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GC-MS Evaluation, Phytochemical and Antinutritional Screening of *Ganoderma lucidum*

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Author's contribution

This work was carried out solely by the author. The author designed the study, performed the statistical analysis, wrote the protocol, managed the analyses, managed the literature searches, and wrote the first draft and final manuscript.

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ABSTRACT

Aims: To qualitatively and quantitatively determine and evaluate the phytochemicals and anti-nutritional components of *Ganoderma lucidum*.
Place and Duration of Study: The study was conducted at Adekulne Ajasin University, Akungba-Akoko, Ondo State and Obafemi Awolowo University, Ife.
Methodology: Twelve mushrooms of *Ganoderma lucidum* were harvested for the study. The mushrooms were assayed for proximate, antinutritional and phytochemical composition using conventional methods. The metabolite composition was estimated using GC-MS.
Results: The mushrooms contain 29.30% crude fibre, 42.10% total carbohydrate, and 6.33% ash while the antinutritive factors present include cyanide 0.008 mg/100 g, and phytate 0.012. Extract of the fungus in the present study do not contain tannins, anthraquinones, and volatile oils while other phytochemicals were found in it. The GC-MS result showed the mushroom contains saturated and unsaturated fatty acids (organic compounds), organometallic compounds, and alkanes. Compounds like 17-Pentatriacontene, 5-Eicosene, 3-Eicosene, 1-Docosene were extracted by the two solvent systems though more compounds were extracted by N-Hexane than Ethyl acetate. The first compounds were extracted at retention time of 19.195 min.

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Conclusion: The study alluded to the fact that unique advantageous compounds abound in the fungus which may be relevant to mans' health and useful for his day to day activities though further research is still needed to better validate the use of the mushroom.

Keywords: Ganoderma; phytochemicals; antinutritive; components.

1. INTRODUCTION

The challenge of global warming, drug resistance, and poor elimination and clearance of residual drug metabolites from the body has made it more than necessary to explore natural plant parts with antimicrobial activities and potentials [1,2]. Phytochemicals are abundant, locally renewable, user-friendly and environmentally safe, and attracts low capital [3,4]. Mushrooms contain a vast amount of compounds among which are polysaccharides and triterpenes. Polysaccharides are regarded for their antitumour and immunostimulating activities and are majorly composed of glucans. The glucan linkages confer antitumour properties which is dependent on their molecular weight composition. Polysaccharides of mushroom origin prevent oncogenesis, tumour metastasis, and angiogenesis [5,6] Polysaccharides extracted from *G. lucidum* have been credited to stimulate macrophages, natural killer cells, T-lymphocytes, and interleukins production and activities [6,7]. These polysaccharides also confers immunomodulating, prophylactic, non-invasive action against tumour metastasis.

Ganoderma lucidum also known as Reishi by the Chinese is a mushroom of the Ganodermataceae family mostly found attached to tree stumps that are at the stage of decomposing. The fungus is cosmopolitan with interwoven hypha that forms a lamellaless mycelium employed in the treatment of various medical conditions ranging from cancer, tumour, hypotension, microbial infections, to inflammatory conditions [8-12]. It has been reported that the fungus apart from its many ailing properties, prolong life and improves immunity. Extract from the mushroom have been reported to contain an array of medicinally important bioactive compounds responsible for its activities and potency; and include triterpenoids, pleurostrin, ganoderic acid among others [13,14]. To this end, the study aims to qualitatively and quantitatively determine, evaluate, and report the phytochemical and nutritional components of *Ganoderma lucidum* found in Ondo State, Nigeria.

2. MATERIALS AND METHODS

2.1 Plant Materials

Twelve *Ganoderma lucidum* mushrooms were collected from the Arimogija Forest Reserve, Ifon, Ondo State. These were identified and authenticated by the Botany Department, Federal University Lafia, Nasarawa State, Nigeria. Identification of the fungus macroscopically was based on colour, odour, morphology, and the cap of the mushroom. The colour of the powdery print (spore print) was also used for identification according to the modified methods of Wasser [15]. Microscopic examinations of spores on a glass slide using lacto phenol in cotton blue was also done. After identification, the mushrooms having been cleaned and washed were dried under shade for twenty one (21) days. The dried samples were separately grounded using a Marlex Electroline electric blender Model IS: 4250, CM/L 7371373, kept in polyethylene bags, labeled and stored at 20°C. Fresh fruiting bodies of *Ganoderma lucidum* were ground and kept for further analysis.

