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Proximate Composition and Chemical Profiles of Reishi Mushroom (*Ganoderma lucidum* (Curt: Fr.) Karst)

T. Garuba[1*], G. S. Olahan[1], A. A. Lateef[1], R. O. Alaya[1], M. Awolowo[2], A. Sulyman[3]

[1]Department of Plant Biology, University of Ilorin, Nigeria

[2]Department of Biology, National Open University of Nigeria, Nigeria

[3]Department of Animal Health Production, Federal College of Wildlife Management, New-Bussa, Nigeria

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Abstract

Ganoderma lucidum is a mushroom commonly used in folk medicine especially Traditional Chinese Medicine (TCM) but information on its nutritional and chemical profiles remains insufficient. This work aimed at evaluating proximate composition and identification of bioactive compounds in ethanolic extract of *G. lucidum*. Pulverized *G. lucidum* was suspended in ethanol in 1:10 and extraction was carried out by rotary evaporation to produce *G. lucidum* extract (GLE). Proximate composition of the sample was analyzed. Fourier Transform Infrared (FTIR) spectroscopy was carried out to identify different functional groups in GLE. Gas Chromatography-Mass Spectrometry (GC-MS) was used to determine the bioactive compounds of the sample. Proximate analysis revealed that the amount of carbohydrate in sample was the highest (44.95%), followed by protein (15.75%). FTIR results showed that OH, C=O, C-OH, N-H 1* and 2* and alkyl halide are functional groups in GLE. A total of twelve (12) bioactive compounds were identified and the most prevailing compound in GLE was ethyl octadeca-9,12-dienoate (45.95%), followed by ethylhexadecanoate / ethyl palmitate (18.09%). Guaiacol (4.95%), octadecanoic acid (5.37%), ethylcyclohexane (3.31%) were also present. It can be inferred from this study that *G. lucidum* is nutritional and contains bioactive compounds that are useful in nutraceutical and pharmaceutical industries.

Keywords: Bioactive compounds; Chemical profile; Functional group; Mushroom.

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1. Introduction

Mushrooms are a group of macro-fungi with conspicuous epigenous or hypogenous fruiting bodies [1] and have been valued throughout the world as both food and medicine for thousands of years [2]. *Ganoderma lucidum* (Curt: Fr.) Karst. (Common names:

* *Corresponding author*: garuba.t@unilorin.edu.ng

Lingzhi or Reishei) belongs to phylum Basidiomycota and Family *Ganodermataceae* of Aphyllophorales [3]. It is called white rot fungus because of its ability to degrade lignocellulosic materials such as wood [4]. The mushroom is popular due to its pharmaceutical importance rather than its nutritional benefits because of its hard texture and bitter taste [5].This group of mushrooms is an underutilize source of bioactive pharmaceutical compounds [6]. The mushroom is used in folk medicine to prevent, manage or cure many diseases such as hepatitis, gastric ulcer, hypertension, bronchitis and hypercholesterolemia [7]. Biomedical investigations in China, Korea, Japan and USA revealed that the extraction of bioactive compounds such as triterpenes and polysaccharides from *Ganoderma* are good in preventing and treating medically important diseases including hypertension, diabetes, cancer and Acquired Immunodeficiency Syndrome (AIDS) [4]. Antibacterial activity of *G. lucidum* extract from basidiocarp against Gram-positive bacteria has also been reported [8]. Chinese believes that *G. lucidum* contributes to longevity and also serve as therapeutic factory [5]. DNA protection from breakage of strand triggered by UV irradiation or hydroxyl radicals is another protective role of *G. lucidum* [9].

Nutritionally, mushrooms provide many of dietary benefits found in meat, bean and grains [2]. Vegetarians take mushroom as an integral part of their meal. Fresh *G. lucidum* contains about 90% moisture and the residual 10% consist of ash (8–10%), protein (10–40%), fat (28%), carbohydrate (3–28%) and fibre (3–32%) [10]. It was reported that wild and artificially cultivated *G. lucidum* have similar nutritional component although extraction significantly increase the amount of carbohydrate and crude protein [11]. Polysaccharides in the mushroom contains glucose, mannose, xylose, fucose galactose and arabinose as monomers that contribute significantly to its antitumor, antioxidant and antibacterial properties [12].

G. lucidum fungus is an important mushroom and majority of researches had been centered on its traditional benefits and role in folk medicine. Information on its chemical profile remains obscure. This study aimed at evaluating the proximate composition and chemical profiles of ethanolic extract of *G. lucidum*.

