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GC-MS Analysis of Bioactive Compounds in Some Wild-Edible Mushrooms from Calabar, Southern Nigeria

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ABSTRACT

Studies to exploit wild mushrooms as a source of biologically active compounds are gaining importance in the recent years. In that view, the present study was carried out to evaluate the bioactive compounds of six wild edible mushrooms: *Lentinus squarrosulus* Mont., *Auricularia auricular-judae* (Bull.) Wettst., *Mycetinis copelandii* (Desjardin) A.W. Wilson & Desjardin, *Baeospora myosura* (Fr.) Singer, *Pleurotus ostreatus* (Jacq. ex. fr) Kummer and *Volvariella volvacea* (Bull. ex. Fr.) Singer. The specimen was subjected to phytochemical screening using gas chromatography mass spectrometer (GCMS) with the view to identify the important volatile constituents. The dried sporocarp was subjected to methanol extraction using a Soxhlet extractor and rotatory evaporator. This led to the identification of 14, 26, 33, 5, 49 and 32 different compounds in *L. squarrosulus*, *A. auricular-judae*, *M. copelandii*, *B. myosura*, *P. ostreatus* and *V. volvacea* respectively. n-Hexadecanoic acid; 9,12-Octadecadienoic acid (Z,Z)- and 2(3H)-Furanone where the most frequently occurring compounds across the studied mushrooms. The identified compound reportedly shows diverse bioactive activities, including antiviral, anticancer, antimicrobial, antioxidant, hypocholesterolemic, anaphylactic, narcotic, neurostimulant, emollient, expectorant, laxative, pesticidal, insecticidal and insectifugal activities. This data may provide baseline information about the bioactive constituents of these species from Nigeria, in providing valuable compounds of substantial medicinal and agricultural importance.

Keywords: Anticancer, Antiviral, Bioactive compounds, GC-MS, Wild-edible mushrooms.

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I. Introduction

Mushroom includes the well-known over 14,000 species of epigeous macrofungi belonging to basidiomycetes and ascomycetes [1]. About 2166 species are known to be edible [2]. The importance of mushrooms as food material is due not only to their pleasant organoleptic properties, but also to the rich content of biologically active substance required for healthy human diet [3]. Mushrooms are known to be rich in protein, vitamins, unsaturated fatty acids, sterols, chitin, fibers and essential minerals but low in calories cholesterol [4]-[6].

In addition to food, there is an increasing interest in developing mushroom bioactive constituents as control agents of several diseases and to delay aging processes [7]-[9]. Friedman [10] reviewed mushroom polysaccharides which have shown therapeutic properties such as anti-obesity, anti-diabetes, anticancer and antibiotic properties. Also, mushrooms endowed with potent antimicrobial and antioxidant properties, among other important bioactivities, have been reported in several studies [7], [11]-[14]. In addition to polysaccharides, mushroom synthesizes and accumulates diverse classes of secondary metabolites such as terpenes, phenolics, alkaloids and steroids in defiance of biotic and abiotic stressors in their environment [15], [16].

Though studies on the potential of mushrooms as sources of potent bioactive compounds abounds, little information exists about the phytochemical constituents of wild edible mushrooms from Nigeria. Moreover, the existing information on beneficial mushroom compounds primarily focus on macromolecules such as polysaccharides, proteins and lipids, without due attention to the smaller, volatile compounds with putative health, agricultural and industrial benefits and detriments. The premium step in resolving this challenge is to screen and identify every wild mushroom species with putative bioactive compounds. Such information would helpful not only in the discovery of new therapeutic agent, but also as cradles for the discovery of noble economic materials like with other industrial applications.

In the last few years, gas chromatography mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species [17]. This study therefore aimed to explore the bioactive volatiles present in the methanolic extract of six wields edible mushrooms: *L.*

squarrosulus, *A. auricular-judae*, *M. copelandii*, *B. myosura*, *P. ostreatus* and *V. volvacea*, obtained from Calabar, Southern Nigeria by GC-MS technique. The output would be useful in guiding future researches on pharmaceutical, nutraceutical and agricultural application of the studied mushroom species.

II. Materials and Methods

A. Survey Area and Collection

The geographical location of Calabar is between 4˚54'00"N to 5˚04'00"N latitude and 8˚18'00"E to 8˚24'00"E longitudes, with an altitude ranging between 2 - 96m. The area is drained by the Calabar River and the Great Kwa River. The Temperatures vary from 25-28 °C and mean annual rainfall is about 4,528.08 mm. The vegetation is typical tropical rainforest, though heavily impacted upon by agriculture and urbanization. The fresh sporocarps of *L. squarrosulus*, *A. auricular-judae*, *M. copelandii*, *B. myosura*, *P. ostreatus* and *V. volvacea* were collected from decaying woods in University of Calabar Staff Quarters during the months of July 2018.

B. Morphological Identification of Macrofungi

The harvested mushrooms were brought to the laboratory and taxonomically studied and identified. Initial identification was done on the basis of macroscopic features according to the published descriptions and manuals. Morphological characters such as colour, size, texture shape and margin of fruit body, other features such as odour, stipe and stipe length, pileus length, gill attachment and spacing were considered as previously used by [18]. The nomenclature was based on the Index Fungorum and Mycobank.

C. Preparation of Mushroom Material

The harvested mushrooms were air-dried at room temperature (27 ºC) for ten days, grind into powder with the aid of an electronic blinder and packed in air-tight zip-lock bags and store under ambient condition of 27 ºC for onward transmission to the laboratory.

D. Solvent Extraction

Extraction and phytochemical screening were carried out in BGI laboratory Port Harcourt, Nigeria. Five (5) g of the mushroom material was packed into a thimble of a Soxhlet apparatus and extracted with 50 ml of methanol. The sample was refluxed three times and the extract transferred into the Rotary Evaporator and heated at 30-40 ºC to rid the sample of any trace of the solvent and the extract concentrated to 2 ml. This was transferred further into a Teflon screw-cap vial and cleaned up with 200 mm mesh silica gel and 3 g of anhydrous sodium sulfate in a well packed column to obtain a clean extract for GC-MS screening [19].