Fig. 1. Mushroom of *Ganoderma lucidum*

2.2 Solvent Extraction

The modified methods of Odey et al. [16] were employed in the extraction process. Two solvent systems were used namely n-hexane and ethyl acetate. Four hundred grams (400 g) of *G. lucidum* powder were weighed and soaked in 2000 mL of ethyl acetate and n-hexane (98% BDH) at a ratio of 1:5 (powder: solvent). The mixtures were kept in air-tight containers and left for 48 hr at room temperature. Residue were removed using double layer muslin clothe and

further filtration done using WhattMan No 1 filter paper (24 cm). The filtrates were then separately concentrated in vacuo using Rotary Evaporator (Model RE52A, China) to 10% of their original volumes at 40°C. The final concentrations to dryness were done by evaporating to dryness in water bath at 40°C. The extracts were stored in a refrigerator from where part of it was used for the proximate, phytochemical, and GC-MS analyses.

2.3 Phytochemical Screening and Proximate Analysis

Qualitative phytochemical screening and proximate analysis were carried out using the methods of Savithramma et al. [17], Onyeike and Osuji [18], and others.

2.3.1 Steroids

Extract of *G. lucidum* (0.5 g) was dissolved in 2 mL acetic anhydride and cooled in ice. Sulphuric acid was carefully added and observed. Colour change from violet to blue to green indicate the presence of steroid.

2.3.2 Terpenoids

To 0.5 g of the fungus extract was added 2 ml chloroform. Sulphuric acid was later added to form a lower layer. A reddish- brown colour at the interface indicate the presence of terpenoid.

2.3.3 Tannins

Five grams of the mushroom extract was stirred in 10 mL distilled water and filtered with Whattman's filter paper. Ferric chloride reagent was then added to the filter. A blue-black or or blue-green precipitate indicate presence of tannins [19,20].

2.3.4 Saponins

The modified methods of Orole et al. [19] and Trease and Evans [20] was employed for the test. Each plant extract (0.5 g) was shaken with water in a test tube. Frothing which persisted on warming was taken as preliminary evidence of the presence of saponins. In order to remove 'false-positive' result, the blood haemolysis' test was performed on those extracts that frothed in water. Half gram of each extract was boiled briefly with 50 ml phosphate buffer of pH 7.4, and allowed to cool, then filtered. Five millilitres of the filtrate was passed for 3 hr through an asbestos disc (1.5 mm thick and 7 mm in diameter), which had been previously soaked with 2-3 drops of 1% cholesterol in ether and dried. After filtration, the disc was washed with 0.5 ml of distilled water, dried and boiled in 20 ml xylol or 2 hr to decompose the complex formed between cholesterol and any saponin in the extract. The disc was then washed in ether, dried and placed on a 7% Blood Nutrient Agar. Complete haemolysis of red blood cells around the disc after 6 hr indicated the presence of saponins.

2.3.5 Phenols

To little portions of the extract was 5 mL distilled water added, followed by the addition of 2 – 5 drops of neutral 5% ferric chloride solution. Appearance of dark green coloration showed the presence of phenol.

2.3.6 Flavonoids

2.3.6.1 Flavonoids

To 5 mL of water was dissolved 2 g of the extract in a test tube. Few drops of sodium hydroxide solution were added to the resulting solution. A yellow colour shows the presence of flavonoids.

2.3.6.2 Glycosides

Five millilitres of 25% H_2SO_4 was transferred into a tube and 0.5 ml of the extract was added and boiled in water bath for 15 mins. Fehling's solution (5 ml) was then added to the boiled mixture. A reddish brown colour indicated the presence of steroidal ring of glycosides.

2.3.7 Anthraquionones

To 5 g of the mushroom extract was added 5 mL benzene and shaken properly until it dissolved. Five milliliters of 10% ammonia solution was then added to the filtrate and the mixture shaken. Pink, red, or violet coloration in the ammoniacal (lower) phase indicate the presence of free hydroxyl anthroquionones.

