanones b) Flavonols with a free 3-OH and with or without a free 5-OH		
Pale yellow Light yellow-purple Dihydroflavonols lacking a free 5-OH		

Table 3: *The visual detection of flavonoids on paper chromatography with ammonia vapor identified and classification according to (Marby 1970) this identification need more investigation.*

Name	Gallic acid	Pyro catechol	Resorcinol		Aloxiprin		P-cumenol	Butaxamine	Quercetin	Rutin
CID	370	289	5054		71586929		7465	18026	5280343	5280805
GC/MS %		6.4	6.4	1.7	1.4	0.9	0.9	0.02		
Retention Time		12.5	12.5	11.5	10.9	13.1	13.4	25.7		
Phenols classes	Simple phenols			Quinone	phenolic acid	Chromones	Chromones		Flavonoid	Glycosidic flavonoids
IUPAC	3,4,5-trihydroxy benzoic acid	1,2-benzenediol	1,3-Benzene diol	α.-methyl-α-propyl cyclopropane-methanol	dialuminum; 2-acetyl oxybenzoic acid, oxygen (2-) or Alsalicylic acid	3,4,7,7-Tetrahydro-3-Methyl-2(3H)-benzofuranone	4-Isopropyl phenol	α- (1-aminoethyl) -2, 5-dime hoxy benzene methanol	Sophoretin, Meletin Xanthaurine	Rutoside, Phytomelin Quercetin 3-rutinoside
MF	$C_7H_6O_5$	$C_6H_6O_2$	$C_6H_6O_2$	$C_8H_{16}O$	$C_9H_8Al_2O_7$	$C_9H_{12}O_2$	$C_9H_{12}O$	$C_{15}H_{25}NO_3$	$C_{15}H_{10}O_7$	$C_{27}H_{30}O_{16}$
MWg/mol	170.12	110.112	110.112	128	282.119	152	136.194	267.369	302.238	610.521

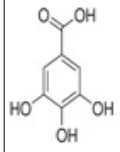
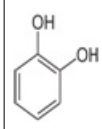
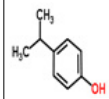
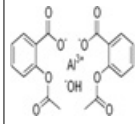
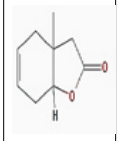
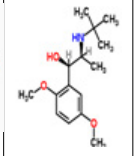
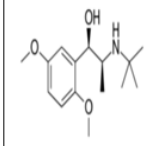
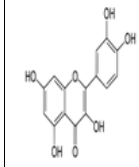
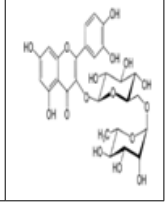
Chemical structure										

Table 4: *Information's about the phenols detected metabolites in methanolic extract of Diutina rugosa MH333102 strain according to Pub Chem citation.*

MF = Molecular Formula
MW = Molecular Weight g/mol

(Table 4) summarizing and clearing that 98 metabolites are detected in all samples and classified into 11 different chemical groups according to their bioactive groups and including one from each (dine and phenol), two from (acids, aldehydes and amide), 5 From each (alkanes and sterols, 7 from each (alcohol and free fatty acids), and 33 from each (esters and ketones).

(Table 1) summarized the results recorded in the basidium samples which recorded fifty-one metabolites according to (Eman & Farghaly2014) including one from each (aldehyde and dine), two from (acids, alkanes. and sterols), 3 alcohols, 4 free fatty acids, 13 ketones, 21 esters and 33 from each (esters and ketones). Fatty acid 9, 12-octadecadienoic or Cis-linoleic recorded 29.9 the highest mass fraction and flowed by their esters recorded 5.4 mass fraction. The basidium recorded the highest numbers of aroma metabolites also it recorded highest numbers of esters 21 and ketones 13 and free fatty acids 4. The aroma metabolites detected in basidium sample fluctuate between 29.9 to 0.01 mass fractions.

