

EVALUATION OF BIO-CHEMICAL MARKERS IN SELECTED EDIBLE WOOD ROTTING MACROFUNGII

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ABSTRACT

The search for natural products that has therapeutic properties with little or no side effects has been the main subject around the world. Different plants have been and are being explored for their medicinal properties. However, higher fungi remain a vast and a large source of new pharmaceutical products yet to be explored. The phyto-constituents in the ethanolic extract of Six edible wood rotten fungi were investigated by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The results showed that a total of 25 phyto-constituents were revealed in *Ganoderma lucidum* and *Pleurotus florida* while 17 compounds were revealed in *Pleurotus eryngii* and *Pleurotus pulmonarius*. *Pleurotus ostreatus* and *Pleurotus sajor-caju* recorded 31 and 30 phyto-constituents respectively. Some constituents were common to all the fungi while some were absent in some fungi. Generally, most of them occurred in trace amount while a few of the constituents occurred in large amount. Some bioactive compounds such as Methyl stearate, Hexadecanoic acid, methyl ester, (Z)-, Hexadecanoic acid, ethyl ester, 11,14-Octadecadienoic acid, methyl ester and 9-Octadecenoic acid methyl ester were common to all the fungi extract. Four of the fungi had cholesterol content which was however in trace amount of 1.37%, 1.22%, 1.16% and 0.68% for *G. lucidum*, *P. eryngii*, *P. ostreatus* and *P. pulmonarius* respectively. Of the compounds, 9-Octadecenoic acid, methyl ester, (E)- had the highest concentration of 35.08% in *P. pulmonarius*. This was closely followed by 11,14-Octadecadienoic acid methyl ester also in *P. pulmonarius*. Although, the concentration varied across the fungi extracts. The bio-active components in these fungi species could serve as a lead for development of novel natural products with therapeutic properties.

Keywords: Natural products; Wood rotters; Bioactive compounds; Fatty acids; Linoleic acids

INTRODUCTION

Wood rotten fungi most importantly edible mushrooms contain various secondary metabolites that contribute to various biological activities apart from being nutrient-rich

food source and non-toxic drugs that have physiological benefits (Mirfat *et. al.*, 2010). The benefits of wood rot fungus secondary metabolites have been reported by several researchers as anti-fungal (Triani *et. al.* 2017) and potentially as an anti-microbial herbal treatment for bacterial and fungal pathogens in humans (Oli *et. al.* 2020). They also have potential as a functional food and natural remedy against hyper-lipidemia and hepatoprotection (Zanga, 2020).

The beneficial effects as well chemical composition of medicinal mushrooms was reviewed by Cateni *et. al.* (2022). There are various known bioactive metabolites synthesized by fungi such as volatile oils, flavonoids, alkaloids, ascorbic and organic acids, fats, polysaccharides, tocopherols, glycosides, minerals, proteins, carotenoids, terpenoids, lectins, enzymes, phenolics, and folates (Venturella *et. al.*, 2021; Herawati *et. al.*, 2021). Yet, higher fungi remain a vast and a large source of new pharmaceutical products yet to be explored. The most well known secondary metabolites produced by fungi are β -lactam antibiotics which was first discovered in 1928 by Sir Alexander Fleming. Another fungal compounds are Statins which are known for their cholesterol lowering activity (Barrios-Gonzalez *et. al.*, 2010). Many indole alkaloids used in medicine originally from Apocynaceae plant species are also being synthesized by fungi (Hoffmann, 2013). Several important terpenes such as aristolochenes, carotenoids, gibberellins, indole-diterpenes and trichothecenes are synthesized by fungi (Keller *et. al.*, 2015) as well as a newly identified terpenoid; lucidone D (Liu *et. al.*, 2013). Sesquiterpenes are common fungal metabolites which are common among the Basidiomycetes. The two most recognized plant hormones; Strobilurins and gibberellins used in Agriculture most importantly Gibberellins are isolated from the species *Gibberella fujikuroi* (Kavanagh, 2011). Also, lovastatin which act as a potent inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is also produced by higher Basidiomycetes mainly *Pleurotus* species (Wasser, 2011).

Excessive intake of saturated fatty acids have various health implications. However, a number of naturally occurring fatty acids such as monounsaturated fatty acids and polyunsaturated fatty acids have positive effects on health (Adeoye-Isijola *et al.*, 2018). In fungi, the major fatty acids are palmitic and stearic acids and their unsaturated derivatives such as oleic, oleic, linoleic and linolenic, lauric acids, aldehydes, acetate, ethyl esters, amide or substituted amides. These compounds are known to have anti-oxidative, cancer inhibitive and cardio-protective properties (Adeoye-Isijola *et. al.*, 2018). In addition, they are known to have demonstrated anti-bacterial, anti-fungal as well as anti-viral activities against various pathogenic micro-organisms (Bergson *et. al.*, 2011). One of the wood rotters in this study is *Ganoderma lucidum*. Although, *G. lucidum* is considered more valuable for its pharmacological properties than nutritional value. However, its anti-tumor, immuno-modulatory, cardiovascular, respiratory, anti-hepatotoxic and pain relieving effects has been reported by Sharma *et al.* (2014). The extract from *G. lucidum* has been reported to have strong anti-bacterial activity against *Klebsiella pneumonia* (Quereshi *et. al.*, 2010) and *E.coli* (Sharma *et. al.*, 2014; Alves *et.*

al., 2012; Quereshi *et al.*, 2010) with ganoderic acid, triterpenes and polysaccharides being the major active compounds (Quereshi *et.al.*2010). Combination of *G. lucidum* extracts with chemotherapeutic agents (ampicillin, cefazolin, oxytetracycline and chloramphenicol) resulted in antagonism against *Bacillus subtilis* and *Klebsiella oxytoca* (Sheena *et. al.*, 2003; Alves *et. al.*, 2012;). Ozen *et. al.*, (2011) reported the sensitivity of *E. coli*, *Enterococcus aerogenes*, *Pseudomonas aeruginosa* and *K. pneumoniae* to several extracts (ethanolic, methanolic and xylene) of *Pleurotus sajor-caju*.

Although, the cultivation as well as the nutritional values of the wood rotten fungi in this study have been reported. Likewise, information on the anti-microbial activities of the extracts from these fungi are available in scientific literatures. However, few data exists on the bio-active compounds in the extracts of these fungi. Generally, a number of fungi produce a range of similar as well as different compounds. Therefore, understanding the bio-active components in these fungi species could serve as a lead for development of novel natural products with therapeutic properties.

