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GC-MS ANALYSIS OF BIOACTIVE PHYTOCHEMICALS PRESENT IN METHANOL EXTRACT OF *PLEUROTUS OSTREATUS* (JACK EX. FR.) P. KUMM: EVIDENCE FOR ITS MEDICINAL DIVERSITY

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ABSTRACT

The research study was aimed to determine the chemical constituents present in the fungus *Pleurotus ostreatus* (Jack ex. Fr.) P. Kumm. methanol extract using Shimadzu P 2010 Ultra Gas Chromatography-Mass Spectrometry. GC-MS analysis of the methanol extract of *P. ostreatus* revealed the existence of thirty (30) different chemical constituents based from the GC-MS chromatogram. The major chemical compounds detected were at peaks 3, 1, 21, 12 and15 with the following retention times of 2.643, 2.057, 6.717, 4.439 and 4.865 in terms of minutes. The identified major compounds were glycerin (23.36%), formamide (10.62%), 2-nonanol (9.92%), cyclobutanone, 2-methyl-4-hydroxy-(9.67%) and 1,1,2-trimethyl-3,8,9-trioxa-bicyclo[4] (5.62%)respectively. The presence of these major components in *P. ostreatus* and in all of the other 25 minor constituents shown in the chromatogram indicates their medicinal potentials which may enhance health and wellness in humans for the preservation of human life

essential for the restoration of human dignity as they can be formulated into useful and quality drugs to alleviate pains and sufferings in men.

KEYWORDS: GC-MS analysis, bioactive compounds, *Pleurotus ostreatus*, methanol extract

INTRODUCTION

Pleurotus ostreatus (Jack ex. Fr.) P. Kumm[1] commonly known as oyster mushroom is from the fungi kingdom and is noted to be an edible variety. It was first cultivated in Germany as a subsistence measure during World War I.[2] The oyster mushroom is one of the more commonly sought wild mushrooms though it can also be cultivated on straw and other media. It has the bittersweet aroma of benzaldehyde (which is also characteristic of bitter almonds).[3] The species genus *Pleurotus* are widespread in hardwood forests around the world. Since the beginning of their commercial cultivation, their popularity has mainly increased because of their ease of cultivation, and nutritional value. Traditional medicine attributed medicinal values to *Pleurotus* species. Scientific evidence supports their importance as producers of substances with antibiotic, antiviral, anticarcinogenic, anti-inflammatory and hypocholesterolemic activities.[4] The samples used in this study was the cultivated variety which was grown and cultivated at the University of Nigeria Nsukka.*P. ostreatus* is among the varieties of natural products. Natural products are extremely an important source of medicinal agents. Although there are some new approaches to drug discovery, such as combinatorial chemistry and computer-based molecular modeling design, none of them can replace the importance of natural products in drug discovery and development.[5] Many non-natural, synthetic drugs cause severe side effects that were not acceptable except as treatments of last resort for terminal diseases such as cancer and that the metabolites discovered in medicinal plants may avoid the side effect of synthetic drugs.[6] It has been shown that in *vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful medicinal properties for further chemical and pharmacological investigations.[7] Since there is no noted GC-MS report on the phytoconstituents of the methanol extract of *P. ostreatus* in Nigeria, it was chosen as the technique to detect the chemical constituents present in the subject of this study, hence, the aim of this study was to determine the organic compounds present in the crude methanol extract of *P. ostreatus* with the aid of GC-MS technique, whereby the results may provide support and insight for its use in traditional medicine. The phytochemicals

present in *P. ostreatus* obtained from the chromatogram indicates its medicinal diversity and posits its further potential pharmacological uses leading to its medicinal diversity.

MATERIALS AND METHODS

Sample collection and authentication

All materials under study should be properly authenticated, as much time and money can be wasted on the examination of a material of doubtful origin.[8] The fully matured fungi *Pleurotus ostreatus* (Jack ex. Fr.) P. Kumm (wild variety oyster mushrooms) were collected in March 2016 from the University of Nigeria Nsukka (UNN). The material was authenticated and identified by Dr. (Mrs.) Adedokun, a mycologist-taxonomist of UNN. A voucher specimen has been deposited at Madonna University Herbarium, Elele, Rivers State, Nigeria under the collection number Mu/PHGSY/16/007.