(Table 2) showing the results recorded in the spent samples which recorded twenty-seven aroma metabolites including one from each (aldehyde, amide, and sterol), two alcohols, 3 alkanes, 6 esters 13 ketones, 2-butenedioic acid-bis (2-ethylhexyl)-ester recorded 36 mass fraction. The aroma metabolites detected in spent fluctuate between 36 to 0.1 mass fractions.

(Tables 3 & 4) clearing that the comparative studding of the aroma metabolites through the growth of *P. ostreatus* and detected that eight aroma metabolites still found in both samples of substrate and basidium but the mass fraction of each detected metabolites is very low but increased and folded in the basidium includes one from (amid, ketone and ester), two sterols [ergosta-5,7,22-trien-3β-ol 1.7 and 47.21 mass fraction in substrate and basidium, respectively but ergosta-5,7,9(11), 22-tetraen-3β-ol 0.1 in the two samples] and three free fatty acids (hexadecanoic or palmitic, dodecanoic or lauric and 9-octadecenoic or oleic).

Six ketones are detected in both basidium and spent samples but the amount in the spent is larger than the amount found in the basidium. One phenol and two esters are found in the substrate and spent with the stable amount. Three esters includemethyl pentadecanoate, ethylhexadecanoate,andethyloleate are stable in all tested samples with the same amount of substrate, basidium and spent samples.

Thirty-six metabolites are found only in substrate and non-appear again in any other tested samples we also neglected. 1, 2-benzenedicarboxylic acidbis-(2-ethylhexyl)-ester (55) recorded in spent at 4.6 mass fraction. It is used in applications such as coating. 11,15-tetramethyl-2-hexadecene-1-ol(78) or phytol's an acyclic-diterpene alcohol recorded in spent at 3.7 mass fraction that used in a synthesis of vitamin E and K and also used in the fragrance and cosmetics industry. 2, 4-bis-(1,1-dimethylethyl)-phenol (48) recorded in spent at 1.3 mass fraction and used as UV stabilizers, antioxidants and light-protection agents. B-caryophyllene oxide (83), is a natural bicyclic-sesquiterpene recorded in spent at 0.1 mass fraction. It has anti-inflammatory, antimicrobial and anti-parasitic, anti ociceptive,

neuroprotective, anxiolytic and antidepressant and anti-alcoholism activity. It is also an approved food flavoring. Maleic acid or 2-butenedioic acid (E)-, bis-(2-ethylhexyl)-ester (54) representing high mass fractions about 36mass fraction.

Terpene is used in the cosmetic and perfumery industries and it giving different flavors and properties like termicidal, insecticidal, antimicrobial and antioxidant. Lactones are responsible for various taste and odor like oily-peachy, creamy, fruity, and nutty, coconut, etc produced by various fungi, coconut aroma is very popular as a food flavor and chemically they include γ-octalactone, γ-non alactone, and linoleic acid. Milky, buttery and coconut-like flavor provided by these lactones are desirable in dairy and milk products. D-decalactones have a fruity and oily flavor that is commonly used in peach, apricot and strawberry aromas. Ester sensory tastes contribute to a fruity aroma to the food industries, esters derivatives like acetate esters, such as ethyl acetate, hexyl acetate, and isoamyl-acetate and 2-phenylethyl acetate provide a typicalflavorof wine and other grape-derived alcoholic beverages. Ethyl or methyl esters of short-chain fatty acids give the fruity flavor (Gupta., *et al*. 2015).

Six wild edible mushrooms detected by HPLC analysis and cleared that malic and ascorbic acids were the most abundant compounds, followed by citric plus ketoglutaric acids and also have succinic, fumaric, oxalic, quinic and shikimic acids (Valentão., *et al.* 2005).