3. RESULTS AND DISCUSSION

3.1 Proximate Analysis

Analysis of the proximate composition of *Ganoderma lucidum* (Table 1) showed that, the mushroom contains 29.30% crude fibre, 42.10% total carbohydrate and 0.012 mg/100 g phytate which are an antinutritive factor.

3.2 Phytochemical Composition

The phytochemical analysis showed the fruiting bodies of *Ganoderma lucidum* extracted with

ethyl acetate and N-hexane had flavonoid, phenol, sterols while volatile oils and tannins were absent in the extract.

3.3 GC-MS Analysis

GC-MS chromograms of the crude extracts (Fig. 2 and Fig. 3) showed the fruiting bodies had similar compound extracted by the two solvents.

Results from the chromograms showed that most of the extracted compounds were saturated and unsaturated fatty acids (organic compounds) and organometallic compounds and alkanes. Compounds like 17-Pentatriacontene, 5-Eicosene, 3-Eicosene, 1-Docosene were extracted by the two solvent systems. The first compounds were extracted at retention time of 19.195 min. From the results of the GC-MS chromogram as shown in Tables 3 and 4, more compound were extracted by N-Hexane when compared to Ethyl acetate.

Figs. 4 – 9 reveals the mass spectrums of Propanoic acid, 2-methyl-, 2, 2 dimethyl -1(-2-hydroxy-1-methylethylpropyl ester, Propanoic acid, 2-methyl-3-hydroxy-2,4,4-trimethylpentyl ester, E- 15-Heptadecenal, 1-Nonadecene, 1-Docosene, and Phenol, 2,6 bis(1,1-dimethylethyl).

4. DISCUSSION

Terpenoids found in the mushroom confer antioxidant, antimicrobial, antifungal, antiviral, antihyperglycemic, antiinflammatory, and antiparasitic activities on *G. lucidum* while the antioxidant properties found in the fungus is induced by phenols and flavonoids in response to damages resulting from oxidative stress on cells and adjoining tissues integrity. These phytochemicals have biomolecules with high affinity for scanvenging reactive free radicals capable of causing cell death and tissue damage which inadvertently result in cancer, emphysema, cirrhosis, atherosclerosis, and arthritis. Phenolic compounds are antimicrobial in nature especially against Gram-positive bacteria where result is dependent on concentration [21]. Shahidi and Wanasundara [22] explained the mechanism of action of phenols to be by terminating free

Table 1. Proximate and antinutritional composition of dried *Ganoderma lucidum*

Proximate composition analysis		Antinutritive factors composition	
Constituents	**Quantity (%)**	**Constituents**	**Quantity (mg/100 g)**
Moisture	9.90	Cyanide	0.008
Ash	6.33	Oxalate	0.42
Crude protein	7.73	Phytate	0.012
Crude fat	4.20		
Crude fibre	29.30		
Total carbohydrate	42.10		

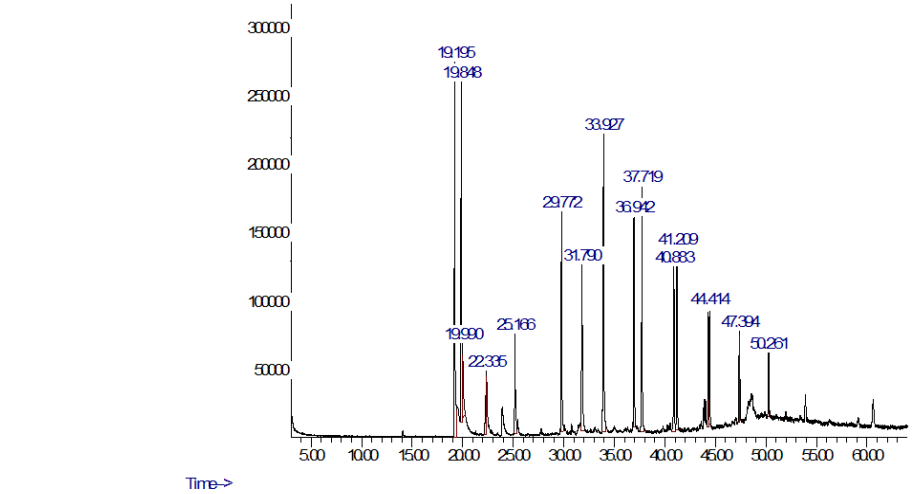

Fig. 2. GC-MS Chromogram for Ethyl acetate extracted *Ganoderma lucidum* fruiting bodies