2. Experimental

2.1. *Sample collection*

The mushroom (*Ganoderma lucidum*) samples were collected at Faculty of Management Science Area, University of Ilorin, Kwara State, Nigeria. The taxonomy of the samples was authenticated in the Herbarium unit, Department of Plant Biology, University of Ilorin. The samples were collected in a polythene bag, sliced and air-dried in the laboratory at 25 ± 2 °C for two weeks to a constant weight. They were pulverized using sterilized pestle and mortar.

2.2. *Preparation of Ganoderma lucidum extract (GLE)*

The pulverized samples of *Ganoderma lucidum* (800 g) were suspended in 8 L of ethanol for 72 h with continuous shaking by orbital shaker at 3000 rpm. The solution obtained was filtered with Whatman No. 4 filter paper and the resulting filtrates were evaporated in rotary evaporator at 50 °C to remove the extractant. The *G. lucidum* extract (GLE) was then stored at 4 °C for further use.

2.3. *Proximate analysis of Ganoderma lucidum*

Proximate composition of *Ganoderma lucidum* was determined using standard procedures of Association of Official Analytical Chemists [13]. Moisture was determined by water loss due to drying at a temperature of 105 °C. While crude protein was estimated by the Kjeldahl method, the crude fat was determined by extraction of a known weight of sample with petroleum ether using Soxhlet apparatus. The defatted sample was used to determine crude fibre. Carbohydrate was determined by difference.

2.4. *Fourier Transform Infrared (FTIR) Spectroscopy*

The GLE was analyzed to identify the types of chemical bonds (functional groups) present in the sample by FTIR (Thermo scientific Nicolet iS5 with iD1 transmission) spectrophotometer using KBr disc in the range of 4000 cm^{-1} to 400 cm^{-1}. A drop of the GLE was encapsulated with KBr pellet in order to prepare a translucent sample disc which was loaded in FTIR spectrometer.

2.5. *Gas Chromatography-Mass Spectrometry (GC-MS)*

The GC-MS analysis of GLE was carried out using Agilent Technologies 7890A Gas Chromatograph equipped to a mass detector Agilent Technologies 5975C inert MSD with Tripple-Axis Detector, 30m x 0.25mm ID x 1µm of capillary column. The instrument was set to an initial temperature of 110 °C and maintained at this temperature for 2 min. At the end of this period the oven temperature was increased up to 280 °C, at the rate 5 °C/min, and maintained for 9 min. injection port temperature was ensured as 250 °C and Helium flow as one mL/min. The ionization voltage was 70eV. The samples were injected in spilt mode as 10:1. Mass spectral scan range was set at 45-450 (m/z).

Using computer searches on a National Institute Standard and Technology (NIST Ver. 2.1 MS) data library and comparing the spectrum obtained through GC-MS, compounds present in the sample were identified. The names, retention time, molecular formula of the bioactive compounds were ascertained.

3. Results and Discussion

3.1 *Proximate composition of Ganoderma lucidum*

The results of the proximate analysis revealed that the amount of carbohydrate in the sample mushroom was the highest (44.95%), followed by protein (15.75%). Ash had the lowest quantity of 4.00%. The amount of crude fibre and moisture were 14.81% and 12.99% respectively (Fig. 1). The results agreed with the report which stated that carbohydrate and crude protein are the most abundant nutrient in *G. lucidum* [12]. The percentage of protein is similar to 15.04% as reported [14] and it is an indication that *G. lucidum* is a good source of protein despite that the fruiting bodies are corky, tough and thick. As a result of this, the mushroom can be used as a protein dietary supplement for both man and livestock [15]. The percentage of crude fat was found to be higher compared to previous studies [14,15]. The difference may be due to the nature of substrate, the part samples, habitat and place of sample collection [16]. Generally, mushrooms are called low fat calorie food which is attributed to high fibre content, no free fatty acids and no cholesterol [17]. The presence of a reasonable amount of fibre in *G. lucidum* facilitates movement of bulk via digestive track and averts constipation [18].