MATERIALS AND METHOD

Materials

The fungi used in this study include *Pleurotus ostreatus*, *Pleurotus pulmonarius*, *Pleurotus florida*, *Pleurotus erynngii*, *Pleurotus sajor-caju* and *Ganoderma lucidum*. *Pleurotus ostreatus*, *Pleurotus pulmonarius*, *Pleurotus erynngii* samples were obtained from the Microbiology Department, University of Ilorin. *Ganoderma lucidum* and *Pleurotus sajor-caju* were obtained from by Plant Biology Department, University of Ilorin while *Pleurotus florida* was obtained from by the Pathology Department, Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria.

Preparation of Growth Medium and Subculturing

Potato Dextrose Agar (PDA) was prepared according to the manufacturer's instruction with 1% Streptomycin added to prevent bacterial growth. After preparation, the sterile medium was aseptically poured into sterile Petri-dishes. The petri dishes were allowed to solidify. Thereafter each of the fungi samples were inoculated onto the plates and were then incubated at of $25 \pm 2^\circ\text{C}$ in the dark. This was done to obtain mycelia not more than 7 days old.

Liquid State Fermentation

For mass production of the fungi mycelia, Potato Dextrose Broth (PDB) were prepared following manufacturer's instruction with 1% Streptomycin to prevent bacterial growth. The PDB were prepared in a 1Litre conical flask. Thereafter each of the fungi samples from the 7 day old stock was sub-cultured into the PDB. The flasks were then incubated for 20 days at an ambient temperature of $25 \pm 2^\circ\text{C}$ in the dark. After the expiration of the 20 days incubation period, the fungi mycelia were harvested, washed free of broth and then air dried to constant weight. The dried mycelia were then grounded to powder to aid

extraction. Each of the pulverized mycelia were then extracted with Ethanol under constant agitation for 48 hours. After extraction, each of the fungi extract was filtered and the residue re-extracted (2x) for complete exhaustion. The combined ethanolic extract was evaporated under reduced pressure using a rotary vacuum evaporator. The extract was stored at 4° C in a refrigerator until further analysis in a glass vial.

Phytochemical Analysis of the Fungi Extracts

The bio-active component analysis in each of the wood rotten fungi was done by GC-MS analysis of the ethanolic extracts of the fungi mycelia. Identification of the components was achieved by comparison of the acquired spectra with the existing NIST library. The sample was analyzed in Perkin Elmer-Clarus-600 instrument using software Turbomass 5.2 version. Capillary standard non-polar column (30 m x 0.25 mm, 0.25 mm film thickness) was used. The volume of injected specimen was 1 µL of ethanol extract, injector temp. 220°C with a split ratio of 25:1 Carrier gas Helium, Solvent Delay=3.00 min, source Temp=180°C, oven temperature program initial temp 60°C for 5 min, ramp 7°C/min to 300°C, hold 15 min, Scan: 50 to 600Da, ionization energy 70 eV, in the electronic ionization mode.

Identification of Compounds

The identification of compounds was done using computer matching of mass spectra with those of standards (NIST library). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Name, Molecular weight and structure of the components of the test materials were ascertained. The test was run in triplicate.

RESULTS

Mushroom samples of *P. ostreatus*, *P. pulmonarius*, *P. florida*, *P. eryngii*, *P. sajor-caju* and *G. lucidum* were analysed and quantified for the presence varieties of bio-chemicals. The bio-chemical composition, the amount (%) of the individual component obtained and chromatographic spectral data obtained are presented in Tables 1 to12 and depicted in chromatogram Figs 1-6.

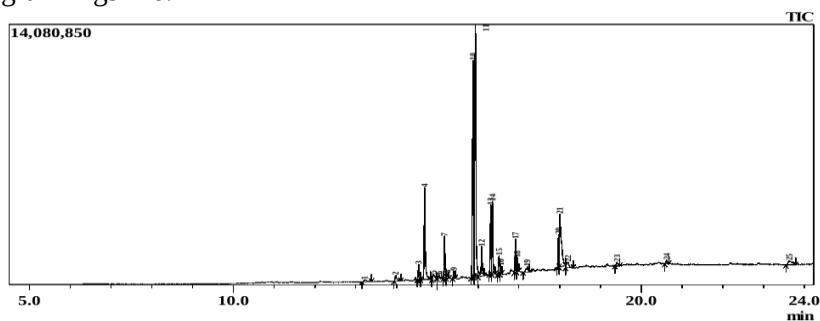


Figure 1: Chromatogram of the Ethanolic Extract of *G. lucidum*
Ganoderma lucidum

The GC-MS analysis of *G. lucidum* sample showed the presence of a total 25 bioactive compounds (Table 1). The time of detection of the compounds ranged from 13.216 min to 23.634 min. The bioactive compounds varied from 11 to 28 carbon atom compounds. The *G. lucidum* recorded five different compound nature. Fatty acid was the most dominant compound which was responsible for about 50% of the total bioactive compounds while oleic acid was 20%, palmitic acid, ophthalmic acid and linoleic acid represented 30% of the compounds.

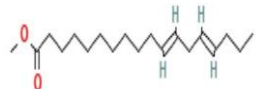
The major compounds identified were: 9-Octadecenoic acid, methyl ester, (E)- (25.18%); 11,14 Octadecadienoic acid, methyl ester (21.31%); Hexadecanoic acid, methyl ester 8.73% and 2,3-Dihydroxypropyl elaidate 8.38%; Ethyl Oleate (5.85%) and n-Propyl 9,12-octadecadienoate (5.52%). These 6 compounds accounted for 74.97% of the total bio-active components while the remaining ones occurred in trace amount.

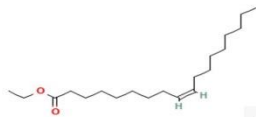
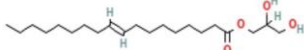
Table 1: Compounds Identified in the Ethanolic Extract of *G. lucidum*

Peak	R _{Time}	Name of compounds	Peak (%)	Area	Height (%)
1	13.216	Methyl tetradecanoate	0.36		0.17
2	13.935	Pentadecanoic acid, methyl ester	0.92		0.58
3	14.510	9-Hexadecenoic acid, methyl ester, (Z)-	1.48		1.47
4	14.690	Hexadecanoic acid, methyl ester	8.73		9.17
5	14.865	Dibutyl Phthalate	1.06		0.56
6	14.897	Ethyl 9-hexadecanoate	0.34		0.42
7	15.040	Hexadecanoic acid, ethyl ester	3.22		4.14
8	15.176	Methyl 9-heptadecenoate	0.52		0.51
9	15.402	Isopropyl palmitate	0.65		0.77
10	15.879	11,14Octadecadienoic acid, methyl ester	21.31		21.68
11	15.933	9-Octadecenoic acid, methyl ester, (E)-	25.18		25.23
12	16.087	Methyl stearate	3.20		2.96
13	16.308	n-Propyl 9,12-octadecadienoate	5.52		6.91
14	16.354	Ethyl Oleate	5.85		7.27
15	16.516	Octadecanoic acid, 17-methyl-, methyl ester	1.64		1.90