Samples preparation and extraction

The choice of extraction procedure depends on the nature of the plant material or sample and the components to be isolated.[9] The fungi *Pleurotus ostreatus* was washed with water, drained and cut into small pieces, drying was done at room temperature, and the dried leaves were powdered and sieved using Sieve Mesh 60. Then, further oven-dried at a controlled temperature of 45^0C. One hundred and fifty grams of the fungi powder was extracted using 500 mL methanol for 72 hours by cold maceration process with agitation. To collect the stock plant extract, the solution was first filtered using a muslin cloth, then finally in a vacuum filtration set-up using Whatman No. 1 filter paper as a filtering medium. The extract was then allowed to concentrate in a rotary evaporator kept at not more than 60^0C. The collected extract of about 20 milliliters is oven-dried at 45^0C producing a semi-liquid but not totally dried extract. The extract used for testing should approximate as closely as possible to that obtained by the traditional process used.[10]

Preparation of the extract for GC-MS analysis

The methanol extract of the fungi *P. ostreatus* was analyzed using Shimadzu P2010 Ultra Gas Chromatography – Mass Spectrometry (GC-MS) for the identification of phytochemicals present. A solvent blank analysis was first conducted using 1 µl of methanol. Then 1µl of the reconstituted methanol extract solution was employed for GC-MS analysis using direct liquid injection.

Analysis

GC-MS analysis was carried out on a GC system comprising a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) instrument; Shimadzu GCMS-QP2010 Ultra, employing the following conditions: Rtx-5ms fused silica capillary column (30x0.25mm IDxIEM df, composed of 100% dimethyl polysiloxane), operating in electron impact mode of 70 eV; helium (99.999%) as carrier gas at a constant flow of 1mL/minute and a sample injection volume of 1 µl which was employed (split ratio of 10:1) injector temperature 250^0C, ion source temperature 280^0C. The oven temperature was programmed from 110^0C (isothermal for 2 minutes), with an increase of 10^0C/minute to 200^0C, then. 5^0C/minute to 280^0C, ending with a 9 minute isothermal at 280^0C. Mass spectra were taken at 70eV and scan interval of 0.5 seconds and fragments from 40 to 550 Da. Total run time was 30 minutes.

Identification of phytochemicals in *P. ostreatus*

The compounds were then identified from the GC-MS peaks, using library data of the corresponding compounds. GC-MS was analyzed using electron impact ionization at 70eV and data was evaluated using a total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library using National Institute of Standards and Technology (NIST) Search. The relative % amount of each component was calculated by comparing its average peak area to the total areas. Measurement of peak areas and data processing were carried out by GC-MS Post Run Solution Software.[11]

RESULTS AND DISCUSSIONS

Free radicals play a crucial role in the development of tissue damage in pathological events. The extraction method presented was simple, rapid and inexpensive, with reduced solvent consumption. The GC-MS method used for the analysis of the obtained extracts can be an interesting tool for testing a number of some active principles in herbs used in cosmetics, pharmaceutical and food products.[12][13] The studies on the active principles in the matured fungi *Pleurotus ostreatus* methanol extract by GC-MS analysis revealed the presence of 30 components. The phytochemicals with their peak number, retention time, peak area, and concentration (peak area %) were presented in Table-1 (Sample information). The GC-MS chromatogram of the 30 compounds detected were shown in Figure-1a (0-6.0 minutes) and Figure-1b (6.0-10 minutes). The five (5) major chemical compounds detected were at peaks 3, 1, 21, 12 and15 with the following retention times of 2.643, 2.057, 6.717, 4.439 and 4.865

in terms of minutes. The identified major compounds were glycerin (23.36%), formamide (10.62%), 2-nonano (9.92%), cyclobutanone,2-methyl-4-hydroxy-(9.67%) and 1,1,2-trimethyl-3,8,9-trioxa-bicyclo[4] (5.62%) The presence of these major components in *P. ostreatus* and all 25 other minor constituents shown in the chromatogram indicates their medicinal potentials.

Niacin (2.43%) is a vitamin B3 and is used to treat and prevent a lack of natural niacin in the body, and to lower cholesterol and triglycerides (types of fat) in the body and to lower the risk of heart attack in people with high cholesterol who already had a heart attack, also sometimes used to treat coronary artery disease (atherosclerosis).[14]