E. GC-MS Analysis

Methanolic extracts of whole mushroom samples was analyzed for the presence of different volatile and semi-volatiles compound by GC-MS technique. The analysis was performed at BGI Laboratory, Port Harcourt, Nigeria. The GC analysis of the extracts was performed using an Agilent 6890N gas chromatography equipped with an auto sampler connected to an Agilent Mass Spectrophotometric Detector was used. One (1) µl of the sample was injected in the pulsed spitless mode onto a 30 m × 0.25 mm id DB 5MS coated fused silica column with a film thickness of 0.15 micrometer. Helium gas was used as carrier gas and the column head pressure was maintained at 20 psi to give a constant rate of 1 ml/min. Other operating conditions were preset. The column temperature was initially held at 55 ºC for 0.4 minutes, increased to 200 ºC at a rate of 25 ºC/mins, then to 280 ºC at a rate of 8 ºC/mins and to final temperature of 300 ºC at a rate of 25 ºC/mins, held for 2 minutes. The identification time was based on retention time of the volatile and semi-volatile components in the column. The relative percentage of each extract constituent was expressed as percentage with peak area normalization [17].

The identification of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored chemical Library of National Institute for Standard and Technology (NIST) library version 2.4 as previously adopted by JeromeJeyakumar [20].

F. Determination of Bioactive Properties

The prediction of the bioactive properties of the identified compounds was based on Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/phytochem/search/list).

III. Result

Fig. 1-6 are the chromatograms of *L. squarrosulus*, *A. auricular-judae*, *M. copelandii*, *B. myosura*, *P. ostreatus* and *V. volvacea* samples respectively whereas the identified compounds and their retention time, molecular formula, molecular weight, peak area (%) and activities related with medicinal uses are given in Tables 1-6 also respectively. From the result, 14, 26, 33, 5, 49 and 32 different compounds were identified in *L. squarrosulus*, *A. auricular-judae*, *M. copelandii*, *B. myosura*, *P. ostreatus* and *V. volvacea* respectively. Hexadecanoic acid was the most reoccurring compound and was identified in four (*L. squarrosulus*, *V.volvacea*, *B. myosura* and *M. copelandii*) of the assayed mushrooms. This was followed by Linoelaidic acid and 9, 12- Octadecadienoic acid (Z, Z) – (conjugated linoleic acid) that were identified in three (*V. volvacea*, *B. myosura* and *M. copelandii*) of the mushroom samples. In terms of percentage composition, n-Hexadecanoic acid (33.56%), 9-Octadecynoic acid (17.44%) and 9,12-Octadecadien-1-ol, (Z,Z)- (9.91%) were the highest in *L. squarrosulu*, just as N-Desmethyltapentadol (8.38%), Benzamide, N-Desmethyltapentadol (7.68%) and N-(1,1 dimethylethyl)-2,4,6-trimethyl- (7.60%) were highest in *A. auricular-judae*. Others in this category were Hexacosane (13.12%), Heptacosane (7.00%) and Tetracosane (6.50%) as the highest in *M. copelandii*, 3-Tetradecene, (E)- (61.74%), 9,12-Octadecadienoic acid (Z,Z)- (18.76%) and n-Hexadecanoic acid (11.66%) in *B. myosura*, Undecanal, 2-methyl- (11.96%), cis-7-Dodecen-1-ol (7.49%) and Octanoic acid (6.28%) in *P. ostreatus* and 9,12-Octadecadienoic acid (Z,Z)- (16.75%), 9,17-Octadecadienal,

(Z)- (14.37%) and 3-Tetradecene, (E)- (9.00%) as the highest in *V. volvacea*. Reported bioactive properties exist for most of the identified compounds (Tables 1 – 6). Generally, among the identified compounds most were reported to have antimicrobial and antioxidant properties. Other bioactive properties reported for the identified mushrooms include antiviral, Antitumor, Anti-inflammatory, cancer-preventive, nematicidal, pesticidal, insecticidal, insectifungal, herbicidal, emollient, laxative; lubricant, expectorant, anaphylactic, narcotic, neurostimulating and hallucinogenic properties. The identified compounds with no reported bioactivity included Propanedinitrile, methylene-; Aminoacetonitrile; 1,6-Heptadiene; 2-Methylenebicyclo[2.1.1]hexane; Furan among others. Across the studied species, *A. auricular-judae*, *P. ostreatus* and *V. volvacea* recorded two compounds each with antiviral properties.

