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Original Research Article

Gas Chromatography-Mass Spectroscopic (GC-MS) Analysis of n-Hexane Extract of *Lentinus tuber-regium* (Fr) Fr (Polyporaceae) Syn *Pleurotus tuber regium* Fr sclerotia

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Abstract

Purpose: *To identify the chemical constituents of the n-hexane extract of the sclerotia of Lentinus tuber-regium (synonym Pleurotus tuber regium) using gas chromatography-mass spectroscopic (GC-MS) techniques.*
Methods: *The n-hexane extract of the sclerotia of Lentinus tuber regium was obtained by exhaustive Soxhlet extraction and analysed using gas chromatography-mass spectroscopic (MS) techniques. The structures of the identified constituents were confirmed on the basis of their fragmentation pattern in comparison with that obtained from the National Institute of Standards and Technology (NIST) reference library.*
Results: *Seven fatty acids derivatives: heptadecenal, n-hexadecanoic acid, 1-eicosene, linoleic acid, oleic acid, linoleic acid ethyl ester, and octadecanoic acid, and five steroidal triterpenoids: cholesterol, α-ergostenol, anthraergostatetraenol, stigmasterol, and alpha-ergosta-4,6,8(14),22-tetraen-3-one. The major constituents characterised are α-ergosta-4,6,8(14), 22-tetraen-3-one (8.56 %) > Anthraergostatetra-enol (7.19 %), > n-hexadecanoic acid (6.29 %) > linoleic acid (3.69 %).*
Conclusion: *This study shows that L tuber-regium is a veritable source of mono- and poly-unsaturated fatty acids, and ergosterol/provitamin D derivatives which may explain, in part, some of its reported nutraceutical benefits.*

Keywords: *Lentinus tuber-regium, Fatty acids, Steroids, Gas chromatography, Mass spectroscopy*

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INTRODUCTION

Lentinus tuber-regium (Fr.) Fr. (Polyporaceae) Syn. *Pleurotus tuber regium* Fr. (Pleurotaceae) commonly called the king tuber mushroom is an edible gilled fungus of the Agaricomycetes class. It grows wild in both tropical and subtropical regions of the world. It is a common mushroom in the southern part of Nigeria growing on dry wood, where it produces the large spherical to ovoid sclerotia which sometimes measure up to 30 cm in diameter [1,2]. The sclerotium is dark brown on the outside and white on the inside. Locally, it is called '*katala*' in Hausa, '*ike usu*' or '*ero usu*' in Ibo, '*awu*' in Igala and '*umoho'usu*' in Igede (Nigeria).

Edible mushrooms are a popular and valuable food, low in fats but high in minerals, essential amino acids, vitamins and fibres [3]. Some of them produce substances having potential medicinal effects attributed to the presence of

bioactive compounds like terpenoids, steroids, phenolics and alkaloids [4,5].

In traditional medical practice in Nigeria, *L tuber-regium* is used in preparation of traditional medications for headache, stomach ailments, colds and fever, asthma, smallpox and high blood pressure as well as for weight gain and malnourished babies [1,2,6-8].

The aim of this present study was to identify the constituents of the n-hexane extract of *Lentinus tuber-regium* using gas chromatography-mass spectroscopic (GC-MS) technique.

EXPERIMENTAL

Sample collection and identification

The sclerotia of *L tuber regium* used for this study were purchased in November 2012 at the Mile I market, Port Harcourt, from sellers from Orsu area, Imo State, Nigeria and authenticated by a mycologist Dr NL Edwin-Wosu of the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. A voucher specimen (OG-Acc-01. UPH-No. C-058) was deposited in the reference herbarium of the University of Port Harcourt. The sclerotia were pulverized using an electric blender prior to extraction.

Extraction of plant materials

The pulverized plant material was extracted exhaustively with n-hexane using the Soxhlet extractor. The n-hexane extract coded PTHF obtained was allowed to air dry in a fume cupboard.

Gas chromatography-mass spectroscopic (GC-MS) analysis

This was done on the PTHF dissolved in chloroform using an Agilent gas chromatograph Model 6890, coupled to a Mass spectrometer equipped with a DB DB-1MS capillary column (30 m long × 320 µm nominal diameter), programmed from 120 °C (5 min) to 250 °C at 3 °C/min, with 5 min hold time. Helium was used as carrier gas (1.0 ml/min) with sample injection in split mode (50:1). Injector and detector temperature were 250 and 280 °C respectively.