Cag˘larırmak, (2007)recorded the aroma metabolites in extract of three genus of mushrooms and that the P*leurotus major-cajun* recorded 2,5-dimethyloctane, 4-ethyloctane, N-octan-3-ol, 2-methoxythiozole, hexadecanoic acid, palmitic acid, 3,4-dimethyldecane, octadecanoic acid, 9-hexadecenoic acid, 9-hexadecenyl ester, 9-dodecanol,palmitic acid, (2-tetradecyl oxy)ethyl ester, 9-octadecanoic acid–octadecyl-ester, methyl-butanol and 3-methyloxolane-3-one). *Lentinulaedodes* recorded L-limonene, octadecenoic acid, 2-propyl ester, 9-octadecenoic acid, cyclohexane, 1-((1, 5-dimethyl-hexyl)-4-(4methylpenthyl), octadecanoic acid, octadecyl-ester, 1, 2-benzenedicarboxylic acid and eicosamethylcyclopentasiloxane. *Pleurotus ostreatus* recorded 1-dodecanal-lauraldehyde, 1, 2-di-chloroacetone-octane, octadecanoic acid, nonadecanoic acid, 2-nitrocyclooctanone, 9, 12-octadecadienoic-1-ol, Cis-linoleic-methyl-ester, akuammilan-17-ol and hexadecadienoic acid, methyl ester.

Many Author's studied aroma compounds in various mushrooms genus extracts such as alkene, alkane, alcohol, aldehydes, ketone, esters, fatty acids, lactones, phenols, terpenoids, thiols and mercapto compounds. Benzaldehyde (almond cherry, malt, roasted peppers), 1- octene-3-on & 1- octene-3-ol (mushroom, sweet, earthy), octanol (citrus odor) and 3-octanone, esters give the different fruity fragrance compounds such as methyl-butyrate (apple and pineapple fragrance), ethylbutyrate (orange and pineapple), pentyl-butyrate (pear) and pentylbutanoate (apricot). These metabolites give desirable fruit fragrances in food, perfume, cosmetic, pharmaceutical industries, and folk medicine (Dudareva, 1975; Misharina., *et al*. 2009; Zawirska-Wojtasiak., *et al*. 2009; Zhang., *et al* 2008; Moliszewska,2014). Pleurotus ostreatus shave many aroma metabolites with fragrance or odor includes hexanal (Fresh herbal scent, apple, oily), 2-octenal & Oct anal (citrus, herbaceous,), non-anal (fruit, herbaceous, spicy), ester isoamyl acetate (banana) and methyl salicylate (wintergreen). Also, he recorded that the L. edodes mushrooms extract recorded3- octanol (oily, sweet, walnut, citrus), 2-ethyl-1-hexanol (rose, herbaceous), trans-2-octene-1ol, Cis-2-octene-1-ol (floral, herbaceous) 2-decanal (orange, fatty, fishy) and 2-octanone (oily, fatty) (Vlasenko., *et al*. 2017).

Pleurotus ostreatus mushroom antioxidant metabolites such as ascorbic acid, total phenols in the spent, fruiting bodies are tested and the ascorbic acid recorded that 0.3 g/100g DW in fruiting bodies and 0.2 g/100g DW in spent. It also has total phenols 0.25 µg/100g DW in fruiting bodies and 0.53 µg/100g DW in spent (Table 5) and (Figure 5). Table 5 showing comparison between the results recorded in this investigation and results recorded by many Author's and (Figure 5).