16	16.560	i-Propyl 11-octadecenoate	0.61	0.78
17	16.920	Tetracontanedioic acid, dimethyl ester	2.96	3.31
18	16.960	Ascorbyl palmitate	1.26	1.40
19	17.206	Methyl 9-eicosenoate	0.78	0.55
20	17.970	9,12 octadecadienoic acid (Z,Z)-, 2,3-dihydro	2.64	3.21
21	18.006	2,3-Dihydroxypropyl elaidate	8.38	5.27
22	18.203	5-.beta. -Card-20(22)-enolide, 3-.beta.	1.20	0.55
23	19.409	3-Oxatricyclo	0.51	0.41
24	20.628	2,2,4-Trimethyl-3-(3,8,12,16-tetranethyl heptanoate	0.32	0.36
25	23.634	Cholesterol	1.37	0.40

Table 2: Phyto-constituents Identified in *G. lucidum* and Reported Biological Activities

Name of compound	MF	MW	Compound nature	Structure	Biological activities
Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.45	Fatty acid		Antioxidant, insecticide, Hemolytic, Hypocholesteromic
11,14-Octadecadienoic acid, methyl ester	C ₁₉ H ₃₄ O ₂	294.5	Fatty acid		Absorption and distribution in human plasma and lipoprotein lipids
9-Octadecenoic acid, methyl ester, (E)-	C ₁₉ H ₃₆ O ₂	296.48	Fatty acid methyl ester		Raises VLDL And Lowers HDL Cholesterol
n-Propyl 9,12-octadecadienoate	C ₂₁ H ₃₈ O ₂	322.5	Linoleic acid		Antioxidant

Ethyl Oleate	$C_{20}H_{38}O_2$	310.5	Fatty acid ester		Additives, lubricant, plasticizer
2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$	356.5	Monoelaidin		Anti-inflammatory

Pleurotus eryngii

The Gas Chromatography Mass Spectrophotometry (GC-MS) analysis of *Pleurotus eryngii* extract revealed the presence of a total 17 bioactive compounds. Fatty acid was the most dominant compound nature present which represented 50% of bioactive compounds, palmitic acid present 10%, oleic acid and linoleic presented 10% while other compound nature like elaidic, lipid, nitrobenzene and isopropyl derivatives recorded 30%.

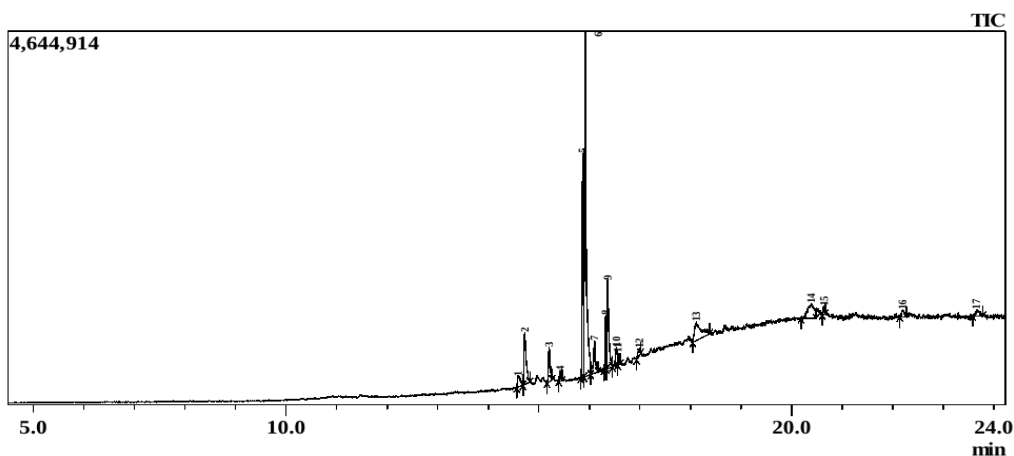


Figure 2: Chromatogram of the Ethanolic extract of *P. eryngii*

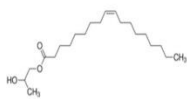
Out of the 17 compounds, 6 compounds occurred in relatively large amount while the remaining 11 compounds occurred in minute quantities. The major compounds identified were; 9-Octadecenoic acid, methyl ester, (E)- (35.08%); Methyl 10-trans, 12-cis-octadecadienoate (16.32%); Hexadecanoic acid, methyl ester (6.59%); (E)-9-octadecenoic acid, ethyl ester (9.50%); Propylene glycol monooleate (6.60%) and 14-Methyl-hexadecane-1,2-diol, isopropyliden (5.56%). The major compounds in *P. eryngii* represented 79.65% of the total phyto-constituents in *P. Eryngii* extract. Cholesterol accounted for 1.22% of the total constituents.

Table 3. Compounds Identified in the Ethanolic Extract of *P. erynqii*

Peak	R _{Time}	Name of compound	Peak (%)	Area	Height (%)
1	14.590	9-Hexadecanoic acid, methyl ester, (Z)-	1.90		1.20
2	14.723	Hexadecanoic acid, methyl ester	6.59		5.46
3	15.204	Hexadecanoic acid, ethyl ester	3.38		3.68
4	15.420	Isopropyl palmitate	0.89		1.06
5	15.869	Methyl 10-trans, 12-cis-octadecadienoate	16.32		23.70
6	15.916	9-Octadecenoic acid, methyl ester, (E)-	35.08		36.48
7	16.101	Methyl stearate	3.99		3.46
8	16.323	n-Propyl 9,12-octadecadienoate	3.99		5.76
9	16.365	(E)-9-octadecenoic acid, ethyl ester	9.50		9.37
10	16.537	Octadecanoic acid, 17-methy-, methyl ester	1.84		1.95
11	16.565	Octadec-9-enoic acid	0.66		1.29
12	16.989	1,3-cyclododecanedione, 2-octyl-	0.86		0.75
13	18.115	Propylene glycol monooleate	6.60		1.97
14	20.390	14-Methyl-hexadecane-1,2-diol, isopropyliden	5.56		1.59
15	20.645	4a,7,7,10a-Tetramethyl-dodecahydrobenzo[f]	0.56		0.84
16	22.199	Dehydroergosterol 3,5-dinitrobenzoate	0.95		0.74
17	23.656	Cholesterol	1.22		0.70