The mass spectrometer worked in electron impact mode at 70 eV with electron multiplier at 1600 V and ion source temperature at 180 °C. Mass spectra data were acquired in the scan mode in m/z range 50-550.

The compounds assayed in PTHF were identified by comparing their retention times with those of reference compounds in the library and by comparison of their mass spectra with those of reference substances from the library with a quality factor > 80 used as criterion for acceptance [9-11].

RESULTS

The n-hexane extract on drying gave a waxy golden yellow solid with a yield of 1.2 ± 0.36 % w/w. The result of the characterised chemical composition expressed as percent peak area response in Table 1 showed seven fatty acid derivatives (15.94 %) and five steroids (20.82 %) corresponding to 36.76 % of the total n-hexane extract.

Constituents corresponding to a total peak area response of 73.24 % though resolved could not be characterised due to insufficient library data and quality factor being less than 80 obtained. Of the total characterised fatty acids compositions, 44.42 % was due to unsaturated fatty acid constituents equivalent to 7.08 % of n-hexane extract.

Oleic acid and linoleic acid derivatives were the major unsaturated fatty acids accounting for 6.14 % of total n-hexane extract, 86.72 % of the total characterised unsaturated fatty acid composition, and 38.52 % of the total characterised fatty acids.

Of the total characterised steroidal constituents (20.82 %), ergosterol derivatives were of high occurrence to a level of 17.91 % of the total n-hexane extract thereby accounting for approximately 68.78 % of the total characterised steroids. Stigmasterol level up to 1.54 % of the total n-hexane extract was also observed.

DISCUSSION

The obtained low yield of 1.2 ± 0.36 % w/w is in line with the reported low fat content of *L tuber regium* [12] and other mushrooms [13]. The presence of linoleic and oleic acid derivatives as the major unsaturated fatty acids is in line with earlier report on mushrooms [14]. Linoleic acid is an essential omega-6 polyunsaturated fatty acid involved in the biosynthesis of arachidonic acid and prostaglandins. Oleic acid an essential omega-9 monounsaturated fatty acid is used as

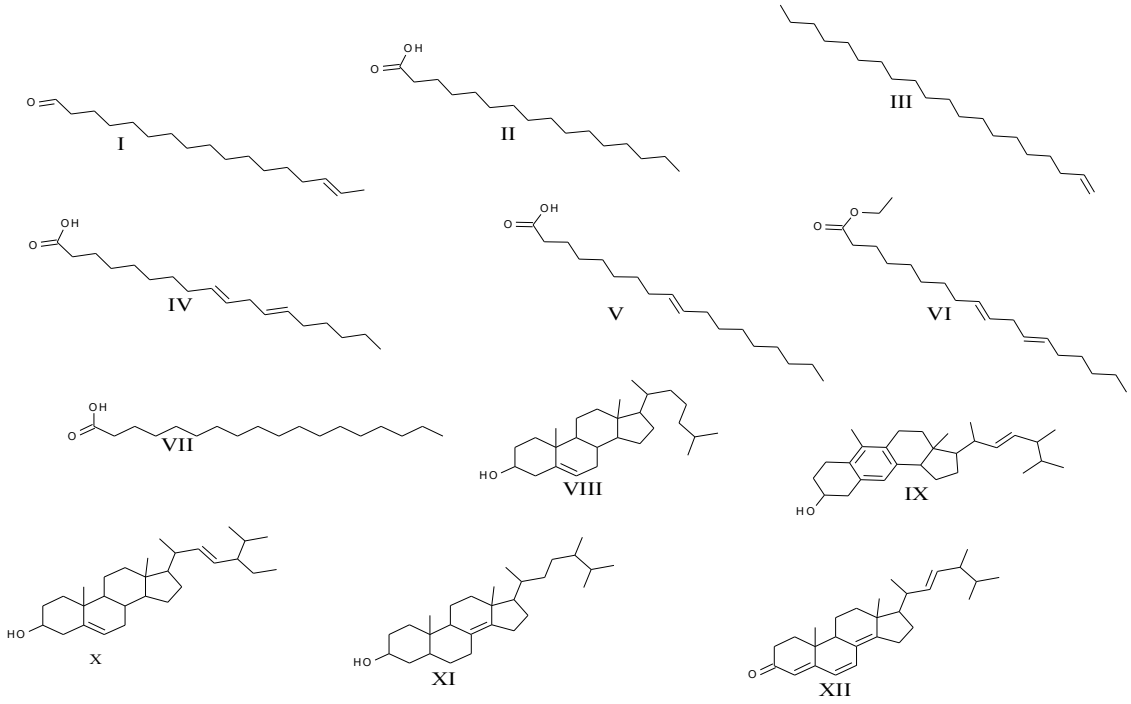

Figure 1: Structure of characterised compounds from the n-hexane extract of *L tuber regium* sclerotia