Table 2. Phytochemical composition of crude N-hexane and ethyl acetate extracts of *Ganoderma lucidum*

Phytochemicals	Ethyl acetate extract	N- hexane extract
Flavonoid	+	+
Tannins	-	-
Phenol	+	+
Saponins	+	+
Sterols	+	+
Terpenoids	+	+
Cardiac glycosides	+	+
Volatile oils	-	-
Anthraquinones	-	-

Key: + showed it is present; - showed it is absent

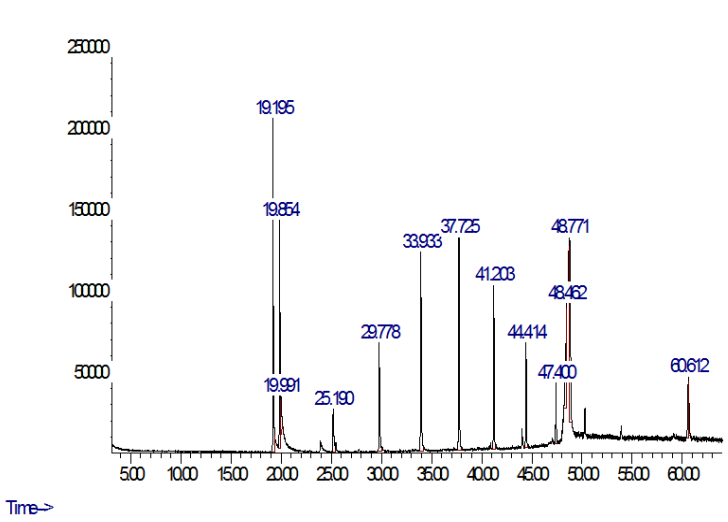

Fig. 3. GC-MS chromogram for N-Haxane extracted *Ganoderma lucidum* fruiting bodies

Table 3. List of compounds in ethyl acetate *Ganoderma lucidum* crude extract fraction detected by GC-MS

S/N	Compound	Chemical formula	Retentionti me	Area %	Class
1	Propanoic acid, 2-methyl-3-hydroxy-2,4,4-trimethylpentyl ester	$C_{12}H_{24}O_3$	19.195	12.33	Saturated fatty acid
2	Butanoic acid, butyl ester	$C_8H_{16}O_2$	19.848	11.88	Saturated fatty acid
3	Propanoic acid, 2-methyl-dodecyl ester	$C_{14}H_{28}O$	19.990	3.36	Saturated fatty acid
4	17-Pentatriacontene	$C_{37}H_{74}$	44.290	3.38	Saturated fatty acid
5	Cyclooctacosane	$C_{28}H_{56}$	47.364	2.05	Saturated fatty acid
6	Tricosyl trifluoroacetate	$C_{25}H_{47}F$	47.394	2.24	Saturated fatty acid
7	Hexacosyl heptafluorobutyrate	$C_{30}H_{53}F_7O_2$	50.261	2.30	Saturated fatty acid
8	5-Eicosene, (E)-	$C_{20}H_{40}$	22.335	2.76	Unsaturated fatty acid
9	Cetene	$C_{16}H_{32}$	25.166	4.12	Unsaturated fatty acid
10	1-Octadecene	$C_{18}H_{36}$	29.772	7.17	Unsaturated fatty acid
11	3-Eicosene, (E)-	$C_{20}H_{40}$	31.790	8.15	Unsaturated fatty acid
12	10-Heneicosene (c,t)	$C_{21}H_{42}$	36.942	7.01	Unsaturated fatty acid
13	1-Docosene	$C_{22}H_{44}$	37.719	8.00	Unsaturated fatty acid

radicals while flavonoid scavenge or chelate free radicals [23,24]. The ability of hexane as a solvent system to extract phytochemicals 1-Docosene and Eicosene fractions agrees with the report of Yogeswari et al. [25] that hexane is a better solvent system in extraction.