Table 4: Phyto-constituents Identified in *P. erynqii* and Reported Biological Activities

Names of compound	MF	MW	Compound nature	Structural	Biological activities
Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.45	Palmitic acid methyl ester		Antioxidant, Insecticide, Hemolytic, Hypocholesteromic

Methyl 10-trans, 12-cis-octadecadienoate	$C_{19}H_{34}O_2$	294.4	Conjugated Linoleic Acid Methyl Ester		Indicates its Activity
9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296.5	Fatty acid methyl ester		Raises VLDL And Lowers HDL Cholesterol
(E)-9-octadecenoic acid, ethyl ester	$C_{20}H_{38}O_2$	310.5	Elaidic acid		Antioxidant, anti-inflammatory activities
Propylene glycol monooleate	$C_{21}H_{40}O_3$	340.5	Oleic acid monoester		Antioxidant
14-Methyl-hexadecane-1,2-diol, isopropylidene	$C_{20}H_{40}O_2$	312.5	Isopropylidene derivatives		

Pleurotus florida

Twenty five (25) compounds were revealed in the ethanolic extract of *Pleurotus florida*. The chromatogram is shown in Figure 3. The compound name, area, retention time (RT), peak area (%) and Height (%) as well as molecular formula, molecular weight (MW), nature of compound, structures of compound and biological activities are presented in Table 5 and 6. Twenty five (25) compounds were revealed in the Extract of *P. florida*. 6 occurred in relatively large amount which included; Isopropyl palmitate (13.09%); Ethyl Oleate (16.72%); 9,12-Octadecadienoic acid, ethyl ester (10.16%); 9-Octadecenoic acid, methyl ester, (E)- (9.71%); Benzoic acid, 2,4-dimethoxy- (7.25%) and Octadecanoic acid, 17-Methyl, methyl ester (6.54%). These compounds accounted for 63.47% of the total phyto-constituents in *P. florida* ethanolic extract. No Cholesterol was revealed in the ethanolic extract of *P. florida*. The *P. florida* recorded nine compound nature of the bioactive compounds detected in the *P. florida* sample. The fatty acid was the most dominant compound nature while other include ophthalmic acid, palmitic acid, aromatic compounds, steroids, oleic acid, linoleic acid etc.

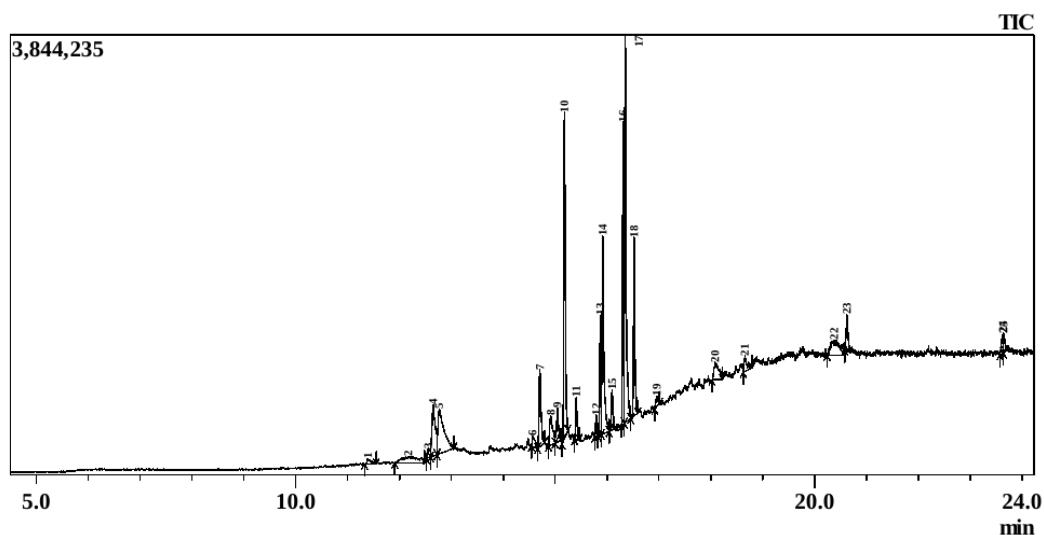


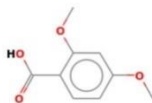
Figure 3: Chromatogram of the Extract *P. florida* Sample

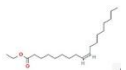
Table 5. Compounds Identified in the Ethanolic Extract of *P. florida*

Peak	R _{Time}	Name of compound	Peak (%)	Area	Height (%)
1	11.389	Phenol, 2,5-bis(1,1-dimethylethyl)-	0.50		0.24
2	11.166	Triethyl 4-phosphonobutaboate	2.86		0.31
3	12.555	3-methoxy-2-(1-methylpropyl)-5-(2-methylpropyl)	0.67		0.35
4	12.649	Benzo[b]tetrahydrofuran 3-one, 5,6-dihydroxyl	4.70		2.16
5	12.766	Benzoic acid, 2,4-dimethoxy-	7.25		2.22
6	14.565	9-Hexadecanoic acid, methyl ester, (Z)-	0.85		0.52
7	14.706	Hexadecanoic acid, methyl ester	4.02		3.83
8	14.919	Dibutyl phthalate	2.33		1.44
9	15.043	Ethyl 9-hexadecanoate	1.76		1.68
10	15.179	Hexadecanoic acid, ethyl ester	1.54		16.26
11	15.406	Isopropyl palmitate	13.09		2.10
12	15.790	Ethyl 9-hexadecanoate	0.73		1.07
13	15.871	11,14 Octadecadienoic acid, methyl ester	4.13		5.97

14	15.917	9-Octadecenoic acid, methyl ester, (E)-	9.71	9.86
15	16.096	Methyl stearate	1.54	2.02
16	16.309	9,12-Octadecadienoic acid, ethyl ester	10.16	15.05
17	16.353	Ethyl Oleate	16.72	19.32
18	16.525	Octadecanoic acid, 17-Methyl, methyl ester	6.54	8.89
19	16.960	Methyl 16-hydroxy-hexadecanoate	0.66	0.53
20	18.080	E,EZ-1,3,12-Nonadecatriene-5,14-diol	2.38	0.87
21	18.660	Bis(2-ethylhexyl) Phthalate	1.10	0.77
22	20.376	Hexadecanoic acid, 1,3-propanediyl ester	3.47	0.74
23	20.626	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetran)	1.73	1.82
24	20.635	17Alphaethynyl-17beta-hydroxy-6beta-methoxyl	0.96	1.02
25	20.645	p-Dimethylaminobenzaldehyde methylpyridine	6- 0.59	0.97

