

BIOACTIVE COMPOUNDS IN ETHANOL EXTRACT OF *LENTINUS SQUARROSULUS* MONT - A
NIGERIAN MEDICINAL MACROFUNGUS

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Abstract

Background: The continuous search for new lead compounds of therapeutic importance has become necessary in the face of treatment failures and multidrug resistance plaguing the world. While many plants and higher fungi are sources of bioactive compounds yet to be fully harnessed, understanding the bioactive components in macrofungus could serve as a lead for investigating its biological activities and medicinal potentials.

Materials and Methods: The bioactive compounds in the ethanolic extract of *Lentinus Squarrosulus*, an edible Nigerian macrofungus, were investigated by Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

Results: There were nine bioactive compounds in this edible macrofungus. Of these compounds, 9,12-Octadecanoic acid ethyl ester (37.39%; RT:39.815) was the highest in quantity, followed by Hexadecanoic acid ethyl ester (14.49%; RT:36.550). Other fatty acids, their ethyl esters and other compounds identified included 2-Butenethioic acid,3-(ethylthio)-S-(1-methylethyl) ester (4.51%; RT:15.866), n-Hexadecanoic acid (4.74%; RT:36.034), 9,12-Octadecadienoic acid (Z,Z)- (11.88%; RT:39.429), 9,17-Octadecadienal,(Z)- (5.01%; RT:39.500), ethyl oleate (5.27%; RT:39.898), 3a,6-Methano-3aH-indene,2,3,6,7 tetrahydro (4.04%; RT:48.379), and 9,12-Octadecadienoic acid (Z,Z)-,2 hydroxy-1-(hydroxymethyl) ethyl ester (12.68%; RT:48.682). Some of these compounds have antimicrobial, antioxidant, hepatoprotective, hypocholesterolemic as well as cancer preventive activities amongst others.

Conclusion: This study showed the bioactive components of therapeutic potentials in *L. squarrosulus* while creating a platform for screening, isolating and identifying many bioactive components which may be useful in the treatment of the various ailments, disorders and diseases in the nearest future.

Keywords: Bioactive constituents; ethanolic extract; *Lentinus squarrosulus*; GC-MS analysis; mushroom; macrofungi.

Introduction

Higher fungi including mushrooms have been part of the human diet for thousands of years (Cooke, 1977). Most mushrooms are very important nutritionally and are rich in protein, minerals and vitamins (Mattila et al., 2001; Rudawska and Leski, 2005). Of more than 200 species existing in nature, about 22 species are rigorously cultivated (Manzi et al., 2001). While many bioactive compounds affecting human and animal physiology have been identified in many mushrooms (Wang and Ng, 2006), many species of mushrooms have therapeutic values including antihemorrhagic, anticholesterol, antitumor and anticancer properties (Zheng et al., 2010).

Lentinus squarrosulus (also referred to as *Lentinus subnudus*) is one of the most common Nigerian edible mushrooms. It grows wild on decaying wood during the raining season from April to October. Its identifying features include its tough mixture of matured sporophores, velvety stipe and funnel-shaped whitish pileus (Jonathan, 2002). While its spores print is white and spore size ranged between 5 – 8 and 2 – 4 µm along the major and minor axis respectively (Jonathan, 2002), *Lentinus subnudus* belongs to the family Polyporaceae which is the largest group of Aphyllophorales (Pegler, 1983). *Lentinus* species grow naturally on some substrates and can be successfully cultivated on others (Gbolagade et al., 2006; Jonathan et al., 2010).

Being edible, it can be prepared into a range of delicacies as they add flavour to food and act as condiments (Oluoye and Etugo, 1993). Its meaty taste is a good alternative to animal protein among rural dwellers and average Nigerians who cannot afford the high cost of meat due to their financial status. Although indigenous mushrooms growing in the wild have been found to be nutritious and very important for medicinal purposes, many have been used as tea and nutritional food for their special fragrance and texture purposes (Manzi et al., 1999). Most of them are very good sources of amino acids, ascorbic acid, glycogen, lipid, sugar and vitamins and minerals such as calcium, iron, phosphorus, potassium, sodium, magnesium and zinc (Moa and Jica, 2000; Oei, 2003; Jonathan et al., 2011; Kumari et al., 2011).