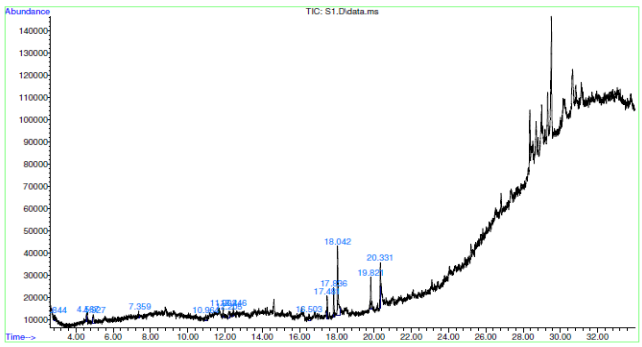

Fig. 1. GC-MS chromatogram of methanolic extract of *L. squarrosulus.*

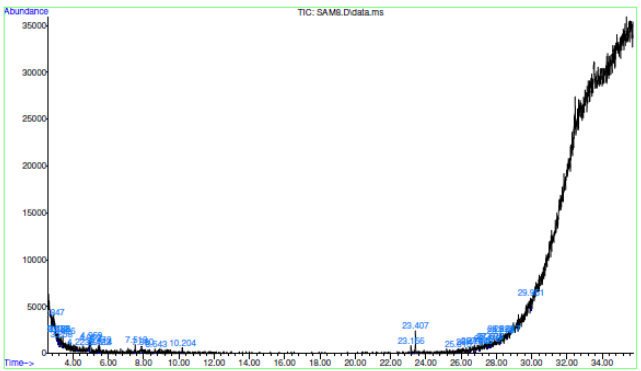

Fig. 2. GC-MS chromatogram of methanolic extract of *A. auricular-judae.*

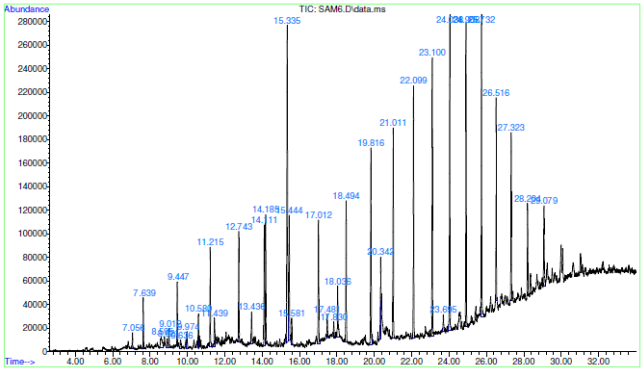

Fig. 3. GC-MS chromatogram of methanolic extract of *Mycetinis copelandii*.

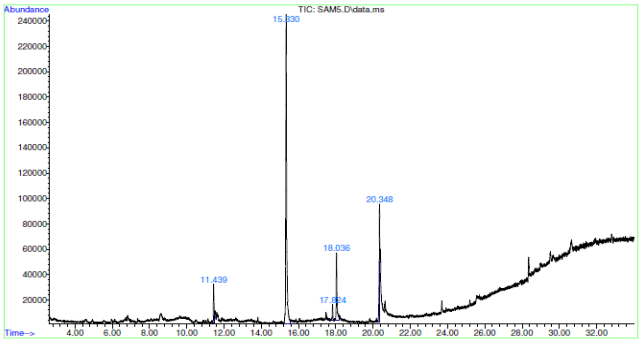

Fig. 4. GC-MS chromatogram of methanolic extract of *Baeospora myosura*.

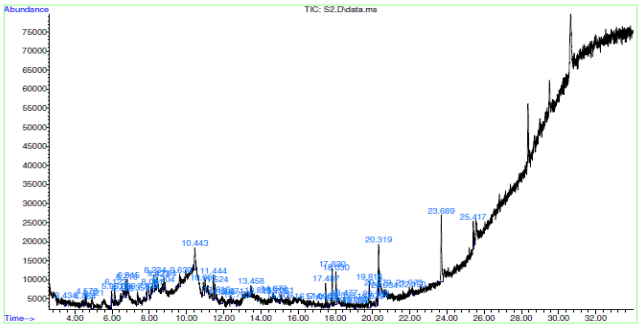

Fig. 5. GC-MS chromatogram of methanolic extract of *P. ostreatus.*

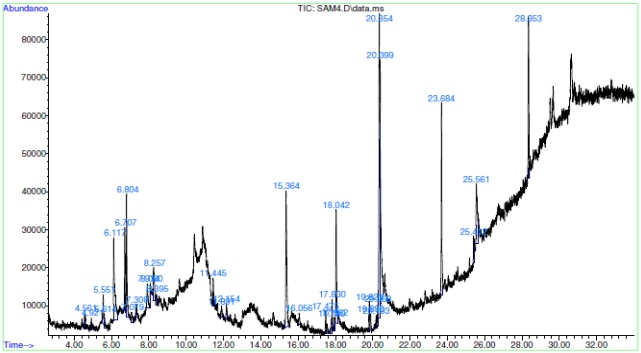

Fig. 6. GC-MS chromatogram of methanolic extract of *Volvariella volvacea.*

IV. Discussion

The mushrooms fruiting body include mixtures of bioactive compounds with putative health and industrial benefits and detriments. Mushrooms endowed with potent antimicrobial and antioxidant properties, among other important bioactivities, have been reported in several studies [14]. However, there are very few studies on mushroom bioactive compounds in Nigeria. Here, six wild edible mushrooms that are commonly available in Calabar, Southern Nigeria was selected, and their volatile organic compound evaluated using GCMS. The result revealed the presence of different compounds, with diverse bioactive properties. n-Hexadecanoic acid; 9,12-Octadecadienoic acid (Z,Z)- and 2(3H)-Furanone occurred most frequently among the studied mushroom, depicting the potential of mushrooms as sources of these compounds [21]. its, e.g., "A·m^2."

TABLE 1: BIOACTIVE COMPOUNDS IDENTIFIED IN THE METHANOLIC EXTRACT OF *LENTINUS SQUARROSULUS*

SN	RT (min.)	Compound	Molecular formula	Molecular weight g/mol	Peak Area %	Activity*
1	2.844	4-Dimethylamino-3,5-dinitrobenzoic acid	C_8H_{10}	106.16	2.12	Antioxidant, Increase Aromatic Amino Acid decarboxylase activity
2	4.567	Bicyclo[2.1.1]hex-2-ene, 2-ethenyl	$C_9H_9N_3O_6$	255.18	2.10	Energizer, Hexokinase stimulator
3	4.927	3-Methyl-4-nitro-5-(1-pyrazolyl)pyrazole	$C_6H_6N_4O_2$	166.14	3.55	Antioxidant
4	7.359	4-Phenylsemicarbazide	$C_7H_9N_3O$	151.17	1.60	No activity reported
5	10.964	4-pentyloxy-1H-pyridin-2-one	$C_{10}H_{15}NO_2$	181.23	1.60	Antiviral, Hallucinogenic, Helicicide, Hematopoietic, Hepatoirritant, Herbicide, Antioxidant, Hyperthermic
6	11.902	2-Propanone, 1-(1-methyl-4-nitro-1H-imidazol-5-yl)-	$C_7H_9N_3O_3$	183.16	2.42	Antiviral, Hallucinogenic, Helicicide, Hemangglutinator, Hematopoietic, Hepatoirritant, Herbicide, Antioxidant, Hyperthermic
7	12.205	2,2-Dianilino-1 phenylethanone	$C_{20}H_{18}N_2O$	302.4	3.15	No activity reported
8	12.446	1,2 Diphenoxyethane-	$C_{14}H_{14}O_2$	214.26	2.08	No activity reported
9	16.503	DL-Gabaculine	$C_7H_9NO_2$	139.15	3.08	No activity reported
10	17.481	Undecanoic acid, 10-methyl-, methyl ester	$C_{13}H_{26}O_2$	214.34	8.37	Antioxidant, Increase Aromatic Amino acid decarboxylase activity
11	17.836	Benzenepropanoic acid, 3,5-bis(1,1 -dimethylethyl)-4-hydroxy-, methyl ester	$C_{25}H_{21}NO_6$	431.4	8.98	Antioxidant, increase aromatic amino acid decarboxylase activity
12	8.042	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	33.56	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic
13	19.821	9,12-Octadecadien-1-ol, (Z,Z)-	$C_{18}H_{34}O$	266.5	9.91	Oligosaccharide Provider, Increase Zinc Bioavailibility
14	20.331	9-Octadecynoic acid	$C_{18}H_{32}O_2$	280.4	17.44	Antioxidant