Table 4. List of compounds in N-Hexane *Ganoderma lucidum* crude extract fraction detected by GC-MS

S/N	Compound	Chemical formula	Retention Time	Area %	Class
1	Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	$C_{12}H_{24}O_3$	19.195	14.10	Saturated fatty acid
2	Propanoic acid, 2-hydroxy-, 2-methylpropyl ester	$C_7H_{14}O_3$	19.854	13.10	Saturated fatty acid
3	Propanoic acid, 2-methyl-, butyl ester	$C_8H_{16}O_2$	19.991	1.90	Saturated fatty acid
4	Behenic alcohol	$C_{22}H_{46}O$	37.725	10.40	Saturated fatty acid
5	Trifluoroacetoxy hexadecane	$C_{18}H_{33}F_3O_2$	41.203	7.47	Saturated fatty acid
6	1-Heneicosanol	$C_{21}H_{44}O$	44.414	4.93	Saturated fatty acid
7	17-Pentatriacontene	$C_{37}H_{74}$	47.400	2.89	Saturated fatty acid
8	Benzenepropanoic acid, 3,5-bis(1,1 -dimethylethyl)-4-hydroxy-, octadecyl ester	$C_{35}H_{62}O_3$	48.462	8.10	Saturated fatty acid
9	5-Octadecene, (E)-	$C_{18}H_{36}$	25.190	3.24	Unsaturated fatty acid
10	1-Nonadecene	$C_{19}H_{38}$	29.778	6.04	Unsaturated fatty acid
11	3-Eicosene, (E)-	$C_{20}H_{40}$	33.933	11.06	Unsaturated fatty acid
12	1-Docosene	$C_{22}H_{44}$	37.725	10.40	Unsaturated fatty acid
13	Butane, 2,2-dimethyl-	C_6H_{14}	19.195	14.10	Alkane
14	Silane, diethylheptyloxyoctadecyloxy-	$C_{29}H_{62}O_2Si$	60.612	2.55	Alkane
15	Zinc, bis[[5,5'-methylenebis[3,4-dihydro-4,4-dimethyl-2H-pyrrol-2-on ato]](1-)-N1,N1']-, (T-4)-	$C_{26}H_{34}N_4O_4Zn$	48.462	8.10	Organometallic
16	Propanamide, 2,2-dimethyl-N-(3-methylphenyl)-	$C_{12}H_{17}NO$	48.771	11.19	Organic
17	9-O-Pivaloyl-N-acetylcolchinol	$C_{25}H_{31}NO_6$	59.599	3.02	Alcohol/Organic
18	Testosterone-17.beta.-cypionate-3-methyloxime	$C_{28}H_{43}NO_3$	60.594	3.01	Organic

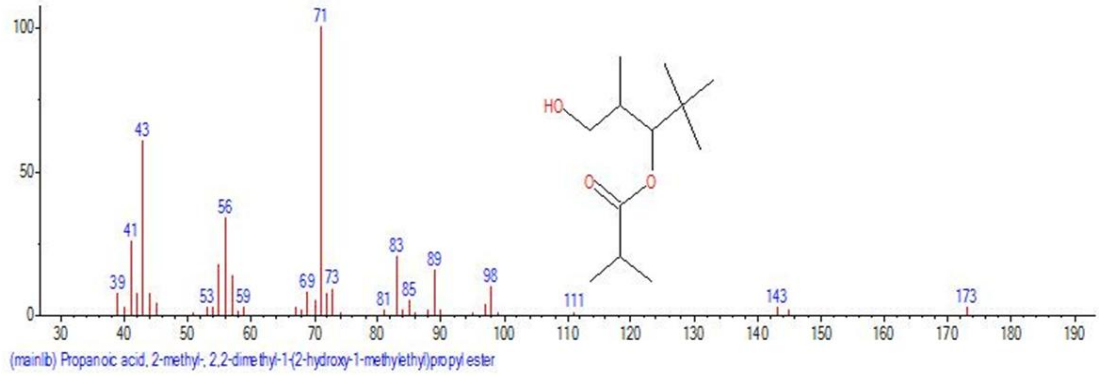

Fig. 4. Mass spectrum of propanoic acid, 2-methyl-, 2, 2 dimethyl -1(-2-hydroxy-1-methylethylpropyl ester