Table. 6: Phyto-constituents identified in *P. florida* and reported biological activities

Compound	MF	M W	Functional groups	Structure	Biological activities
Benzoic acid, 2,4-dimethoxy-	C ₉ H ₁₀ O ₄	182	Aromatic		Antimicrobial
Isopropyl palmitate	C ₁₉ H ₃₈ O ₂	298.5	Palmitic acid		Antioxidant, nematocides, pesticides
9-Octadecenoic acid, methyl ester, (E)-	C ₁₉ H ₃₆ O ₂	296.5	Fatty acid methyl ester		Raises VLDL And Lowers HDL Cholesterol
9,12-Octadecadienoic acid	C ₂₀ H ₃₆ O ₂	308.4	Linoleic acid		Antiinflammatory, Nematicide, Insectifuge,

noic acid, ethyl ester			ethyl ester		Antiacne,Hypocholesterolemic ,Cancer preventive,Hepatoprotective, Antihistaminic,Antiarthritic, Antieczemic,	
Ethyl Oleate	$C_{20}H_{38}O_2$	310.5	Fatty acid ester		Additives, plasticizer	lubricant,
Octadecanoic acid, 17-Methyl, methyl ester	$C_{20}H_{40}O_2$	312.5	Fatty acid methyl ester		Antimycotic	

Pleurotus ostreatus

P. ostreatus ethanolic extract revealed the presence of 31 bio-active compounds. Ten compound nature of the bioactive compounds were also detected. The fatty acid was the most dominant compound nature while other include ophthalmic acid, palmitic acid, benzoic acid, monoelaidin, lipid, oleic acid, linoleic acid, Docosahexadecanoic acid etc.

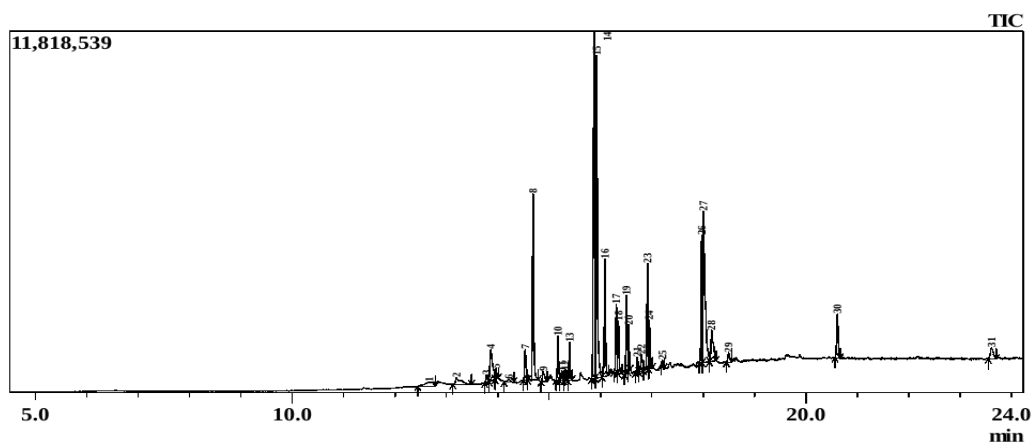


Figure 4: Chromatogram of the Extract *Pleurotus ostreatus*

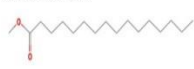
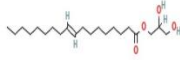
Five compounds occurred in relatively large amount. The compounds included; 11, 14-Octadecanoic acid, methyl ester (17.38%); 9-octadecenoic acid, methyl ester, (E)- (15.52%); 2,3-Dihydroxypropyl elaidate (10.63%); Hexadecanoic acid, methyl ester (8.08%) and n-Propyl 9,12-octadecadienoate (5.54%). Cholesterol accounted for just 1.16% of the constituents.

Table 7. Compounds identified in the ethanolic extract of *P. ostreatus*

Peak	R _{Time}	Name of compound	Peak (%)	Area	Height (%)
1	12.666	2-Methoxy-4-methylbenzoic acid	1.24		0.22
2	13.198	Methyl tetradecanoate	1.22		0.35
3	13.780	Anthracene, 1,2,3,4-tetrahydro-9-propyl-	0.56		0.37
4	13.866	Acetamide, N-(2,4-dimethoxyphenyl)-	2.85		1.57
5	13.965	Pentadecanoic acid, methyl ester	0.52		0.54
6	14.219	Phthalic acid, di (3-(2-methoxyethyl) heptyl) ester	0.19		0.14
7	14.537	9-Hexadecenoic acid, methyl ester, (Z)-	1.38		1.55
8	14.689	Hexadecanoic acid, methyl ester	8.08		9.35
9	14.887	Dibutyl phthalate	0.83		0.50
10	15.174	Hexadecanoic acid, ethyl ester	1.76		2.21
11	15.249	Palmitoleic acid	0.51		0.47
12	15.318	Methyl 9-heptadecenoate or 9-17:1	0.40		0.52
13	15.398	Isopropyl palmitate	1.53		1.91
14	15.877	11,14-Octadecanoic acid, methyl ester	17.38		17.44
15	15.925	9-octadecenoic acid, methyl ester, (E)-	15.52		16.19
16	16.085	Methyl stearate	4.82		5.90
17	16.307	9,12-octadecanoic acid, ethyl ester	2.77		3.45
18	16.352	Ethyl oleate	2.18		2.63
19	16.510	Iso-propyl 9-cis., 11-.trans.-octadecanoate	3.22		3.87
20	16.551	i-Propyl 11-octadecenoate	1.54		2.31
21	16.716	Isopropyl stearate	0.62		0.78
22	16.801	Butyl 9-hexadecenoate	1.00		0.83
23	16.918	Tetracontanedioic acid, dimethyl ester	4.90		5.32
24	16.950	Ascorbyl palmitate	2.06		2.43
25	17.208	Methyl 9-eicosinoate	0.23		0.32
26	17.970	n-Propyl 9,12-octadecadienoate	5.54		6.39
27	18.006	2,3-Dihydroxypropyl elaidate	10.63		7.61

28	18.163	Octadecanoic acid, 3-hydroxypropyl ester	2.46	1.56
29	18.495	Methyl 4,7,10,13,16,19-docosaheptaenoate	0.70	0.50
30	20.616	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetran)	2.17	2.20
31	23.615	Cholesterol	1.16	0.58

Table 8: Phyto-constituents identified in *P. ostreatus* and reported biological activities