* Source: Dr. Dukes Phytochemical and Ethnobotanical Databases

TABLE 2: BIOACTIVE COMPOUNDS IDENTIFIED IN THE METHANOLIC EXTRACT OF *AURICULARIA AURICULAR-JUDAE*

SN	RT (min.)	Compound	Molecular formula	Molecular Weight g/mol	Peak Area %	Activity*
1	2.847	2-Nonenoic acid	$C_9H_{16}O_2$	156.22	4.79	Antioxidant
2	3.073	Propanedinitrile, methylene-	$C_4H_2N_2$	78.07	3.67	No activity reported
3	3.132	Aminoacetonitrile	NH_2CH_2CN	56.07	2.68	No activity reported
4	3.166	1,6-Heptadiene	C_7H_{12}	96.17	3.81	No activity reported
5	3.258	2(3H)-Furanone	$C_4H_4O_2$	84.07	2.91	Antiviral, Hallucinogenic, Helicicide, Antioxidant, Hematopoietic, Hepatoirritant, Herbicide, Hyperthermic
6	3.426	2-Methylenebicyclo[2.1.1]hexane	C_7H_{10}	94.15	2.74	No activity reported
7	4.223	Acetyl cyanide	C_3H_3NO	69.06	2.17	Acetylcholinergic, Antioxidants
8	4.902	Bicyclo[1.1.0]butane	C_4H_6	50.09	5.83	No activity reported
9	4.969	Ethylbenzene	C_8H_{10}	106.16	6.55	No activity reported
10	5.422	1,3-Butadiene	C_4H_6	54.09	3.16	No activity reported
11	5.473	Propiolonitrile	C_3HN	51.05	3.03	No activity reported
12	5.514	Acetamide, 2-fluoro-	C_2H_4FNO	77.06	2.25	No activity reported
13	7.519	Propiolamide	C_3H_3NO	69.06	2.20	No activity reported
14	7.880	1-Penten-3-yne	C_5H_6	66.1	5.29	No activity reported
15	8.643	Furan	C_4H_4O	68.07	2.28	No activity reported
16	10.204	Methane, isocyanato-	C_2H_3NO	57.05	2.73	No activity reported
17	23.156	1-Propanol, 2-methyl-	$C_4H_{10}O$	74.12	3.08	Antioxidant
18	23.407	Benzamide, N-(1,1 dimethylethyl)-2,4,6-trimethyl-	$C_{11}H_{15}NO$	177.24	7.60	Antioxidant, Anaphylactic, Antitumor, Narcotic, Natriuretic, Nematicide, Neurostimulant
19	25.815	6-Nitro-8-methoxy-2H-chromene	$C_{10}H_9NO_4$	207.18	2.39	Antiviral, Hallucinogenic, Helicicide, Antioxidant, Hematopoietic, Hepatoirritant, Herbicide, Hyperthermic
20	26.478	Cyclohexane-1,3-dione, 2-allylaminomethylene-5,5-dimethyl-	$C_{12}H_{17}NO_2$	207.27	3.22	No activity reported
21	26.746	N-Desmethyltapentadol	$C_{13}H_{21}NO$	207.31	3.97	Antitumor, Anaphylactic, Neurostimulant, antioxidant, Nematicide
22	27.040	N-Desmethyltapentadol	$C_{13}H_{21}NO$	207.31	2.26	
23	27.476	N-Desmethyltapentadol	$C_{13}H_{21}NO$	207.31	2.15	
24	27.635	1,3,5-Triazine, 2-chloro-4,6-bis(methylthio)-	$C_5H_6ClN_3S_2$	207.7	2.45	No activity reported
25	27.677	1,3,5-Triazine, 2-chloro-4,6-bis(methylthio)-	$C_5H_6ClN_3S_2$	207.7	2.37	
26	28.214	Pyridine, 1,2,3,6-tetrahydro-1-methyl-4-[4-chlorophenyl]-	$C_{12}H14ClN$	207.7	2.28	Antioxidant
27	28.239	4-Allyl-5-furan-2-yl-2,4-dihydro-1,2,4]triazole-3-thione	$C_9H_9N_3OS$	207.25	4.21	No activity reported
28	28.617	3-METHYL-N-(5-METHYL-4, 5-DIHYDRO-1, 3-THIAZOL-2-YL) PYRIDIN-2-AMINE	$C_{10}H_{13}N_3S$	207.3	2.29	Antitumor , Anaphylactic, Neurostimulant, antioxidant, Nematicide
29	29.951	(5-ISOPROPYL-2-METHYLPHENOXY)TRIMETHYLSILANE	$C_{13}H_{22}OSi$	222.4	5.65	No activity reported

* Source: Dr. Dukes Phytochemical and Ethnobotanical Databases.