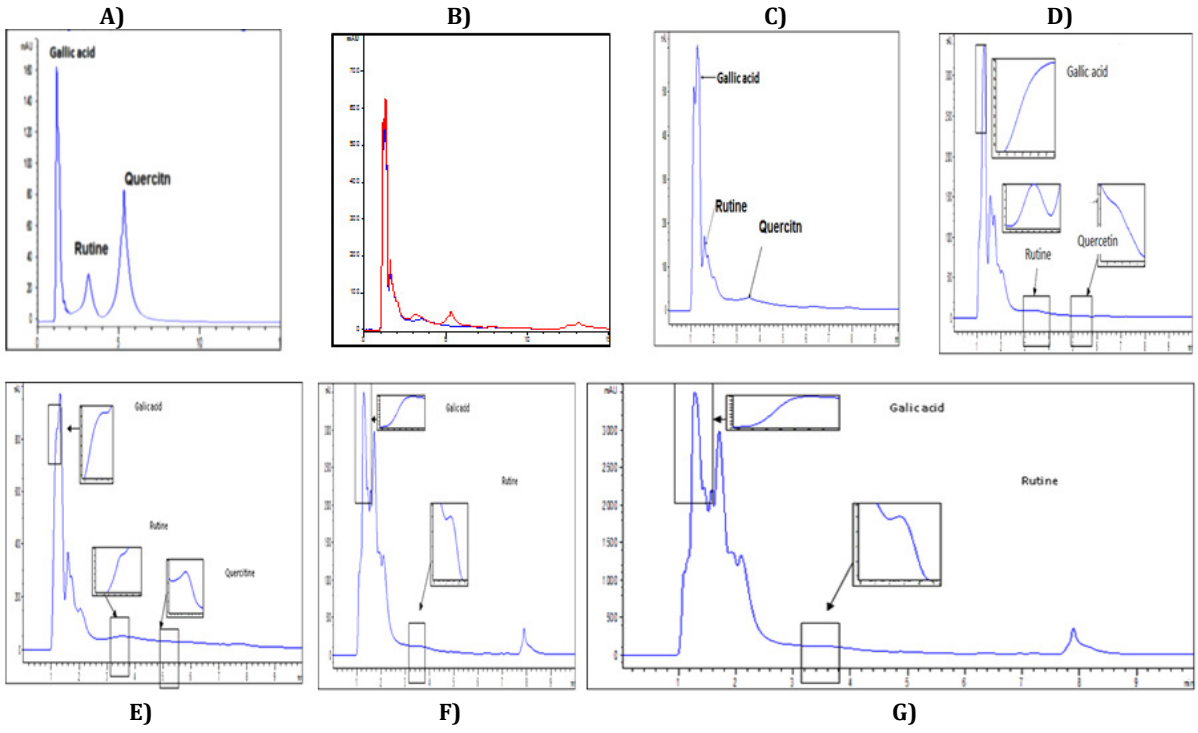


Figure 5: *Representative chromatogram for a standard mixture of gallic acid, rutin, and quercetin at 5mg/L detected by HPLC analysis in five yeast methanolic extracts.*

A. HPLC standard curve *B. Diutina rugosa (1:1) spiked with mix. Of standard at 5 mg/ L*

C. D. rugosa MH333102; *D. R. mucilaginosa MH298827;*

E. R. mucilaginosaMH333091; *F. R. mucilaginosa KR264902;*

G. R. mucilaginosa MH333100.

Serial	No. of C atoms	Group	Examples
1.	C_6	Simple phenols or Benzoquinones	Catechol, valnnilic acid, hydroquinone, syringic acids &2,6-dimethoxybenzoquinone
2.	C_7 (C_6 - C_1)	Phenolic acids, phenolic aldehydes	Gallic & salicylic acids
3.	C_8 (C_6 - C_2)	Acetophenones, Tyrosine derivatives & phenylacetic acids	3-Acetyl-6-methoxybenzaldehyde, tyrosol, p-Hydroxyphenylacetic acid&homogentisic acid
4.	$c_9 = C_6 - C_3$	Hydroxycinnamic acids, Phenylpropenes, Coumarins, Isocoumarins, Chromones	Caffeic, ferulic acids, myristicin, eugenol, umbelliferone, aesculetin, bergenon, eugenin
5.	$C_{10} = C_6 - C_4$	Naphthoquinones	Juglone & plumbagin
6.	$C_{13} = C_6$-C_1 - C_6	Xanthonoids	Mangiferin
7.	$C_{14} = C_6 - C_2$ -C_6	Stilbenoids, Anthraquinones	Resveratrol & emodin
8.	C15 = C6 - C3 -C6	Flavonoids, Isoflavonoids, Neoflavonoids	Quercetin, rutin, catechin & epicatechin