Although the nutritional values and taste components of many mushrooms have been reported by Yang et al. (2001), there is a dearth of information on the bioactive compounds in extracts of mushrooms especially as regards those of *Lentinus* species. While plants and higher fungi are large sources of bioactive compounds serving as lead to develop novel drugs (Cowan, 1999) and about 80% of the world population depends on natural products for their health maintenance due to minimal side effects and cost effectiveness (Jagtap et al., 2009), understanding the bioactive components in this macrofungus could serve as a lead for investigating its biological activities and medicinal potentials. Consequently, determining the bioactive compounds of the extract by using Gas Chromatography-Mass Spectrometry (GC-MS) becomes inevitable. The combination of the separation technique (GC) with the best identification technique (MS) makes GC-MS an ideal technique for qualitative and quantitative analysis for volatile and semi-volatile compounds. This study, therefore, aims at utilizing a rapid method, Gas Chromatography-Mass Spectrometry (GC-MS) technique, for quantitative determination of bioactive compounds in *Lentinus squarrosulus* Mont. in view of the fact that higher fungi remain a vast and a large source of new pharmaceutical products yet to be explored. This is to ultimately discover and identify new safe drugs which are devoid of side effects and are active against a wide range of diseases.

Materials and Methods

Collection of mushroom materials and identification

Several batches of fresh fruiting body of the macrofungus, *L. squarrosulus*, growing on a decaying tree stump of *Mangifera indica* were collected from Odogbolu, Ogun state, Nigeria between April and June, 2014 and identified using the standard descriptions of Alexopoulos et al. (1996). This identification was further authenticated by Professor S.G. Jonathan, of the Department of Botany, University of Ibadan, and Ibadan, Nigeria. A voucher specimen (BU001/0817) was deposited at the Herbarium of Babcock University. The mushroom samples were dried, powdered and stored in air-tight amber bottle and refrigerated at 04°C for future uses.

Extraction procedure

Two hundred gram (200 g) of the crude powdered sample of *L. squarrosulus* (shown in Figure 1) was soaked in ethanol usually used traditionally in preparing herbal medicines and agitated continuously over 72 h. It was then filtered through Whatman No. 1 filter paper. The extract was then concentrated using a rotary evaporator.



Figure 1: *Lentinus squarrosulus* (Mont) growing on a decaying tree stump of *Mangifera indica* in Nigeria

Gas Chromatography-Mass Spectrum analysis (GC-MS) of mushroom ethanolic extract

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system with an AOC-20I auto sampler and gas chromatograph interfaced to a mass spectrophotometer instrument employing the following conditions: Column Elite-1 fused silica capillary column (30 mm × 0.25 mm I.D × 1 μ M df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 0.5 μl was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. Total GC running time was 60 min.

Identification of components

To identify the name, structure and molecular weight of the bioactive compounds in the mushroom, the spectra of the bioactive components in the extract were compared with the spectra of known components in the database of National Institute of Standard and Technique (NIST) with more than 62,000 patterns.

Results

In this study, nine bioactive compounds were identified in the ethanolic extract of the fruiting body of *Lentinus squarrosulus* by GC-MS analysis. The compounds identified were 2-Butenethioic acid, 3-(ethylthio) -, S-(1-methylethyl) ester, n-Hexadecanoic acid (Palmitic acid); Hexadecanoic acid ethyl ester (Palmitic acid ethyl ester), 9,12-Octadecadienoic acid (Z,Z)- (Linoleic acid), 9,17-Octadecadienal, (Z)-, 9,12 Octadecadienoic acid ethyl ester (Linolelaidic acid, ethyl ester), Ethyl Oleate (Oleic acid ethyl ester), 3a,6-Methano-3aH-indene,2,3,6,7-tetrahydro- and 9,12-Octadecadienoic acid (Z-Z)-,2-hydroxy-1-(hydroxymethyl) ethyl ester (2-monolinolein). While the chromatogram of the different bioactive components of the ethanolic extract of *Lentinus squarrosulus* is shown in Figure 2, the Retention Time (RT), name of the bioactive compounds, Peak Area (%), Molecular Formula (MF) and molecular weight (MW), are as shown in Table 1.