TABLE 3: BIOACTIVE COMPOUNDS IDENTIFIED IN THE METHANOLIC EXTRACT OF *MYCETINIS COPELANDII*

SN	RT (min.)	Compound	Molecular formula	Molecular Weight g/mol	Peak Area %	Activity*
1	7.056	Octane, 2,3,7-trimethyl-	$C_{11}H_{24}$	156.31	0.35	No activity reported
2	7.639	Tridecane	$C_{13}H_{28}$	184.36	0.99	No activity reported
3	8.595	Silacyclopentane, 1,1-dichloro	$C_4H_8Cl_2Si$	155.11	0.81	No activity reported
4	8.921	6-Nitroundec-5-ene	$C_{11}H_{21}NO_2$	199.29	0.37	Energizer
5	9.012	Dodecane, 2,6,10-trimethyl-	$C_{15}H_{32}$	212.41	0.46	No activity reported
6	9.447	Tridecane, 6-methyl-	$C_{14}H_{30}$	198.39	1.44	Antioxidant
7	9.636	Naphthalene, 1,3-dimethyl-	$C_{12}H_{12}$	156.22	0.34	No activity reported
8	9.974	Naphthalene, 2,3-dimethyl	$C_{12}H_{12}$	156.22	0.55	
9	10.580	Undecane	$C_{11}H_{24}$	156.31	0.86	Antimicrobial, carcinogenic
10	11.215	Hexadecane	$C_{16}H_{34}$	226.44	2.14	No activity reported
11	11.439	Silane, [(1,1-dimethyl-2-propenyl) oxy]dimethyl-	$C_7H_{16}OSi$	144.29	0.90	No activity reported
12	12.743	Pentadecane, 7-methyl-	$C_{16}H_{34}$	226.44	2.14	Antioxidant
13	13.436	10-Methylnonadecane	$C_{20}H_{42}$	282.5	0.85	No activity reported
14	14.111	Heptadecane	$C_{17}H_{36}$	240.5	2.44	No activity reported
15	14.185	Pentadecane, 2,6,10-trimethyl-	$C_{18}H_{38}$	254.5	2.93	No activity reported
16	15.335	3-Tetradecene (E)-	$C_{14}H_{28}$	196.37	10.90	Antitumor, cancer-preventive, antioxidant, Ectoparasiticide, Embryocide, Emollient, Energizer, Erythrocytogenic, Enterostimulant, Expectorant, Euphorigenic, Fertility-Enhancing
17	15.444	Heptadecane, 8-methyl-	$C_{18}H_{38}$	254.49	3.02	Antioxidant
18	15.581	Octacosane	$C_{28}H_{58}$	394.8	0.78	No activity reported
19	17.012	Nonadecane	$C_{19}H_{40}$	268.5	3.12	No activity reported
20	17.481	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	0.57	Anti-inflammatory, Hypocholesterolemic, Cancer Preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antioxidant, Acidulant
21	17.830	OXALIC ACID, 2-ETHYLHEXYL ISOHEXYL ESTER	$C_{16}H_{30}O_4$	286.41	0.39	Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antioxidant, Acidulant
22	18.036	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	1.60	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic
23	18.494	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	282.55	3.58	No activity reported
24	19.816	Heneicosane	$C_{21}H_{44}$	296.6	4.98	No activity reported
25	20.342	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	2.69	Anti-inflammatory, Hypocholesterolemic, Cancer Preventive, Insectifuge, Antiarthritic, Antieczemic Hepatoprotective, Antiandrogenic, Nematicide, Antihistaminic
26	21.011	Docosane	$C_{22}H_{46}$	310.6	5.07	No activity reported
27	22.099	Hentriacontane	$C_{31}H_{64}$	436.8	5.87	No activity reported
28	23.100	Tetracosane	$C_{24}H_{50}$	338.7	6.50	No activity reported
29	23.695	1-Octadecanesulphonyl chloride	$C_{18}H_{37}ClO_2S$	353.0	0.47	No activity reported
30	24.038	Heptacosane	$C_{27}H_{56}$	380.7	7.00	No activity reported
31	24.908	Hexacosane	$C_{26}H_{54}$	366.7	6.62	No activity reported
32	25.732	Hexacosane	$C_{26}H_{54}$	366.7	6.50	
33	26.516	Nonadecane, 9-methyl-	$C_{20}H_{42}$	282.5	4.22	Antioxidant
34	27.323	10-Methylnonadecane	$C_{20}H_{42}$	282.5	3.71	No activity reported
35	28.204	Eicosane	$C_{20}H_{42}$	282.5	2.29	No activity reported
36	29.079	Eicosane	$C_{20}H_{42}$	282.5	2.26	

* Source: Dr. Dukes Phytochemical and Ethnobotanical Databases.

TABLE 4: BIOACTIVE COMPOUNDS IDENTIFIED IN THE METHANOLIC EXTRACT OF *BAEOSPORA MYOSURA*

SN	RT (min.)	Compound	Molecular formula	Molecular Weight g/mol	Peak Area %	Activity*
1	11.439	2, 2-DIMETHYL-1-DIMETHYL (ISOPROPYL) SILYLOXYPROPANE	$C_{10}H_{24}OSi$	188.38	5.86	No activity reported
2	15.330	3-Tetradecene (E)-	$C_{14}H_{28}$	196.37	61.74	Antitumor, cancer-preventive, antioxidant, Ectoparasiticide, Embryocide, Emollient, Energizer, Erythrocytogenic, Enterostimulant, Expectorant, Euphorigenic, Fertility-Enhancing
3	17.824	HEXYL 2-(4-ETHYLANILINO)-2-OXOACETATE	$C_{16}H_{23}NO_3$	277.36	1.99	No activity reported
4	18.036	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	11.66	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic
5	20.348	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	18.75	Anti-inflammatory, Hypocholesterolemic, Cancer Preventive, Insectifuge, Antiarthritic, Antieczemic Hepatoprotective, Antiandrogenic, Nematicide, Antihistaminic

* Source: Dr. Dukes Phytochemical and Ethnobotanical Databases.