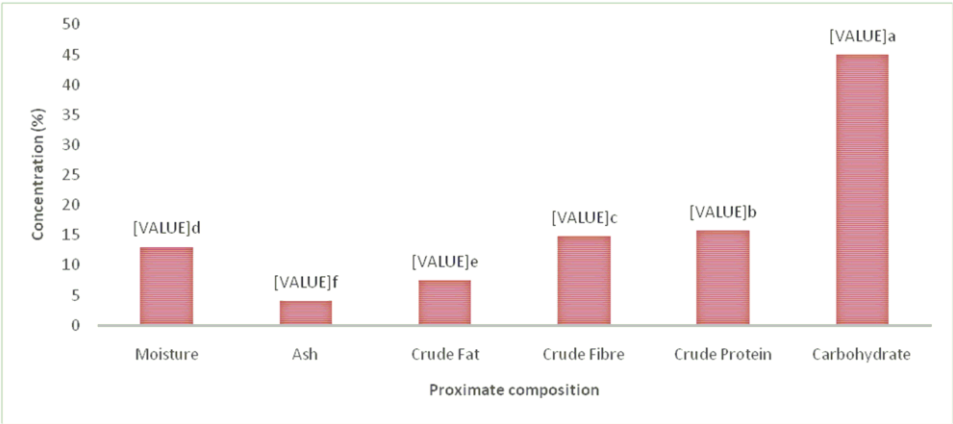

Fig. 1. Proximate composition of *Ganoderma lucidum.*

3.2. *Fourier Transform Infrared Spectrophotometry (FTIR) of GLE*

The observed peak positions from the FTIR spectroscopy of *Ganoderma lucidum* are summarized in Table 1. The peak at 3563 cm^{-1} is assigned to the –OH bond of water. The peak at 2926.65 cm^{-1} correspond to –CH stretch of alkane or –OH bond of carboxylic acid. The peak at 1720.32 cm^{-1} is due to C=O stretch of carbonyl. C=C stretch is responsible for the peak at 1460.69 cm^{-1}. The observed peak at 1042.70 cm^{-1} may be due to C-OH of carboxylic acid. The peak at 735.62 cm^{-1} is associated to primary and secondary amine while alkyl halide is responsible for the peak at 637.47 cm^{-1}. These

results indicate that sample contain alkyl halides, alcohols, esters, carboxylic acids, ethers, alkenes and amine compounds. The presence of carboxylic acids, amine and halogens may be responsible for medicinal properties of the extract [19].

Table 1. FTIR peaks of *Ganoderma lucidum* and the assigned bonds.

Wave number (cm)	Assigned bonds
3563.00	OH of alcohol
2926.65	CH stretching alkane
1720.32	C=O stretch of carbonyl
1460.69	C=C stretching
1042.74	C-OH of carboxylic acid
735.62	N-H 1* and 2* amine
637.47	Alkyl halide

3.3. *Gas Chromatography-Mass Spectrometry (GC-MS) of GLE*

The GC-MS chromatogram showed the absorbance level of each compound along with time (Fig. 2). The GC-MS analysis of GLE revealed twelve (12) compounds from the gas chromatography fraction of ethanolic extract of the sample. While the bioactive compounds with their retention time, peak or concentration (%), molecular formula and molecular weight are presented in Table 2, the biological activities and chemical nature of each bioactive compound are summarized in Table 3. The major and most prevailing active compound in GLE was ethyl octadeca-9,12-dienoate at RT of 39.99 with the peak of 45.95%. This polyenoic fatty acid compound is biologically recognized for its lowering cholesterol level and ability to prevent liver damage [20]. Ethyl hexadecanoate (ethyl palmitate) was the second most abundant compound (18.09%) in GLE at RT of 38.89. It is a stearic acid with antimicrobial property [21]. *G. lucidum* had previously reported to contain arrays of bioactive compounds that are therapeutically important such as alkaloids, steroids, polysaccharides and fatty acids [22]. This quality makes this Reishi mushroom gain ground in Tradition Chinese Medicine (TCM) [7].