Abundance

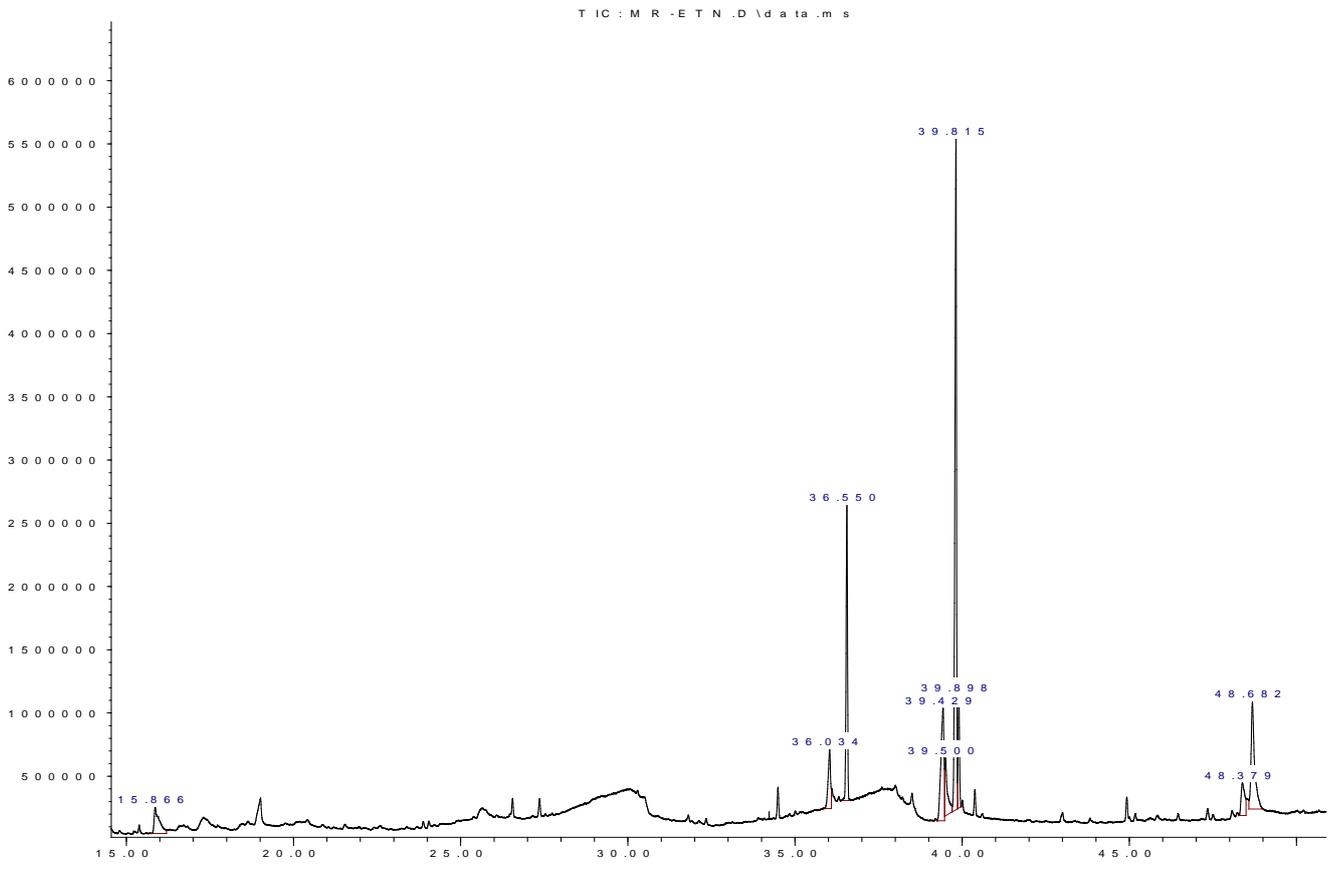


Figure 2: GC-MS spectrum of the ethanolic extract of *Lentinus squarrosulus*

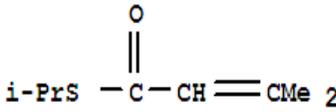
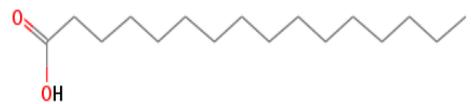
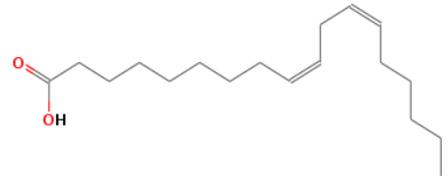
Table 1: Chemical composition of *L. Squarrosulus* using Gas Chromatography Mass Spectrometry (GC-MS)