TABLE 5: BIOACTIVE COMPOUNDS IDENTIFIED IN THE METHANOLIC EXTRACT OF *PLEUROTUS OSTREATUS*

SN	RT (min.)	Compound	Molecular formula	Molecular Weight g/mol	Peak Area %	Activity*
1	3.434	Oxirane, 2,2'-(1,4-butanediyl)bis-	$C_8H_{14}O_2$	142.2	0.62	No activity reported
2	4.464	2-Chloro-2-methylhexane	$C_7H_{15}Cl$	134.65	0.49	No activity reported
3	4.578	Ephedrine	$C_{10}H_{15}NO$	165.24	1.31	No activity reported
4	4.921	1,3,5,7-Cyclooctatetraene	C_8H_8	104.15	2.24	No activity reported
5	5.951	Butyl 2-(2-(2-methoxyethoxy)ethoxy)acetate	$C_{11}H_{22}O_5$	234.29	2.37	No activity reported
6	6.123	Carbamic acid, methyl-, ethyl ester	$C_4H_9NO_2$	103.12	2.36	Antioxidant
7	6.478	N-Acrylonitrylaziridine	$C_5H_6N_2$	94.11	1.16	Anaphylactic, Antitumor, Antioxidant, Nematicide, Neurostimulant
8	6.598	4-Cyanocyclohexene	C_7H_9NO	123.15	0.70	No activity reported
9	6.718	Cycloheptane	C_7H_{14}	98.19	1.78	No activity reported
10	6.815	Cycloheptane	C_7H_{14}	98.19	1.48	
11	7.359	Nonane, 4-ethyl-5-methyl-	$C_{12}H_{26}$	170.33	1.66	Antioxidant
12	7.874	Allyl methallyl ether	$C_7H_{12}O$	112.17	1.43	No activity reported
13	8.091	2-Nonanone	$C_9H_{18}O$	142.24	1.17	No activity reported
14	8.234	Cyclopentanecarboxylic acid, 2-amino-, trans-	$C_6H_{11}NO_2$	129.16	3.21	Antioxidant
15	8.423	Ethoxy(methoxy)methylsilane	$C_4H_{12}O_2Si$	120.22	0.56	No activity reported
16	8.704	2-Pentyn-1-ol	C_5H_8O	84.12	1.12	Provide Oligosaccharides
17	8.784	2-Chloro-2-methylhexane	$C_7H_{15}Cl$	134.65	1.55	No activity reported
18	9.630	1-Guanidino-2-propanol	$C_4H_{11}N_3O$	117.1	1.87	No activity reported
19	10.443	1-Isopropoxy-2,2,3-trimethylaziridine (sin)	C_8H_{17}	143.23	4.06	No activity reported
20	10.901	4-Acetylpyrimidine	$C_6H_6N_2O$	122.13	2.50	No activity reported
21	11.444	L-Alanine, 3-[(aminocarbonyl)amino]-	$C_4H_9N_3O_3$	147.13	3.88	Antioxidant, cancer preventive, Antitumor, Larvicide, laxative, leptospiricide, Leucocytogenic, Lubricant
22	11.524	Ethyl(dimethyl)isopropoxysilane	$C_7H_{18}OSi$	146.3	1.77	No activity reported
23	11.616	Ethoxy(methoxy)methylsilane	$C_4H_{12}O_2Si$	120.22	0.59	No activity reported
24	11.896	2-Methylenecyclohexanol	$C_7H_{12}O$	112.17	1.33	No activity reported
25	12.371	Hydrazine, 1,2-bis(1-methylethyl)-	$C_{18}H_{24}N_2$	268.4	0.87	Antidote
26	12.703	Cyclobutanone, 2-methyl-2-oxiranyl	$C_7H_{10}O_2$	126.15	0.68	Antioxidant
27	13.458	1,5,7-Octatrien-3-ol, 2,6-dimethyl	$C_{10}H_{16}O$	152.23	1.61	Provide Oligosaccharides
28	13.825	Furan, 2,5-dihydro-3-methyl-	C_5H_8O	84.12	0.61	Antioxidant
29	14.677	Furan, 2,5-dihydro-3-methyl-	C_5H_8O	84.12	0.77	
30	14.775	Chloroacetic acid, 4-methylpentyl ester	$C_8H_{15}ClO_2$	178.65	0.50	Antioxidant
31	15.009	2-Pentene, 3-methyl-, (Z)-	C_6H_{12}	84.16	0.55	Antioxidant
32	15.061	2H-Pyran, 3,4-dihydro-	C_5H_8O	84.12	0.60	Antiviral, Hallucinogenic, Antioxidant, Helicicide, Herbicide, Hirudicide, Hypercholesterolemic, Hyperglycemic,
33	16.514	2(3H)-Furanone	$C_4H_4O_2$	84.07	1.25	Antiviral, Hallucinogenic, Antioxidant, Helicicide, Hepatostimulant, Herbicide, Hirudicide, Hypercholesterolemic, Hyperglycemic
34	17.098	Cyclobutanone, 2-methyl-`2-oxiranyl	$C_7H_{10}O_2$	126.15	0.72	Antioxidant
35	17.487	Nonanoic acid, methyl ester	$C_{10}H_{20}O_2$	172.26	3.63	Antioxidant
36	17.595	1,3-Cyclopentanediol, cis-	$C_5H_{10}O_2$	102.13	0.50	No activity reported
37	17.830	Oxalic acid, monoamide, N-(4-ethyl phenyl)-, isohexyl ester	$C_{16}H_{23}NO_3$	277.36	4.32	Antioxidant, Anaphylactic, antitumor, Narcotic, Nematicide, Neurostimulant
38	18.030	Octanoic acid	$C_8H_{16}O_2$	144.21	6.28	Antioxidant
39	18.356	Cyclobutanone, 2-methyl-2-oxiranyl	$C_7H_{10}O_2$	126.15	0.59	Antioxidant
40	18.477	2,4,6,8-Tetramethyl-1-undecene	$C_{15}H_{30}$	210.4	1.40	No activity reported
41	19.032	Cyclopentanone	C_5H_8O	84.12	0.49	No activity reported
42	19.095	3-Hexene, (Z)-	C_6H_{12}	84.16	0.57	Increase Zinc Bioavailability
43	19.816	8-Dodecenol	$C_{12}H_{24}O$	184.32	3.91	No activity reported
44	19.879	2,4,6,8-Tetramethyl-1-undecene	$C_{15}H_{30}$	210.4	0.66	No activity reported
45	20.182	2,4,6,8-Tetramethyl-1-undecene	$C_{15}H_{30}$	210.4	1.32	
46	20.279	1-Dodecyne	$C_{12}H_{22}$	166.3	2.09	No activity reported
47	20.319	cis-7-Dodecen-1-ol	$C_{12}H_{24}O$	182.34	7.49	Provide Oligosaccharides
48	20.634	1,12-Tridecadiene	C13H24	180.33	0.78	No activity reported
49	21.973	1-Methoxy-3-hydroxymethyloctane	$C_{10}H_{22}O_2$	174.28	0.83	No activity reported
50	22.150	Pentadecafluorooctanoic acid, dodec-2-en-1-yl ester	$C_{20}H_{23}F_{15}O_2$	580.4	0.56	Antioxidant, Encephalopathic, Endocrinactive, Energizer, Fertility-Enhancing
51	23.689	Undecanal, 2-methyl-	$C_{12}H_{24}O$	184.32	11.96	Antioxidant
52	25.417	2-Pentene, 2-chloro-, (E)-	C_5H_9Cl	104.58	3.26	Anticancer, Anaphylactic, Antitumor, Ectoparasiticide, Edemagenic, Embryocide, Emetic, Emmenagogue, Endoanesthetic, antioxidant, Expectorant