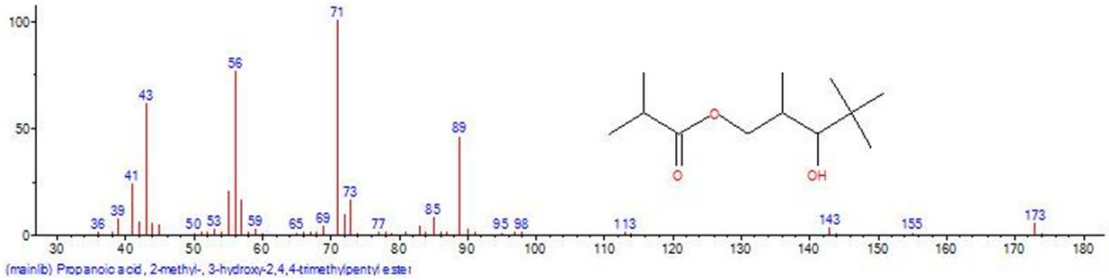

Fig. 5. Mass spectrum of propanoic acid, 2-methyl-3-hydroxy-2,4,4-trimethylpentyl ester

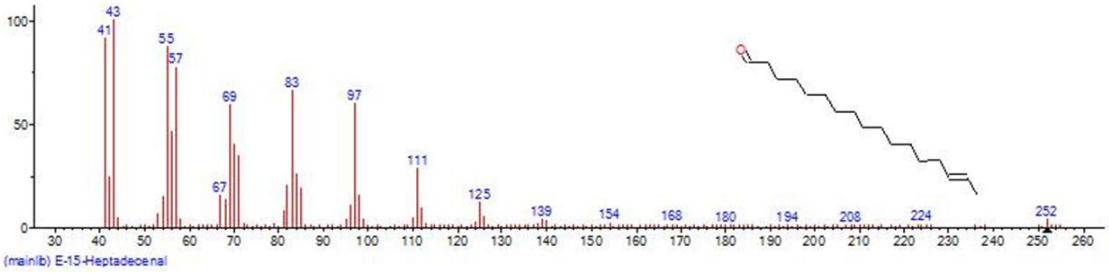

Fig. 6. Mass spectrum of E- 15-Heptadecenal

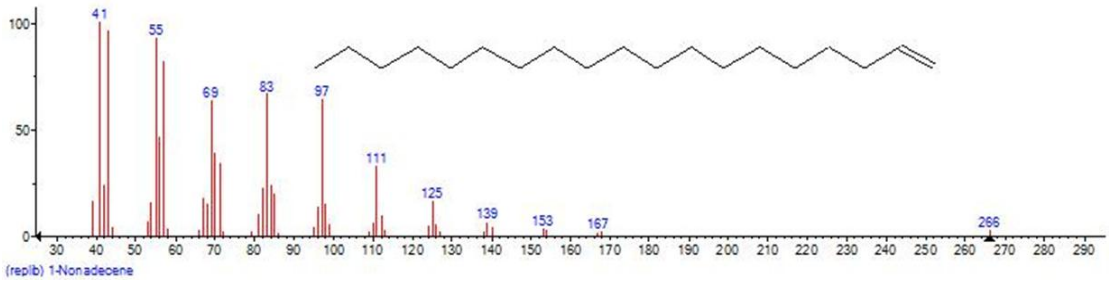

Fig. 7. Mass spectrum of 1-Nonadecene

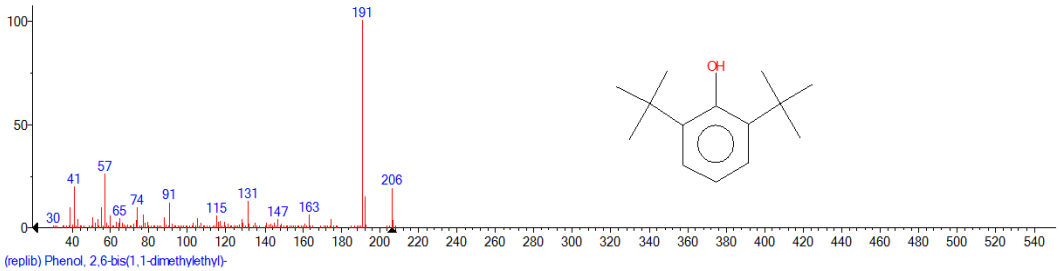

Fig. 8. Mass spectrum of phenol, 2,6 bis(1,1-dimethylethyl)-

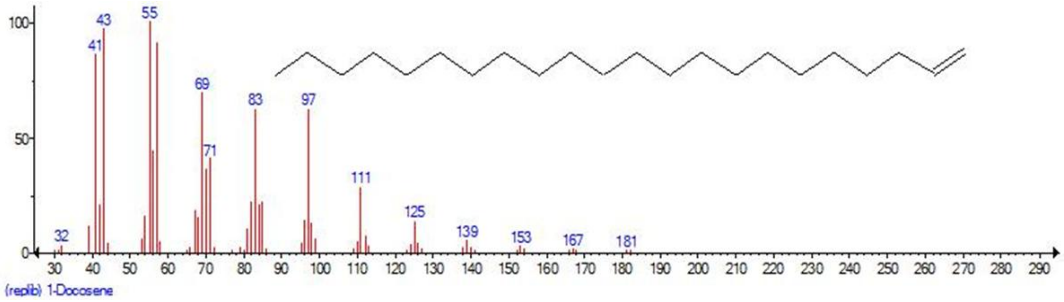

Fig. 9. Mass spectrum of 1-Docosene

The qualitative and quantitative Anti-Nutrient composition showed that *Ganoderma lucidum* contains Phytate, Oxalate, and cyanide. Phytate stores elemental phosphorous and myoinositol; and forms crystals which are excreted in urine when it combines with divalent metallic ions like Calcium and Iron [26]. Also excreted in urine is oxalate which binds calcium.

Metabolites and compound present in fungi extract have been reported to be of immense therapeutical importance to man, animals and plants. Extraction of the constituent compounds is dependent on a host of factors among which are temperature, time, and very critical is the solvent system. 1-Nonadecene, 9-eicosene extracted are long-chain fatty acids with anti-tuberculosis and antimicrobial activities [27], 5-Octadecene induces sexual attraction in lactating mothers [28], while Tricosyl trifluoroacetate found in the GC-MS result is reported to possess exfoliating activities thus conferring keratolytic potential on it. 1-docosanol, an antiviral agent also extracted is an alcohols which exhibits cytotoxic and hemolytic activities against viral cell wall; it is non-toxic, with teratogenic properties [29].

1-Octadecene present in *G. lucidum* is also present in other organisms and studies showed that the compound has anticancer, antioxidant and antimicrobial activities [30,31]. Yogeswari *et al*. [25] reported the abilities of 1-docosene, 1-octadecene, (E)-5-eicosene, 1-nonadecene to prevent the growth of bacteria. These compound belonging to unsaturated fatty acid group act as oxygen receptors thus they are strong radical scavengers [32]. Silane, diethylheptyloxyoctadecyloxy- a phytosterol present in the fungus has been reported to possess the ability to lower blood cholesterol level [33]. Benzenepropanoic acid, 3,5-bis(1,1 -dimethylethyl)-4-hydroxy-, octadecyl ester is toxic to green algae at EC50 > 30ppm for 72h, inhibitory to bacteria at concentration at IC20, IC50, IC80 > 100 ppm.

4. CONCLUSION

Ganoderma lucidum is therapeutically important as more metabolites and uses to which it could be put continue to surface coupled with extensive work already carried out on it. The result of this findings collaborate earlier work done on this fungi species and alluded to the fact that it harbors various bioactive compounds which confer its many importance resulting in various uses to which it is put. However, further investigative studies to better understand and unravel the mechanisms involved in its action should be pursued.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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