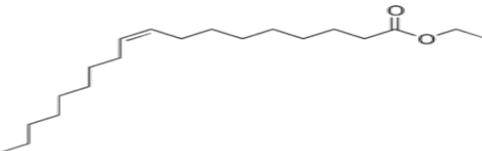
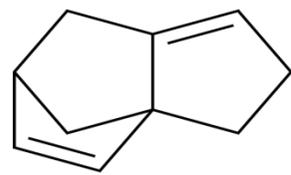
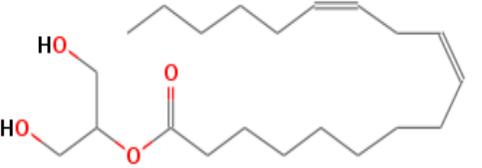
S/no.	RT (min)	Name of the Compound	(MF)	(MW)	PA (%)
1.	15.866	3-(ethylthio), S-(1-methylethyl) - 2-Butenethioic acid ester	C ₈ H ₁₄ OS	158.26	4.51
2.	36.034	n-Hexadecanoic acid (Palmitic acid)	C ₁₆ H ₃₂ O ₂	256.42	4.74
3.	36.550	Hexadecanoic acid ethyl ester	C ₁₈ H ₃₆ O ₂	284.48	14.49
4.	39.429	9,12-Octadecadienoic acid (Z,Z)- (Linoleic acid)	C ₁₈ H ₃₂ O ₂	280.45	11.88
5.	39.500	9,17-Octadecadienal, (Z)-	C ₁₈ H ₃₂ O	264.45	5.01
6.	39.815	9,12-Octadecadienoic acid, ethyl ester (Linoleic acid ethyl ester)	C ₂₀ H ₃₆ O ₂	308.50	37.39
7.	39.898	Ethyl Oleate (Oleic acid ethyl ester)	C ₂₀ H ₃₈ O ₂	310.51	5.27
8.	48.379	3a,6-Methano-3aH-indene,2,3,6,7-tetrahydro-	C ₁₀ H ₁₂	132.20	4.04
9.	48.682	9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1-(hydroxymethyl) ethyl ester (2-Monolinolein)	C ₂₁ H ₃₈ O ₄	354.52	12.68

Key: RT = Retention Time; MW = Molecular weight; MF = Molecular Formular; PA = Peak Area

From Table 2, the biological/therapeutic activities of the different component of the extracts are presented. While the nature and therapeutic potentials of 3a,6-Methano-3aH-indene,2,3,6,7 tetrahydro and 2-Butenethioic acid, 3-(ethylthio)-, S(1-methylethyl) ester compounds are unknown, other bioactive compounds in the extracts have been categorized as fatty acid, polyunsaturated fatty acid, fatty acid ester, and long chain unsaturated aldehyde. These compounds have been known as anti-inflammatory, anti-androgenic, antioxidant, antitumor, antihistaminic, antimicrobial, chemopreventive, haemolytic, hypocholesterolemic, 5- α reductase inhibitor, pesticide, lubricant, flavour, cancer preventive, immunostimulant, hepatoprotective, antieczemic, antiacne, antiarthritic, antiandrogenic, anticoronary, lipo-oxygenase inhibitor, insectifuge and nematocide.

Table 2: Therapeutic activity of the phytochemicals identified from the ethanolic extract of *L. squarrosulus* by GC-MS.