Glycerin (23.36%) is used to treat eye disorders that are caused by increased intraocular pressure, such as glaucoma. Intravenous preparations of glycerin may be used to treat excessive intracranial pressure. It draws fluid from tissues in the body into the bloodstream and also acts as a diuretic by preventing water re-absorption in the kidneys. These actions dehydrate the tissues while reducing blood volume, thereby diminishing intracranial pressure. Glycerin is a primary constituent of nitroglycerin which is used as a treatment for angina, a painful condition caused by constriction of the blood vessels in the heart. Glycerin works as a softening agent and lubricant in cases of constipation. Glycerin is frequently used as an ingredient in other pharmaceutical preparations. It is used in tinctures and elixirs, such as theophylline, which is used to treat asthma. Glycerin is also used in ointments and creams to prevent them from drying out and can act as a preservative.[15] 2-Pyrrolidinone (3.51%) is used in the synthesis of pharmaceutical drugs e.g Cotinine, Doxapram, Piracetam, Povidone and Ethosuximide. These are some of the chemical constituents to mention a few, with known medicinal and pharmaceutical uses. Some other constituents still need to be isolated and tested for their therapeutic uses. Hence, the performance of GC-MS is a simple, effective and eloquent technique for drug research and discovery.

Table 1: Phytochemicals identified in *Pleurotus ostreatus*(Jack ex. Fr.) P. Kumm.

Peak No.	Retention Time (min)	Area	Area %	Name of Component
1	2.057	3683006	10.62	Formamide
2	2.383	270837	0.78	1,4-Pentanediol
3	2.643	8103665	23.36	Glycerin
4	2.775	263521	0.76	2(5H)-Furanone, 3-methyl-
5	2.882	1396374	4.02	3-Hexyn-2-ol
6	3.247	210001	0.61	4-Heptanone, 3-methyl-
7	3.507	1218161	3.51	2-Pyrrolidinone
8	3.615	982685	2.83	Butanedioic acid, monomethyl ester
9	3.735	966496	2.79	Cyclopropanecarboxylic acid, 1-amino-
10	3.923	163255	0.47	Pentanal, 2,3-dimethyl-
11	4.010	363955	1.05	Succinimide
12	4.439	3355329	9.67	Cyclobutanone, 2-methyl-4-hydroxy-
13	4.560	318289	0.92	Heptanoic acid, 2-methyl-2-butyl ester
14	4.662	509553	1.47	5-Methoxypyrrolidin-2-one
15	4.865	1948428	5.62	1,1,3-Trimethyl-3,8,9-trioxa-bicyclo[4.
16	5.200	410027	1.21	1,4-Dioxaspiro[4,4]nonane-7-carboxy
17	5.260	841626	2.43	Niacin
18	5.486	339872	0.98	Propanedioic acid, phenyl-
19	6.301	430811	1.24	2-Dodecanone
20	6.542	291641	0.84	2-Heptanone, 5-methyl-
21	6.717	3443004	9.92	2-Nonanol
22	6.771	1090217	3.14	2-Undecene, 4,5-dimethyl-, [R*,R*-(E
23	7.065	1886737	5.44	2-Butyn-1-ol, 4-methoxy
24	7.210	314116	0.91	DL-Proline, 5-oxo-, methyl ester
25	7.251	643852	1.86	Acetic acid, 2-propyltetrahydropyran-3
26	7.330	181753	0.52	3-Tetradecene, (E)
27	7.420	239801	0.69	4-Heptanone, 3-methyl
28	7.457	380450	1.10	Niacinamide
29	7.736	212763	0.61	Acetic acid, 2-(1-buten-3-yl)-2-nitro,
30	9.801	225843	0.65	9-Eicosene, (E)
Total		34695978	100.00	