Name of compound	MF	MW	Compound	Structural	Biological activities
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	Palmitic acid methyl ester		Antioxidant, hemolytic, insecticide, hypocholesteromic
11,14-Octadecanoic acid, methyl ester	$C_{20}H_{34}O_2$	294.5	Fatty acid		Absorption and distribution in human plasma and lipoprotein lipids
9-octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296.4	Fatty acid methyl ester		Raises VLDL And Lowers HDL Cholesterol
n-Propyl 9,12-octadecadienoate	$C_{21}H_{38}O_2$	322.5	Linoleic acid		
2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$	356.5	Monoelaidin		Anti-inflammatory

Pleurotus pulmonarius

Similarly as *P. Eryngii*, 17 compounds were revealed in ethanolic extract of *P. pulmonarius* (Table 9). The chromatogram is shown in Figure 5 while the biological activities of the identified major compounds are presented in Table 10. The *P. pulmonarius* recorded seven compound nature of the bioactive compounds detected in the *P. pulmonarius* sample. The fatty acid was the most dominant compound nature while

other includes phthalic acid, palmitic acid, oleic acid, linoleic acid, elaidic acid, isopropyl derivatives, Petroselinic, carboxamide. 11,14-Octadecanoic acid, methyl ester had the largest concentration of 28.24% followed by 9-octadecenoic acid, methyl ester, (E)- (24.81%). Others includes; n-Propyl 9,12-octadecadienoate (5.23%); Butyl 9,12-octadecadienoate (12.01%) and Hexadecanoic acid, methyl ester (8.80%).

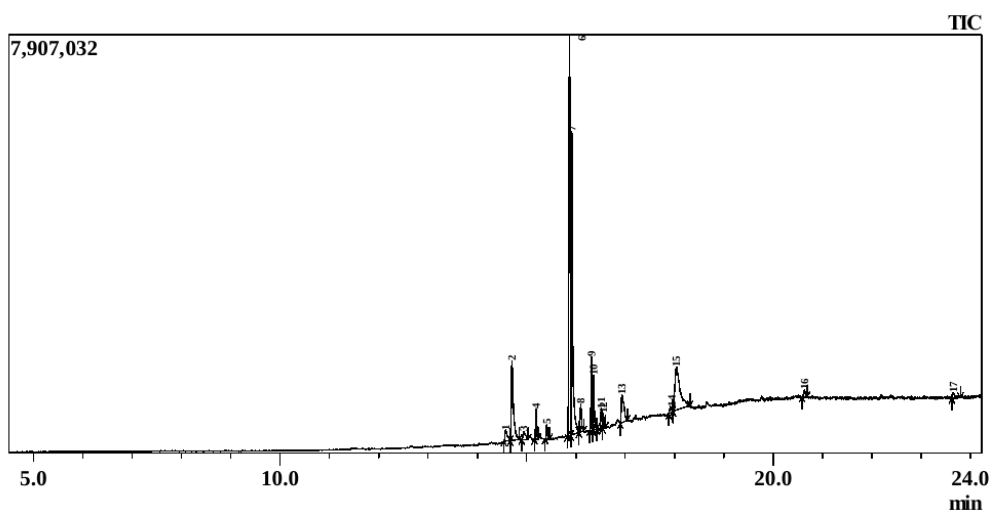


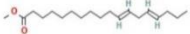
Figure 5: Chromatogram of the Ethanolic Extract of *P. pulmonarius*.

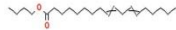
Table 9. Compounds identified in the ethanolic extract of *P. pulmonarius*

Peak	Retention time	Name of compound	Peak (%)	Area (%)	Height (%)
1	14.580	9-Hexadecenoic acid, methyl ester, (Z)-	1.46		0.96
2	14.705	Hexadecanoic acid, methyl ester	8.80		7.06
3	14.945	Dibutyl phthalate	1.10		0.75
4	15.194	Hexadecanoic acid, ethyl ester	2.16		2.68
5	15.410	i-Propyl 14-methyl-pentadecanoate	1.07		1.29
6	15.869	11,14-Octadecanoic acid, methyl ester	28.24		35.56
7	15.916	9-octadecenoic acid, methyl ester, (E)-	24.81		26.91
8	16.096	Methyl stearate	2.20		2.47
9	16.316	n-Propyl 9,12-octadecadienoate	5.23		6.58

10	16.359	(E)-9-octadecenoic acid, ethyl ester	3.99	4.84
11	16.519	Iso-propyl 9-cis 11-trans-octadecenoate	1.37	1.75
12	16.556	6-Octadecenoate	0.96	1.32
13	16.933	Tetracontanedioic acid, dimethyl ester	4.16	2.48
14	17.945	5.beta., 7.beta.H, 10.alpha. -Eudesm-11-en-1.alp	0.72	0.44
15	18.038	Butyl 9,12-octadecadienoate	12.01	3.80
16	20.639	2,2,4-Trimethyl-3-(3-8,12,16-tetramethyl-hept	0.70	0.63
17	23.660	Cyclopentanecarboxamide, ethenyl-2-(3-pen	3- 0.90	0.46

Table 10. Phyto-constituents identified in *P. pulmonarius* and reported biological activities

Name of compound	MMF	MW	Compound nature	Structure	Biological activities
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4	Fatty acid		Antioxidant, hemolytic, hypocholesteromic
11,14-Octadecanoic acid, methyl ester	$C_{19}H_{34}O_2$	294.5	Fatty acid		Absorption and distribution in human plasma and lipoprotein lipids
9-octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296.4	Fatty acid methyl ester		Raises VLDL And Lowers HDL Cholesterol
n-Propyl 9,12-octadecadienoate	$C_{21}H_{38}O_2$	322.5	Linoleic acid		No known activity yet

Butyl octadecadienoat e	9,12- 2	C ₂₂ H ₄₀ O 2	336. 6	Linoleic acid		No activity yet	known
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Pleurotus sajor-caju

Thirty (30) bioactive compounds were revealed in the extract of *P. Sajor-caju* comprising 8 compound nature. The fatty acid was the most dominant compound nature while other include phthalic acid, palmitic acid, oleic acid, linoleic acid, elaidic acid, lipid, aromatic and polyunsaturated hydrocarbon. The major compounds were 11,14-Octadecanoic acid, methyl ester which had the highest concentration of (22.07%) followed by 9-Octadecenoic acid, methyl ester, (E)- (21.88%). The remaining 2 were Hexadecanoic acid, methyl ester (11.70%) and Methyl stearate (6.39%).