9.	$C_{16} = C_6 - C_4 - C_6$	Halogenated algal phenolic compounds	Kaviol A & colpol
10.	$C_{18} = (C_6 - C_3)_2$	Lignans, Neolignans	Pinoresinol & eusiderin
11.	$C_{30} = (C_6 - C_3 - C_6)_2$	Biflavonoids	Amentoflavone
12.	• Poly • $(C_6\text{-}C_3)_{n>12}$ • $(C_6)_n$ • $(C_6 - C_3 - C_6)_n$	Lignins, Catechol, melanins, Flavolans (Condensed tannins), Polyphenolic proteins, Polyphenols	Raspberry ellagitannin, Tannic acid

Table 5: *Classification of fungal phenols and flavonoids derived from shikimic (from phenyl alanine) and ACOA (poyketides) pathway according to their No. of C atoms with some examples of fungal producer and their biological activity. [Turner 1971, Pandey., et al. 2016, Sarker & Nahar 2012].*

(Table 5) and (Figure 6) clearing the results of antioxidant activity were confirmed in both fruiting bodies and spent ethanolic extracts against DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay. The RSA values of mushroom extracts in different concentrations (0.5, 1, 2.5, 5 mg/ml) were expressed as the ratio of sample absorbance decrease in DPPH solution (0.1 mM in test solution) and the absorbance of DPPH solution in the absence of extract at 517 nm. The antioxidant activity was measured in relation to quercetin that was used as a reference antioxidant (positive control). The DPPH scavenging potential of both fruiting bodies and spent ethanolic extracts of the mushroom was dependent on concentration as shown in (Table 2). Antioxidant activities were recorded at high concentrations (5 and 2.5 mg/ml) for both extracts with no significant difference between the antioxidant activities of both extracts. Around 93% inhibition was recorded for 5 mg/ml while about 80% inhibition was recorded for 2.5 mg/ml for both fruiting bodies and spent ethanolic extracts, respectively. At lower concentrations, the ethanolic extracts of fruiting bodies showed relatively higher antioxidant activities in comparison to that of spent ethanolic extracts. At 1 mg/ml, the percentage of inhibition was recorded to be 52% and 36% for the ethanolic extracts of fruiting bodies and spent extracts, respectively. While at 0.5 mg/ml the antioxidant activity of ethanolic extract of fruiting bodies was two folds the antioxidant activity of spent ethanolic extract "34 and 15% of inhibition (Table 2 and Figure 2). Ethanolic extracts from *P. ostreatus* mushrooms reducing the effect on DPPH free radical scavenging increased with the increased concentrations and the best results are recorded at 5mg/ml.