* Source: Dr. Dukes Phytochemical and Ethnobotanical Databases.

TABLE 6: BIOACTIVE COMPOUNDS IDENTIFIED IN THE METHANOLIC EXTRACT OF *VOLVARIELLA VOLVACEA*

SN	RT (min.)	Compound	Molecular formula	Molecular Weight g/mol	Peak Area %	Activity*
1	4.561	Pentanoic acid, 4-methyl-	$C_6H_{12}O_2$	116.16	0.72	Antioxidant
2	4.921	1,3-Propanediamine, N,N-dimethyl-	$C_5H_{14}N_2$	102.18	0.52	Anaphylactic, Antitumor, antioxidant, Neurostimulant, Narcotic, Nematicide
3	5.551	Piperidine, 1-methyl-	$C_6H_{14}ClN$	135.63	1.13	Antioxidant
4	5.614	Acetic acid, nonyl ester	$C_{11}H_{22}O_2$	186.29	0.85	Antioxidant
5	6.117	Allyl(ethoxy)dimethylsilane	$C_7H_{16}OSi$	1442.29	6.38	No activity reported
6	6.707	1-Butanamine, 2-methyl-N-(2-methyl butylidene)-	$C_{10}H_{21}N$	155.28	2.57	Anaphylactic, Antitumor, antioxidant, Neurostimulant, Narcotic, Nematicide
7	6.804	1-Butanamine, 3-methyl-N-(3-methyl butylidene)-	$C_{10}H_{21}N$	155.28	4.94	
8	7.079	*N*,1-dimethylpiperidin-3-amine	$C_7H_{16}N_2$	128.22	0.70	Anaphylactic, Antitumor, antioxidant, Neurostimulant, Narcotic, Nematicide
9	7.308	2(5H)-Oxepinone, 6,7-dihydro-	$C_6H_8O_2$	112.13	0.58	Antiviral, Anaphylactic, Helicicide, Hepatostimulant, Herbicide, Hirudicide, Humectant, Hydrocholerectic, Hypocholesterolemic, Hypotensive,
10	7.914	2, 4, 6-TRIPHENYL-1, 3, 5-TRIAZINE	$C_{21}H_{15}N_3$	309.4	1.65	No activity reported
11	8.080	Pentanedioic acid, ethyl methyl ester	$C_8H_{14}O_4$	174.19	0.66	Antioxidant
12	8.257	L-Azetidine-2-carboxylic acid	$C_4H_7NO_2$	101.1	2.47	Anticancer, antioxidant, antitumor, lactafuge, larvicide, Laxative, Lepidopterocide, Lubricant
13	8.395	Thiophene, tetrahydro-3-methyl-	C5H10S	102.20	0.51	Antioxidant
14	11.445	Allyl(isoproxy)dimethilsane	$C_8H_{18}OSi$	158.11	1.14	No activity reported
15	11.891	Heptanoic acid, propyl ester	$C_{10}H_{20}O_2$	172.26	0.50	Antioxidant
16	12.154	(+)-Diethyl L-tartrate	$C_8H_{14}O_6$	206.19	0.55	Anticancer, antioxidant, Antidote, antitumor, lactafuge, larvicide, Laxative, Lepidopterocide, Lubricant, Leucocytogenic
17	15.364	3-Tetradecene, (E)-	$C_{14}H_{28}$	196.37	9.00	Anticancer, antioxidant, Antidote, antitumor, lactafuge, larvicide, Laxative, emollient, Expectorant, Enterostimulant, Ectoparasiticide
18	16.056	Octane, 4-chloro-	$C_8H_{17}Cl$	148.67	0.58	No activity reported
19	17.475	Nonanoic acid, methyl ester	$C_{10}H_{20}O_2$	172.26	1.00	Antioxidant
20	17.790	4-Decene, 3-methyl-, (E)-	$C_{11}H_{22}$	154.29	0.63	Anticancer, antioxidant, Antidote, antitumor, lactafuge, larvicide, Laxative, Energizer, Emollient, Expectorant, Enterostimulant, Ectoparasiticide, Emmenagogue
21	17.830	TERT-BUTYLDIMETHYLSILYL 2-(2, 4-DICHLOROPHENOXY) ACETATE	$C_{14}H_{20}Cl_2O_3Si$	335.3	1.19	No activity reported
22	17.962	4-Methyl-2-oxopentanenitrile	C_6H_9NO	111.14	0.66	Antioxidant
23	18.042	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	5.83	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic
24	19.821	1,13-Tetradecadiene	$C_{14}H_{26}$	194.36	1.26	No activity reported
25	19.890	1-Dodecene	$C_{12}H_{24}$	168.32	0.66	No activity reported
26	20.193	Cyclobutanone, 2-methyl-2-oxiranyl	$C_7H_{10}O_2$	126.15	0.53	Antioxidant
27	20.268	Undec-10-ynoic acid	$C_{11}H_{18}O_2$	182.26	0.74	Antioxidant
28	20.354	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	16.75	Anti-inflammatory, Hypocholesterolemic, Cancer Preventive, Insectifuge, Antiarthritic, Antieczemic, Nematicide, Antihistaminic
29	20.399	9,17-Octadecadienal, (Z)-	$C_{18}H_{32}O$	264.4	14.37	Antimicrobial
30	23.684	Noxiptiline	$C_{19}H_{22}N_2O$	294.4	7.92	Antidepressant, Cytotoxic, Anticancer, Antimicrobial, Antiviral, Antiprotozoal, Insecticidal
31	25.412	trans-4-Aminocyclohexanol, trimethylsilyl ether	$C_6H_{13}NO$	115.17	0.75	Antioxidant
32	25.561	5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-thione	$C_{11}H_{13}NOS$	207.29	4.54	Antioxidant
33	28.353	(2R,3R,4aR,5S,8aS)-2-Hydroxy-4a,5-dimethyl-3-(prop-1-en-2-yl)octahydronaphthalen-1(2H)-one	$C_{15}H_{24}O_2$	236.35	7.71	Antiviral, Stimulant, Insectifuge, avetifuge, Raticide, Hallucinogenic, Helicicde, Hemopoietic, Herbicide, Hydrocholerectic, Scabicide, Scolicide, Schizonticide, Sedative, Sequestrant, slugicide, Spermicide, Surfactant, Suppurative,