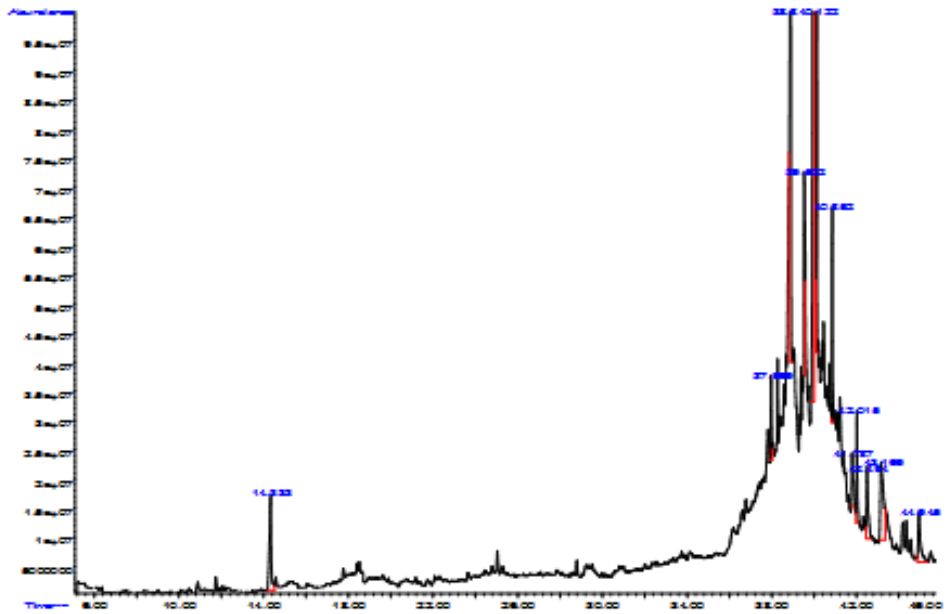

Fig. 2. Gas chromatogram of *Ganoderma lucidum* ethanolic extract.

Table 2. Bioactive compounds identified in *Ganoderma lucidum* ethanolic extract.

S/N	Retention Time	Peak (%)	Name of Compound	Molecular Formula	Molecular Weight (g/mol)
1	14.33	4.95	Guaiacol	$C_7H_8O_2$	124.14
2	37.97	1.81	Ethyl Pentadecanoate	$C_{17}H_{34}O_2$	270.45
3	38.87	18.09	Ethyl hexadecanoate (Ethyl palmitate)	$C_{18}H_{36}O_2$	284.48
4	39.53	3.13	Methyl 9-cis,12-cis-octadecadienoate (Methyl lineolate)	$C_{19}H_{34}O_2$	294.48
5	39.99	45.95	Ethyl octadeca-9,12-dienoate	$C_{20}H_{36}O_2$	308.50
6	40.12	5.37	Octadecanoic acid (Stearic acid)	$C_{18}H_{36}O_2$	284.48
7	40.85	3.31	Ethylcyclohexane	C_8H_{16}	112.22
8	41.79	2.06	Hexadecanoic acid, 2-hydroxyl-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.50
9	42.05	2.45	Bis (2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	390.56
10	42.49	3.15	Creatinine	$C_4H_7N_3O$	113.12
11	43.17	6.57	9,12-Octadecadienoyl chloride (z, z)	$C_{18}H_{31}ClO$	298.90
12	44.95	3.17	3, alpha, 5-cyclo-5, alpha, -ergosta-6, 8(14), 22t-triene	$C_{28}H_{42}$	378.64

Table 3. Biological activities of bioactive compounds in *Ganoderma lucidum* ethanolic extract.

	Name of Compound	Chemical Nature	Activities
1	Guaiacol (2-Methoxyphenol)	Phenolic compound	Antioxidant (superoxide free radicals), Stimulant expectorant, flavoring and gumming agent [23,24]
2	Ethyl Pentadecanoate	Fatty acid ester	Food additive as flavoring agent [25]
3	Ethyl hexadecanoate (Ethyl palmitate)	Stearic acid	Antifungal, Antitumor and Antibacterial [21]
4	Methyl 9-cis,12-cis-octadecadienoate (Methyl lineolate)	linoleic methyl ester	Oxidation/peroxidation assay, Antioxidant additive in biodiesel [26]
5	Ethyl octadeca-9,12-dienoate	Polyenoic fatty acid	Hepatoprotective, Antihistaminic, Hypocholesterolemic, Anti-eczemic [20]
6	Octadecanoic acid (Stearic acid)	Fatty acid	Production of detergent, soap, cosmetics such as shampoos [27]
7	Ethylcyclohexane	Cycloalkane	Organic solvent [28]
8	Hexadecanoic acid,2-hydroxyl-1-(hydroxymethyl)ethyl ester	Amino compound	Hemolytic, pesticide, flavor and antioxidant [21]
9	Bis (2-ethylhexyl)phthalate	Phthalate	Plasticizer
10	Creatinine	Creatine	Antimicrobial and Immunosuppressive [29,30]
11	9,12-Octadecadienoyl chloride (z, z)	Linoleic acid chloride	Nematicide, Antihistaminic, Anticoronary, Insectifuge, Antieczemic, Anticancer and Hypocholesterolemic agents [31].
12	3, alpha., 5-cyclo-5, alpha, -ergosta-6, 8(14), 22t-triene	Not Available	Not Available

4. Conclusion

Ganoderma lucidum is nutritionally important as it contains essential dietary elements. The quality makes the mushroom to be used as dietary supplement. GC-MS analysis unveils the bioactive compounds in GLE which revealed ethyl octadeca-9,12-dienoate as the dominant bioactive compound. If intensively explored, the mushroom will be a good candidate in pharmaceutical and nutraceutical fields. Further research is suggested to ascertain the toxicity profile of GLE so that its essentiality will be fully ascertained.

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