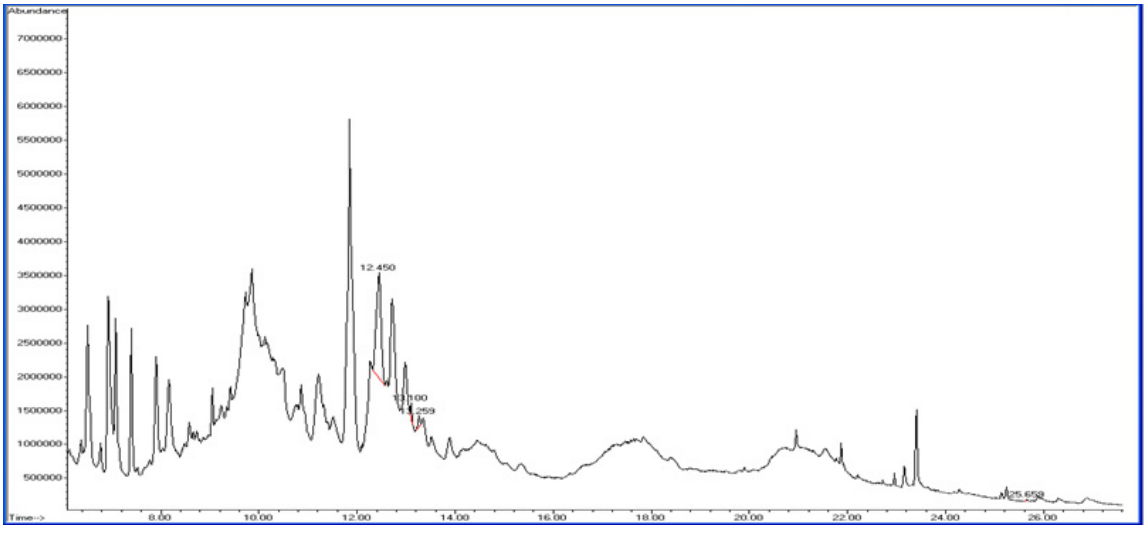


Figure 6: *Chromatogram of the phenolic metabolites detected by GC/MS analysis in methanolic extracts of yeast strain Diutina rugosa MH333102 highest phenols and flavonoids producers.*

Several medicinal mushrooms recorded that at 0.64 mg/mL, scavenging effects were 67.6-74.4% for *Ganoderma* and 24.6% for *C. Versicolor.* It was anticipated that scavenging effects would be excellent for Ganoderma and higher for *C. Versicolor* at concentrations >0.64 mg/ml. However, the scavenging effects of BHA and R-tocopherol at 20 mM (3.6 and 8.6 mg/mL) were 96 and 95%, respectively. Excellent scavenging effects (96.3-99.1 and 97.1%) were observed with methanolic extracts from *A. camphorata* and Brazilian mushrooms at 2.5 mg/mL, respectively.

At 6.4 mg/mL, the methanolic extract from basket stinkhorn scavenged DPPH radical by 92.1%, whereas scavenging effects of methanolic extracts from other specialty mushrooms were 63.3-67.8%. At 6.4 mg/mL, the methanolic extract from tree oyster mushrooms scavenged DPPH radical by 81.8%, whereas scavenging effects of extracts from other commercial mushrooms were 42.9-69.9%. In addition, at 1 mg/mL, methanolic extracts from black and red ear mushrooms scavenged DPPH radical completely (100%), whereas those from snow and jin ear mushrooms scavenged DPPH radical by 94.5% at 0.4 mg/mL and 95.4% at 3 mg/mL, respectively. However, silver ear mushrooms were not effective in scavenging DPPH radical (71.5% at 5 mg/ml) Mau., *et al*. (2002).

Edible mushroom contains many bioactive antioxidant molecule profiles include phenols > flavonoids > ascorbic acid > tocopherols > carotenoids (Gezer., *et al*. 2006, Reis., *et al*. 2011).

Popular edible and medicinal mushrooms have high antioxidant value and stronger antioxidant bio activite metabolites includes carotene; "and phenols (gallic acid, caffeic acid, Cinnamic acid, catechin, gentisic, tannic acid, rutin, quercetin and chrysin) flavonoid; ascorbic acid or vitamin c; trace element (selenium and znic); carotenoids. plasma, triglyceride, low-density lipoprotein, total lipid, phospholipids, polysaccharide (pleuran, glucan, D-and sugar alcohol mannitol); organic acids; glycosphingolipid, lipopolysaccharides; thioacetamide proteins specially hemolysin and ubiquitin; antioxidant enzymes (superoxide dismutase, catalase and peroxidase. Young or closed basidium have 24% higher antioxidant activity than old or opened basidium. Antioxidant activity depends upon many parameters such as tested mushroom part, extraction solvent, source wild or cultivated and the substrate used in cultivated species, kind of antioxidant analysis(Patel., *et al*. 2012; Khatua., *et al*. 2013; Eman & Fargally, 2014).