Table 1: Characterised chemical constituents of the n-hexane extract of *L. tuber-regium* sclerotia

Rt (min)	Compound	Chemical class	Observed diagnostic m/z (intensity) fragmentation peaks	Quality factor	Relative composition (% Area)
14.971	E-15-Heptadecenal [I]	MUH	$[M^+]$252(10),[M-CO]224(10), 97(100)	98	0.94
20.33	n-Hexadecanoic acid [II]	SFA	[M+H]257(12), $[M^+]$256(50), [M-44+H]213(47), 73(100)	96	6.29
21.21	1-Eicosene [III]	MUH	$[M^+]$280(8), 55(100)	95	1.31
24.99	Linoleic acid [IV]	PUFA	[M+H]281(5),$[M^+]$280(26), [M-45]235(3), 67(100)	99	3.69
25.24	Oleic acid [V]	MUFA	[M+H]283(5), $[M^+]$282(12), [M-45-H]236(12), 55(100)	99	1.47
25.44	Linoleic acid ethyl ester [VI]	PUFA	$[M^+]$308(7),[M+H-C_2H_4]280(12), [M-C_2H_5OH45]234(18), 55(100)	98	0.98
26.03	Octadecanoic acid [VII]	SFA	[M+H]285(15),$[M^+]$284(62), [M-44+H]241(30), 73(100)	99	1.26
46.75	Cholesterol [VIII]	Steroid	[M+H]387(25),$[M^+]$386(100), [M-H_2O]368(30),[M-113+2H] 275(33),[M-113+H]274(12), [M-113]273(13)	96	1.37
47.67	Anthraergostatetra-enol [IX]	Steroid	$[M^+]$394(24),[M-H_2O]376(25), [M-125]269(25),[M-125-H_2O]251(100)	91	7.19
48.63	Stigmasterol [X]	Steroid	$[M^+]$412(5),[M-15+H]398(47), [M-29]383(25),[M-iPr-15+H]355(5),[M-140-H]271(100), [M-140-OH]255(47)	99	1.54
49.98	α-Ergostenol [XI]	Steroid	[M+H]401(25),$[M^+]$400(100), [M-15]385(26),[M-15-H_2O] 367(5),[M-127]273(18), [M-127-H_2O]255(73)	91	2.16
52.107	α-Ergosta-4,6,8(14),22-tetraen-3-one [XII]	Steroid	[M+H]393(10),$[M^+]$392(28), [M-125]268(100),[M-15-H_2O] 367(5),[M-127]273(18), [M-127-H_2O]255(73)	98	8.56

Key: *MUFA - Mono-Unsaturated Fatty Acids, SFA = Saturated Fatty Acids, MUH-Mono-Unsaturated Hydrocarbon, Rt- = retention time*

an emulsifying agent, reported to be hypotensive [15] and to hinder the progression of adrenoleukodystrophy, a fatal disease that affects the brain and adrenal glands [16]. The occurrence of ergosterol derivatives (17.91 %) in the n-hexane extract as the predominant steroids could be due to the reported occurrence of ergosterols in yeast and fungal cell membranes functioning in same manner cholesterol does in animal cells. Research has shown ergosterol may exhibit some degree of antitumor properties [17,18]. Ergosterols are known to act as biological precursors of vitamin D2 hence can be classified as provitamins [19]. Stigmasterol level up to 1.54 % of the total n-hexane extract was also observed. Stigmasterol also known as the anti-stiffness factor is a phytosterol reported to have some anti-cancers, antioxidant, hypoglycemic, hypocholesterolemic and thyroid inhibiting properties [20,21].

CONCLUSION

The identified secondary metabolites may explain in part some of the reported medicinal uses of *L tuber regium* [1,2,6-8]. However, it would be premature at this stage to attribute the nutraceutical benefits of *L tuber regium* to any of these characterised constituents, and also to infer chemotypes that could serve as basis for chemotaxonomic studies. Further work is on-going to extend this study to other species of mushrooms with the view of establishing chemotypes variations among them, and also to isolate and characterise these secondary metabolites in pure forms using chromatographic and spectroscopic techniques with the view of establishing their potentials as biologically active entities for drug discovery and development.

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