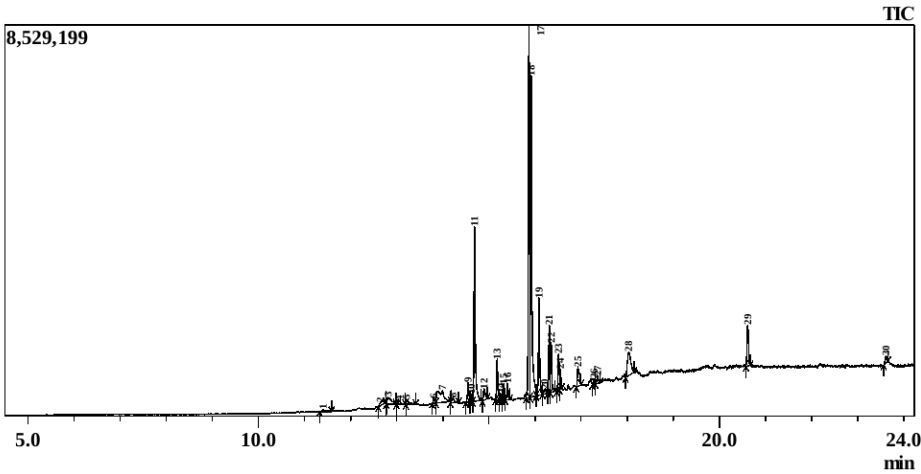


Figure 6: Chromatogram of the Extract *Pleurotus sajor-caju*

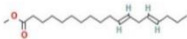
Table 11. Compounds identified in the Ethanolic Extract of *P. sajor-caju*

Area	Retention time	Name of compound	Peak Area (%)	Area (%)	Height (%)
1	11.405	Phenol, 2,4-bis(1,1-dimethylethyl)-	0.56		0.17
2	12.645	Benzo[b] tetrahydrofuran 3-one, 5,6-dihydroxyl	0.74		0.24
3	12.813	3-Ethyl- 1,2-dihydro-6-methyl-5-nitro-2-oxopy	1.39		0.46
4	13.049	Bergamotol, Z-alpha -trans-	0.42		0.27
5	13.210	Dodecanoic acid, 10-methyl-, methyl ester	0.74		0.35
6	13.800	Isophthalic acid, ethyl propyl ester	0.46		0.31
7	13.990	Methyl 9-methyltetradecanoate	3.82		0.84

8	14.218	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl	0.55	0.31
9	14.550	9-Hexadecenoic acid, methyl ester, (Z)-	1.42	1.41
10	14.617	Methyl hexadec-9-enoate	0.52	0.5
11	14.690	Hexadecanoic acid, methyl ester	11.70	12.41
12	14.898	Dibutyl phthalate	1.12	0.80
13	15.178	Hexadecanoic acid, ethyl ester	2.45	2.9
14	15.249	Cis-10-heptadecenoic acid, methyl ester	0.46	0.4
15	15.319	Methyl 9-heptadecenoate or 9-17:1	0.96	1.12
16	15.404	Isopropyl palmitate	1.07	1.16
17	15.858	11,14-Octadecanoic acid, methyl ester	22.07	26.50
18	15.917	9-Octadecenoic acid, methyl ester, (E)-	21.88	22.84
19	16.085	Methyl stearate	6.39	6.39
20	16.205	6,9,12-Octadecatrienoic acid, methyl ester	0.61	0.31
21	16.309	Butyl 9-12-octadecadienoate	3.72	4.77
22	16.353	(E)-9-octadecenoic acid, ethyl ester	3.13	3.52
23	16.513	10-Undecenoic acid, ethyl ester	2.17	2.59
24	16.551	i-Propyl 11-octadecenoate	0.96	1.44
25	16.934	Hencosanedioic acid, dimethyl ester	2.00	1.38
26	17.282	Cholestan-26-oic acid, 3,7-12-tris(acetyloxy)-,	0.37	0.38
27	17.361	Methyl 18-methylnonadecanoate	0.60	0.50
28	18.031	Butyl 9,12-octadecadienoate	3.96	1.69
29	20.618	Squalene	3.08	2.90
30	23.617	Cholesterol	0.68	0.51

Table 12: Phyto-constituents identified in *P. sajor-caju* and reported biological activities

Name of compound	MF	MW	Compound's nature	Structure	Biological activities
Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.4	Palmitic acid methyl ester		Antioxidant, hemolytic, insecticide,

					hypcholesteromi c
11,14- Octadecanoic acid, methyl ester	$C_{19}H_{34}O_2$	294. 5	Fatty acid		Absorption and distribution in human plasma and lipoprotein lipids
9- Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296. 4	Fatty acid methyl ester		Raises VLDL and Lowers HDL Cholesterol
Methyl stearate	$C_{16}H_{32}O_2$	298. 5	Fatty acid		Anti-diarrheal, cytotoxic and Anti-proliferative activity

DISCUSSION

The phyto-constituents in the ethanolic extract of the Six edible wood rotten fungi showed that a total of 25 phyto-constituents were revealed in *G. lucidum* and *P. florida* while 17 compounds were revealed in *P. eryngii* and *P. pulmonarius*. *P. ostreatus* and *P. sajor-caju* recorded 31 and 30 phyto-constituents respectively. The major compound nature in the ethanolic extract of all the wood rotten fungi in the study are fatty acids and their methyl and ethyl esters. Others included palmitic acid, oleic acid and linoleic acid. No phthalic acid was found in *P. eryngii*. However, only *P. eryngii* had nitrobenzene compound. Isopropyl derivatives were present in *P. eryngii* and *P. pulmonarius*. Petroselenic and carboxamide compounds were present in *P. pulmonarius*. Benzoic, monoelaidic as well as docosahexadecanoic acids were present only in *P. ostreatus*. Elaidic acid was present in *P. pulmonarius*, *P. sajor-caju* and *P. eryngii*. Two of the fungi; *P. florida* and *P. sajor-caju* contained aromatic compounds. Lipids were revealed in *P. ostreatus*, *P. sajor-caju* and *P. eryngii* while only *P. sajor-caju* had poly unsaturated hydrocarbons. Only *P. eryngii* did not reveal phthalic acid. The phyto-constituent profiles were similar to those reported by Lata and Atri (2021) for *Lentinus sajor-caju*.