Highlights

1. Screening of yeast flavonoids by different analytical methods.
2. Yeast phenols and flavonoids act as anticancer, anti-aging, antimicrobial and antioxidant.
3. Yeast promising, interesting for research, industry, medicine and pharmacology.

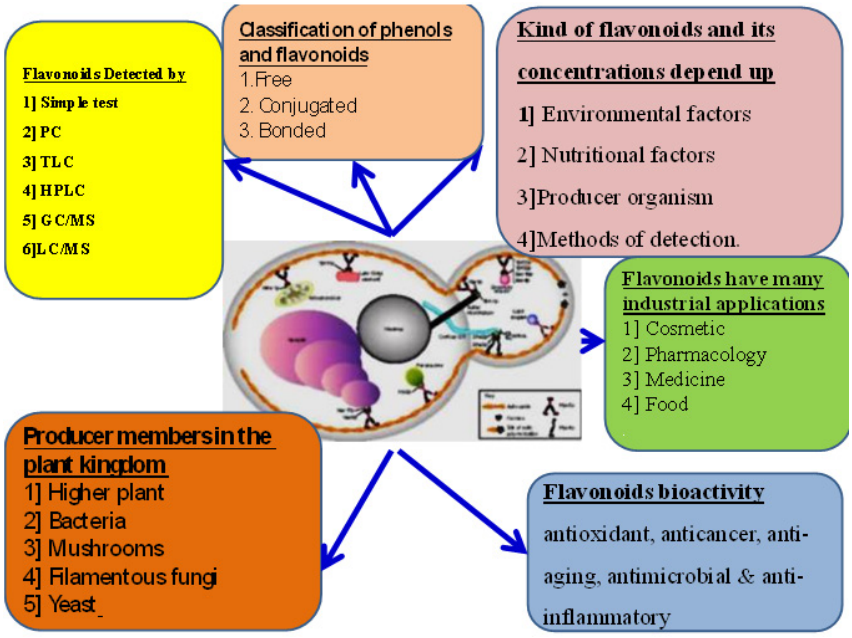
All the chemical materials and standard materials used in this investigation are obtained from Sigma Company imported by Prof: Abo- El -Maaly head of (ACU) Analytical Chemistry Unit, Assiut University, Egypt,

Name		
Commercial	IUPAC	Formula
Gallic acid	3,4,5-Trihydroxybenzoic acid	$C_7H_6O_5$
Quercetin	2-(3,4-dihydroxyphenyl)-3,5,7-trihydr- oxy-4H-chromen-4-one	$C_{15}H_{10}O_7$
Rutin	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamno pyranosyl-(1→6)-β-D-gluco pyranosyloxy]-4H-chromen-4-one	$C_{27}H_{30}O_{16}$

Used Interments

Abbreviation	Descebision
Spect	Spectrophotometer electronic Genesy 5 (Waltham, Mass)
HPLC	High-Performance Liquid Chromatography Analysis occurred by follow-up the following chromatographic conditions includes HPLC apparatus is Agilent Technologies 1200 Series, G1315D. The column is Zorbax Extend C18.
GC/MS	Gas Chromatography Mass Spectrophotometer (7890A-5975B). Column: DB-5ms

Author contribution



Conclusions

Pleurotus ostreatus has high value of flavoring (134 aromametabolites) and antioxidant (phenols and ascorbic acid) which confirmed by DPPH free radical scavenging assay. It must be recommended for using in the medical centers as a healthy food, food additives, and cosmetics. Their spent must be used as food wastes is a wealth to be recycled because it has the high flavoring and bioactive metabolites than the fruiting bodies so recommended for many application.

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