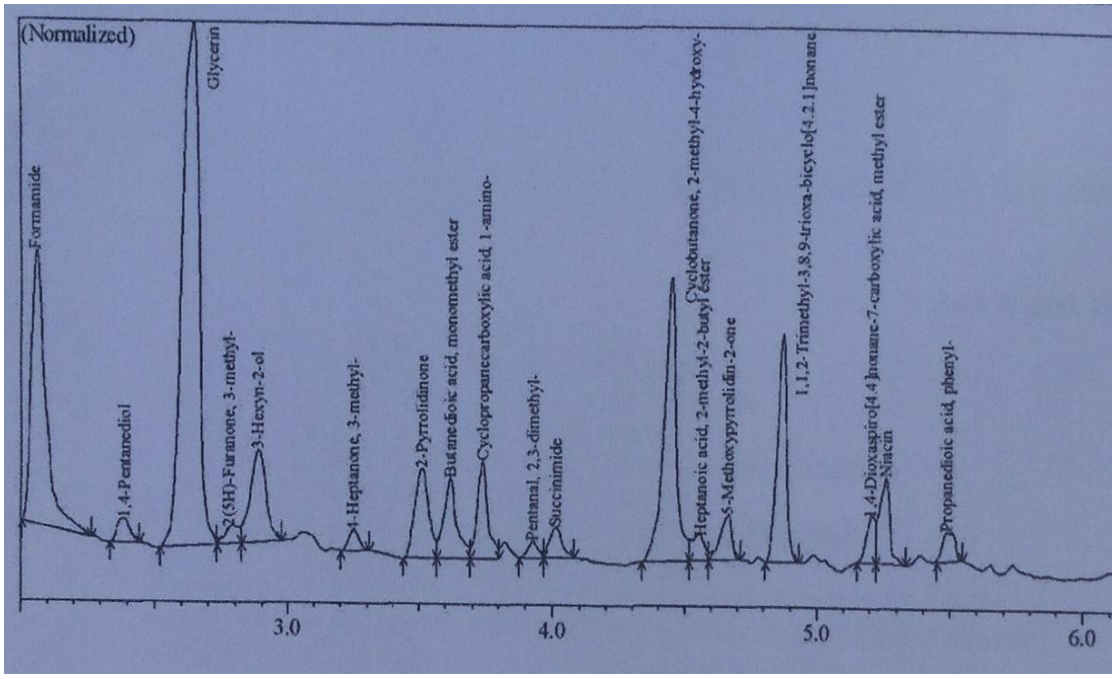

Figure 1a. Chromatogram obtained from the GC-MS analysis of *P. ostreatus* (denoting 0 up to 6 minutes run time).

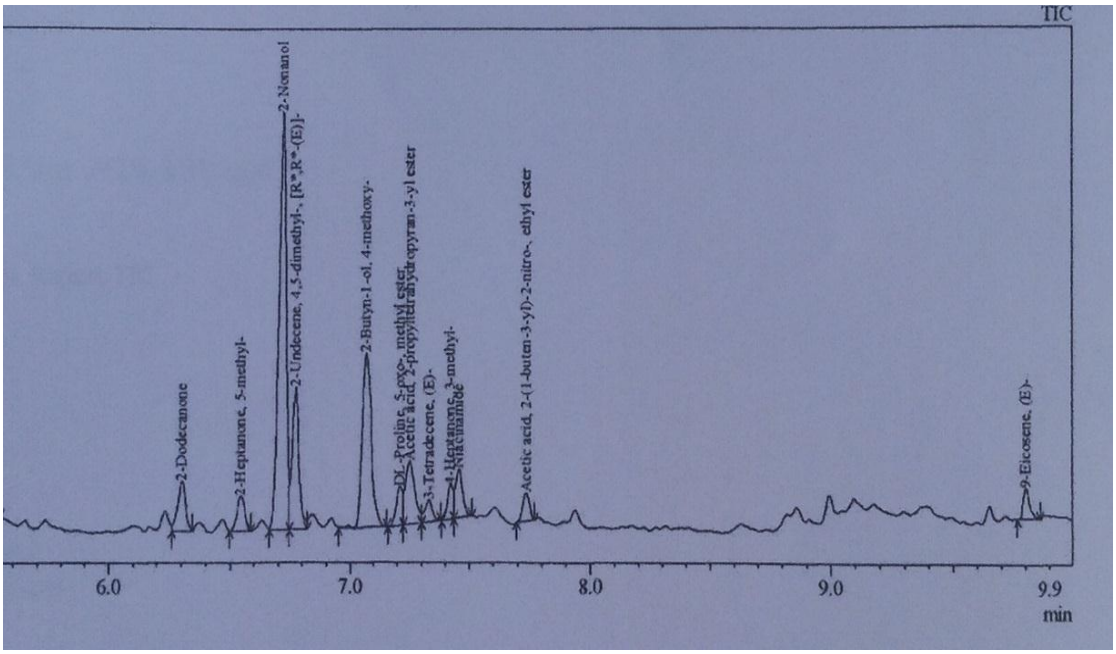

Figure 1b. Chromatogram obtained from the GC-MS analysis of *P. ostreatus* (denoting 6 up to 10 minutes run time).

CONCLUSION

The GC-MS analysis of the methanol extract of *Pleurotus ostreatus* (Jack ex. Fr.) P. Kumm. revealed presence of 30 different acids, alcohols, aldehydes, heterocyclic compounds and certain esters, ketones and aldehydes. These chemical components in *P. ostreatus* are

evidences of the wide array of its medicinal uses leading to its medicinal diversity. The use of GC – MS in drug research and production is currently one of the most reliable tools for drug identification and discovery.

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