The chemical composition of the ethanolic extracts of the six fungi species showed some similarities in their profile. Some constituents were common to all the fungi extracts such as Methyl stearate, Hexadecanoic acid, methyl ester, (Z)-, Hexadecanoic acid, ethyl ester, 11,14-Octadecadienoic acid, methyl ester and 9-Octadecenoic acid methyl ester while some constituents were either present or absent in some fungi. Dibutyl phthalate was revealed in all the extracts except *P. eryngii*. Isopropyl palmitate was revealed in all except *P. pulmonarius*. 9-Hexadecenoic acid, methyl ester, (Z)- was revealed in all except *P. eryngii* and *P. florida*. n-propyl, 9,12-Octadecadienoate was revealed in 4

except *P. florida* and *P. Sajor-caju*. Some of the constituents occurred in large amount while most of them occurred in trace amount. Cholesterol was present in four of the fungi except *P. florida* and *P. Pulmonarius*. Although, the cholesterol was in trace amount. The cholesterol level were 1.37%, 1.22%, 1.16% and 0.68% for *G. lucidum*, *P. eryngii*, *P. ostreatus* and *P. pulmonarius* respectively. Of the bioactive compounds, 9-Octadecenoic acid, methyl ester, (E)- had the highest concentration of 35.08% in *P. pulmonarius*. This was closely followed by 11,14-Octadecadienoic acid methyl ester also in *P. pulmonarius*. Although, the concentration varied across the fungi extracts.

The bioactivity of the phyto-constituents have reported 9-Octadecenoic acid, methyl ester, (E) (an oleic acid compound as well an essential monounsaturated fatty acid) to be very important in human diet. It helps in reducing the low-density cholesterol and glycemic index (Rubio et al., 2009). It is cancer preventive, favour hypocholesterolemic, 5-Alpha reductase inhibitor, anti-androgenic, perfumery, insectifuge, anti-inflammatory, anemiagenic, dermatitigenic and choleric (Rajalakshmi and Mohan, 2016) as well as raises VLDL and lowers HDL Cholesterol. Oleic acid has also been reported to help modify the membrane structure and alters its biophysical properties in addition with lipids (Cassiano et al., 2016). It has also be found useful in pathogen control, treatment of prostate and breast cancer (Binukumar et al., 2005). 9, 12-octadecadienoic acid, methyl ester (a linoleic acid), had been reported to possess anti-inflammatory, nematocide, insectifuge, anti-acne, hypocholesterolemic, anti-cancer, hepatoprotective, anti-histaminic, anti-arthritis and anti-eczemic activities (Ayoola, et. al., 2020).

Sunita et al., (2017) reported that Hexadecanoic acid, methyl ester showed antioxidant, hypocholesterolemic and hemolytic properties. Hexadecanoic acid, methyl ester also showed insecticidal properties and Hexadecanoic acid, ethyl ester was anti-androgenic and 5-alpha reductase inhibitor (Sunita et al., 2017). Likewise, (Jegadeeswari et. al., 2012; Awonyemi et. al., 2020) reported the anti-inflammatory, hypocholesterolemic, cancer, preventive, hepatoprotective, nematocide, insectifuge, anti-histaminic, anticorona, antiarthritic of Hexadecanoic acid, methyl ester: n-Hexadecanoic acid was reported to possess antioxidant, hypocholesterolemic, nematocide, anti-androgenic, pesticides as well as hemolytic activities (Awonyemi et. al., 2020).

Octadecanoic acid, 17-methyl-, methyl ester possess anti-fungal, anti-tumor and anti-bacterial properties (Sunita et al., 2017; Awonyemi et. al., 2020). 9,12-Octadecadienoic acid (Z,Z) was reported to have anti-inflammatory, hypocholesterolemic, cancer preventive, insectifuge, antiarthritic, hepatoprotective, antiandrogenic, nematocide, antihistaminic, antieczemic (Rajeswari et. al., 2013; Awonyemi et al., 2020). Although, antifungal, anti-inflammatory, antioxidants and anti-bacterial properties of Oleic acid were reported by Sales-Campos et. al. (2013). Oleic acid have been found to have therapeutic importance in decreasing the concentration of triacylglycerols (TAG), LDL-cholesterol and increasing the concentration of HDL-cholesterol and regulating insulin sensitivity (Lata and Atri, 2021). Oleic acid was also found to influence the anti-

inflammatory response and reported to possess a protective role in carcinogenesis (Lata and Atri, 2021; Tvrzicka *et. al.*, 2011). Linoleic acid is known to act as a precursor of alcohol in fungi (1-octen-3-ol) and is one of the principal aromatic compounds in most fungi contributing to mushroom flavour (Lata and Atri, 2021). According to Cai *et. al.* (2005), Palmitic acid methyl ester inhibits phagocytosis and decreases cell viability and also decreases secretion of interleukin-10, TNF- α , nitric oxide, and prostaglandin E2. It may also be a lead compound of anticancer drugs (Tomaz *et. al.*, 2012).

The biological activities of other bioactive compounds were Anti-diarrheal, cytotoxic and anti-proliferative activity (Methyl stearate), topical skin lubrication and protection (squalene), hypocholesteromic (cholesterol), use in quantification of fatty acid in dairy products (Hencosanedioic acid dimethyl ester), vulcanization properties (Tetracontanedioic acid, dimethyl ester), were mainly food additives (Methyl tetradecanoate), plasticizer, teratogenic agent, a metabolites, an inhibitor (Dibutyl phthalate), anti-inflammatory, improving insulin activity (palmitoleic acid), moisturizer, anti-static agent (isopropyl palmitate), additives, lubricant, plasticizer (ethyl Oleate), anti-inflammatory, nematicide, insectifuge, anti-acne, hypocholesterolemic, cancer preventive, hepatoprotective, anti-histaminic, anti-arthritic (9,12-octadecanoic acid, ethyl ester among others), Dehydroergosterol 3, 5-dinitrobenzoate the bioactive principles was hypocholesteromic (Nitrobenzene), antioxidant, EC inhibitor, anti-mycotic, antiomycetes (Octadec-9-enoic acid Propylene glycol monooleate and 4a, 7, 7,10a-Tetramethyl-dodecahydro-benzo, antioxidant (Ethyl 9-hexadecenoate), Hepatoprotective, Anti-histaminic, (hexadecenoic acid, ethyl ester) and moisturizer and anti-static agent (Isopropyl palmitate), anti-fungal and anti-microbial activities (Pentadecanoic acid, methyl ester).

CONCLUSION

The study revealed that *P. ostreatus* and *P. sajor-caju* are very diverse in their chemical contents. Although, most of the phytochemicals occurred in trace amount. However, few of the constituents occurred in fairly large amount. Some of the phytoconstituents such as Methyl stearate, Hexadecanoic acid, methyl ester, (Z)-, Hexadecanoic acid, ethyl ester, 11,14-Octadecadienoic acid, methyl ester and 9-Octadecenoic acid methyl ester are common to all the fungi extract. These compounds could be considered as marked components in the ethanolic extract of these selected edible fungi.

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