* Source: Dr. Dukes Phytochemical and Ethnobotanical Databases.

The identified compounds in the present study are known to be biologically active, and protect the mushrooms from microbes, insects, and other herbivores attack [22], [23]. This suggests that the studied mushrooms have pharmaceutical, agricultural and confectionery potentials [24], [25]. The mushroom compounds identified in this study are said to play diverse pharmaceutical and nutritive roles including as antiviral, anticancer, antimicrobial, antioxidant, hypocholesterolemic, anaphylactic, narcotic, neurostimulant, emollient, expectorant, laxative, and other prophylactic agents [26].

Several compounds including n-Hexadecanoic acid; Undecanoic acid, 10-methyl-, methyl ester; 9-Octadecynoic acid, 2-Nonenoic acid; 2(3H)-Furanone and Pyridine, 1,2,3,6-tetrahydro-1-methyl-4-[4-chlorophenyl], detected in this study reacts with free radicals and neutralize them, thus

helping stop or limit damages caused by those reactive species [3], [7], [27]. Hence, the studied mushrooms can be used for minimizing or preventing oxidation in food products, retarding the formation of toxic oxidation products, maintaining nutritional quality, and prolonging the shelf life of foods and pharmaceuticals.

Similarly, compounds detected in *L. squarrosulus, A. auricular-judae, P. ostreatus and V. volvacea* has pedigree of antiviral activities against Human Immune Deficiency Virus (HIV) virus while *A. auricular-judae, M. copelandii, B. myosura, P. ostreatus* and *V. volvacea* synthesizes compounds with anticancer, cancer preventive, antitumor and anti-inflammatory activities. This suggests the potential of these mushrooms in foiling infectious diseases caused by viruses [28]-[31], as well as an immunomodulators in cancer immunotherapy and anti-hyperplasia and hypertrophic cell growth [32]-[34]. Similarly, the presences of Undecane; Noxiptiline and 9,17-Octadecadienal, (Z) with reported antimicrobial activities in *M. copelandii* and *V. volvacea*, reveals the potential of this species as a source of probiotic compounds in the management of infectious disease [25], [35], [36]. Also, the pesticidal, insecticidal and insectifugal activities of the compounds detected in *M. copelandii, B. myosura* and *V. volvacea* as well as herbicidal, nematicidal, larvicide detected in this aligned with the potential these mushrooms have in agriculture [37]. Studies had showed that mushroom-based agrochemicals are not toxic to humans, pollinators, fish, birds, or any other non-targeted animal as against synthetic chemical [38].

Although a number of synthetic agents have been isolated or developed to improve health, nutrition and control agricultural pest effectively, global environmental and health complications arising from the use of these substances is still an increasing human problem. Therefore, novel bioactive agents from biological sources are continuously sought. Extracts of *L. squarrosulus, A. auricular-judae, M. copelandii, B. myosura, P. ostreatus* and *V. volvacea* used in the present study lead to the identification of various compounds known to possesses antiviral, anticancer, antimicrobial, antioxidant, hypocholesterolemic, anaphylactic, narcotic, neurostimulant, emollient, expectorant, laxative, pesticidal, insecticidal and insectifugal activities, and could be considered as potential sources of natural bioactive substances to improve health and agricultural output.

V. Conclusion

The present study was able to characterize the phytochemical fingerprint of six wild edible mushroom species using GCMS, thus enriching and providing additional information on mushroom bioactive compounds in Nigeria. The study also showed the bioactive potentials of the *L. squarrosulus, A. auricular-judae, M. copelandii, B. myosura, P. ostreatus* and *V.volvacea* collected in Calabar, Southern Nigeria, thus giving credence to the therapeutic and agricultural uses of the mushrooms in medicine and food security. Findings from this study are encouraging; however, previous study has reported considerable differences in the contents of chemical compounds produced at different stages of mushroom growth and different substrates, implying that bioactive studies need to take into account the phenology and growth conditions of the mushroom material. Also, clinical and field studies are needed to establish the usefulness of the isolated compounds of these mushrooms in the treatment or prevention of many human diseases as well as pest control.

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