S/n	Name of Compound	Nature of Compound	Chemical Structures	***Medicinal Properties/Therapeutic activities
1.	2-Butenethioic acid, 3-(ethylthio)-, S(1-methylethyl) ester		 <p style="text-align: center;"> $i\text{-PrS} - \overset{\text{O}}{\parallel}{\text{C}} - \text{CH} = \text{CMe}_2$ </p>	Still Unknown
2.	n-Hexadecanoic acid (Palmitic acid)	Fatty acid	<p style="text-align: center;">CAS No. 34365-79-2</p>  <p style="text-align: center;">CAS No. 57-10-3 PubChem CID: 985</p>	Antioxidant, antibacterial, nematocide, anti-inflammatory, hypocholesterolemic, pesticide, lubricant, anti-androgenic, antitumor, flavour, cancer preventive, immunostimulant, chemopreventive, haemolytic 5- α reductase inhibitor, lipoxygenase inhibitor.
3.	Hexadecanoic acid ethyl ester (Palmitic acid ester)	Fatty acid ester	 <p style="text-align: center;">CAS No. 628-97-7 PubChem CID: 12366</p>	Antioxidant, hypocholesterolemic, flavour, nematocide, pesticide, lubricant, anti-androgenic, haemolytic 5- α reductase inhibitor
4.	9,12-Octadecadienoic acid (Z,Z)- (Conjugated Linoleic acid)	Polyunsaturated Fatty acid	 <p style="text-align: center;">CAS No. 60-33-3 PubChem CID: 3931</p>	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematocide, insectifuge(cide), antihistaminic, antieczemic, antiacne, 5- α reductase inhibitor, antiandrogenic, antiarthritic, anti coronary, antimicrobial.
5.	9,17-Octadecadienal,(Z)-	Long chain unsaturated aldehyde		Anti-inflammatory, antioxidant.

6.	9,12-Octadecadienoic acid, ethyl ester (Linolelaidic acid ethyl ester)	Fatty acid ester	<p>CAS No. 56554-35-9 PubChem CID: 5365667</p> 	<p>Hypocholesterolemic, nematicide, antiarthritic, hepatoprotective, antiandrogenic, 5-α reductase inhibitor, antihistaminic, anticoronary, insectifuge, antieczemic, antiacne, antimicrobial. Antimicrobial</p>
7.	Ethyl oleate (Oleic acid ethyl ester)	Fatty acid ester	<p>CAS No. 7619-08-1 PubChem CID: 5365672</p> 	Antimicrobial
8.	3a,6-Methano-3aH-indene,2,3,6,7 tetrahydro		<p>CAS No. 111-62-6 PubChem CID: 5363269</p> 	Still Unknown
9.	9,12-Octadecadienoic acid (Z,Z)-, 2 hydroxy-1-(hydroxymethyl) ethyl ester (2-monolinolein)	Fatty acid ester	<p>CAS No.: 98640-29-0</p>  <p>CAS No. 3443-82-1 PubChem CID: 5365676</p>	<p>Hypocholesterolemic, antieczemic, nematicide, hepatoprotective, antioxidant, antiacne, haemolytic, pesticide, flavour.</p>

Discussion

Mushrooms are macrofungi with a distinctive fruiting body large enough to be seen with the naked eyes. It includes both edible and non-edible species. Some macrofungi serve as food because of their nutrient content while some have been used extensively in traditional medicine (Nwachukwu and Uzoeto, 2010). The compounds in the ethanolic extract of the macrofungus used in this study are fatty acids and their ethyl esters. Others include compounds whose biological activities are still unknown. In searching for new therapeutic alternatives, macrofungi have shown different therapeutic activity including anti-plasmodium, anti-diabetic, anti-inflammatory, anti-carcinogenic, anti-viral, anti-microbial activities and immune-suppression (Imtiaj and Lee, 2007; David et al., 2012). While medicinal properties of higher fungi have been studied extensively, literature is sparse in documenting the bioactive components of pharmacological importance in *Lentinus Squarrosulus* (Mont) Singer, a Nigerian edible macrofungus. In this study, *L. squarrosulus* was found to have bioactive compounds with great therapeutic activities such as antioxidant, hypocholesterolemic, antimicrobial, antitumor, anti-inflammatory, antiarthritic, anticoronary, antieczemic, nematocidal, antiacne and hepatoprotective amongst others.

The GC-MS spectra of the ethanolic extract of *Lentinus squarrosulus* showed different bioactive compounds of varying pharmacological relevance. These compounds include palmitic acid and its ethyl ester, linoleic acid and its ethyl ester as well as oleic acid ethyl ester. Quantitatively, linoleic acid and its ester was the highest in quantity (58%), followed by the ethyl ester of hexadecanoic acid (14.49%) ethyl ester of 9,12-Octadecadienoic acid (Z,Z)-,2-hydroxy-1-(hydroxymethyl) ethyl ester (12.68%). While 3a,6-Methano-3aH-indene,2,3,6,7-tetrahydro- was the least in quantity (4.01%) and 3-(ethylthio), S-(1-methylethyl) - 2-Butenethioic acid ester was (4.51%), n-Hexadecanoic acid was 4.71% and 9,17-Octadecadienal, (Z)- was 5.01%. Although the pharmacological activities of 3-(ethylthio), S-(1-methylethyl) - 2-Butenethioic acid ester and 3a,6-Methano-3aH-indene,2,3,6,7-tetrahydro- are still unknown, there are significant differences between linoleic acid and its ethyl ester and those of palmitic acid and its ethyl ester.

Although fatty acids have various therapeutic activities including anti-inflammatory and antioxidant activities (Kapoor and Huang, 2006; Galli and Calder, 2009; Poudel-Tandukar et al., 2009), omega-6, -7, -9 (N-6, N-7, N-9) fatty acids are important dietary nutrients providing numerous health benefits for humans (Santos et al., 1995; Harris, 2010). While N-6 fatty acids cannot be produced *de novo* in the body, the N-6 polyunsaturated fatty acids can be obtained from vegetable oils (Linoleic acid), egg yolk and organ meats (Whelan and McEntee, 2004). The N-7 mono-unsaturated fatty acids such as palmitoleic acid occur naturally in healthy skin (Spahis et al., 2008). The N-9 mono-unsaturated fatty acid such as oleic acid is a major fatty acid in olive oil. It is able to lower cholesterol levels and reduce atherosclerosis (Nicolosi et al., 2004).

Considering the therapeutic potentials of these bioactive compounds, it is important to indicate that diets in the western world lacking advantageous amounts of N-3 and N-9 fatty acids and contributing to an unhealthy diet rich in N-6 poly-unsaturated fatty acids are gaining popularity in Nigeria (Simopoulos, 2002). Therapeutically, N-3 and N-6 polyunsaturated fatty acids play multiple roles in blood clotting, blood pressure lowering, controlling of inflammation, cell membrane structure and lipid metabolism (Wijendran and Hayes, 2004). An unacceptable balance of these omega fatty acids could contribute to occurrence of different chronic diseases including heart disease, cancer, asthma and arthritis (Darveau et al., 2000; Huang and Ebersole, 2010).

The polyunsaturated fatty acid, 9,12-Octadecadienoic acid (Z-Z)-,2-hydroxy-1-(hydroxymethyl) ethyl ester (LA), is a conjugated linoleic acid known as an oxidant which can protect membranes from harm (Ha et al., 1990). Clinical studies have demonstrated that gamma linoleic acid (GLA), found primarily in evening primrose oil, may reduce joint pain, swelling and morning stiffness associated with rheumatoid arthritis (Allayee et al., 2009). This study, therefore, suggests that in the absence of evening primrose oil, a diet in which *L. squarrosulus* is incorporated could perform the same therapeutic activities.

Conclusion

In conclusion, the major components of the ethanolic extract of *L. Squarrosulus*, as indicated in this study, the extract of *Lentinus squarrosulus* contains bioactive compounds of numerous biological or therapeutic importance. The nine phytochemical constituents identified by the GC-MS analysis are, therefore, medicinally valuable and possess various pharmaceutical applications. That n-Hexadecanoic acid can inhibit haemolytic 5- α reductase implies that *L. squarrosulus* might have a liver disorder curing effect while the anti-inflammatory activity of some of these bioactive compounds would relieve sprained joints and general body pains. The findings strongly suggest that incorporating the extracts of *L. squarrosulus*, a Nigerian edible macrofungi, into the diet and drug formulations will have tremendous health benefits. A toxicological analysis of the identified bioactive compounds would be of necessity to develop safe drugs while further research to optimally extract the bioactive compounds in *L. squarrosulus* is ongoing. It is obvious from this study that *L. squarrosulus* holds a promise for the isolation and purification of novel pharmaceuticals.

Conflict of Interest: The authors hereby declare that there is no conflict of interest

Authors' contributions: All authors contributed equally to this study and have agreed together to submit this manuscript to AJTCAM.

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