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Proximate Composition, Phytochemical and Elemental Analysis of Some Organic Solvent Extract of The Wild Mushroom- *Ganoderma lucidum.*

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Abstract

Brief about the title: The crude extract powder of *Ganoderma lucidum* harvested from Lafia, Nassarawa State of Nigeria during the rainy season was analyzed for proximate contents, phytochemical constituents and mineral composition using various standard methods. Description of the experiment and procedures: The harvested *Ganoderma lucidum* was air dried at 37^0C and grinded to powder; this was preliminary analyzed for proximate contents, phytochemical constituents. The crude powder was subjected to soxhlet extraction at 40^0C using Methanol, Ethylacetate and N-butanol to obtain different organic solvent fractions, these were then concentrated *in vacuo* at 24^0C for 48 hours to obtain different solvent extract fractions. These extracts were then analyzed for phytochemical contents using standards methods. Results: Analysis for proximate constituent showed Moisture contents was 10.54%, Total ash 5.93%, Protein 17.55%, Crude Fats 2.60%, Crude Fiber 30.25%, Carbohydrates 33.13%, and Nitrogen 23.52%. Phytochemical screening from unprocessed *G.lucidum* powder reveals the presence of: Alkaloids, Flavonoids, Reducing sugars, Tannins, Cardiac glycosides, Anthraquinones, Saponins, Volatile oils and Steroids. However, variations in the presence and concentrations of these phytochemicals were observed in the partitioned portions separated by Methanol, Ethyl acetate and N-butanol. Analysis of Total ash reveals in (mg/kg) the presence of Ca (322.6), K (317.1), P (197.1), and Na (193.5) in high quantity, while C (68.2), Fe (44.6), and Zn (14.65) and Mg (8.7.0), were found to be in moderate quantity. However, Si (4.10), Mn (1.83), As (1.23), Cu (0.84), Cr (0.14), Pb (0.106), Mo (0.09), Ni (0.095), F (0.0039), Al (0.20) and Co (0.026) are very low in concentration, but Se (0.00) was completely not detected. Conclusion: The presence of these essential nutrients and minerals found in *G.lucidum* implies that it can be utilized for its medicinal values in healthcare delivery systems, and the medicinal importance are thus highlighted in this work.

Keywords: *Ganoderma lucidum,* Fruiting body, proximate analysis, Phytochemical and Mineral, Healthcare

1. Introduction

Ganoderma lucidum is the common name for the mushroom called *"Lingzhi"* in Chinese, "*Reishi"* by the Japanese, *"Hangul"* or *"Yeongji"* in Korea, is also called *"Glossy ganoderma"* or *"shiny polyporus"* in English, and commonly called *"Leman kwado"* or *"Burtuntuna"* in Hausa. It is found rarely growing in the rainy season at the base of stumps of deciduous trees (National autobon society, 1993). This mushroom belongs to the kingdom "*fungi"* from the phylum "*Basidiomycota"* and in the class of *"Agaricomycetes"* and order *"polyporales"* , family *"Ganodomataceae"* and genus *"Ganoderma"* (Karsten, 1881), while its specie and binomial name is *"Ganoderma lucidum"*. (Wikipedia. 2011*)*.This mushroom is a polypore that is soft (when fresh) corky and flat with conspicuous red-vanished kidney shaped cap, and depending on age, white to dull brown pores are found underneath (Arora, 1986). It has a worldwide distribution and found growing in both tropical and temperate geographical regions including North and South America, Africa, Europe and Asia. The mushroom is normally found growing as parasite or saprotroph on a wide variety of trees. (Arora, 1986). *Ganoderma lucidum* has been used as medicinal mushroom in traditional chinese medicine (TCM) for more than 2,000 years (Kenneth, 1990), thus making it one of the oldest mushrooms known to have been used medicinally. Its ultimate herbal substance is based on its presumed health benefits and absence of side effects (Engelbrecht and Volks 2005). Among its many medicinal benefits, the mushroom is claimed to have antitumor activity, immunomodulatory and immunotherapeutic application. It is also said to have activity against hypertension and diabetes, with microbial activity on such micro-organisms as

Aspergilus niger, Bacillus cereus, Candida albicans and Escherichia coli. This mushroom was also reported to have antiviral activity with specific action on *HSV-1 and HSV-2, Influenza virus, Vessicular stomatitis* and HIV *type1* or a " fix it all" remedy for maladies (Lindequist *et al.,* 2005; Paterson, 2006), (Liu *et al.,* 2006), (Chinese Herbal Medicine, *Materia medica,* 2004), (Wang and Ng, 2006), (Moradelli *et al.,*2006), (el-Mekkawy *et al.,* 1998), (Engelbrecht and Volks 2005). Although these reports were mostly based on crude or hot water extracts, purification and specific identification of few chemicals, there is yet, the need to analyze the chemical compositions using various organic solvents soluble fractions from the extracts of the of the wild mushroom-*G.lucidum* (Mizuno, 2011) to assess its chemical composition.

2. Material and methods

2.1 Plant collection and identification

Fresh *Ganoderma lucidum* fruits were collected from Lafia in Nassarawa State, North-Central Nigeria in the rainy season, between the months of August-September, 2008, and it was transported to Maiduguri, North- Eastern Nigeria, using a clean polythene bag. The plant was identified and authenticated at the Department of Botany, University of Maiduguri, Nigeria. The mushroom was air dried to reduce the moisture content, on a clean shelf in the Laboratory of the Department of Veterinary Physiology, Pharmacology and Biochemistry at the faculty of Veterinary Medicine, University of Maiduguri, Nigeria. The dried mushroom was ground to fine powder using clean pestle and mortar, this powder was stored in an air tight black polythene bag at room temperature, until required for use.

2.2 Chemicals

Organic chemical solvents used for the extraction include reagent graded chemicals such as Methanol, Ethyl acetate and N-butanol.

2.3 Extract preparation

The prepared dried *Ganoderma lucidum* was weighed *(*1.5kg) using a metler balance (Toledo-PB 153, Switzerland) and placed in a thimble then into a soxhlet chamber, to this was added 7.5 litres of Methanol and the mixture was steamed at 40^{o}C for 24 hours until the methanol fraction was completely extracted, the filtrate was evaporated within a period of 24 hours, using electric evaporator. using the same procedure, the residue thereof was then washed with 7 .5L of ethyl acetate to obtain ethyi acetate soluble fraction, and the remains (residue) there from was also washed with 7,5Lof n-butanol reagents to equally obtain the n-butanol soluble fraction. The different organic solvent filtrates realized were concentrated *in vacuo* at 20^{0}C and the extracts realized from the different organic solvents were weighted and observed for their colors, textures, tastes and various pH values they were kept in a clean beaker and covered airtight with foil paper until needed for use.

2.4 Proximate analysis

Analysis for proximate contents of the dried powder of *Ganoderma lucidum* was done by methods described by American Organization for Analytical Chemistry-AOAC (1998). The sample was weighed (0.1g) and was analyzed for moisture contents, Carbohydrates, Crude fiber, Crude protein, Total ash, Crude fats (lipids). Nitrogen was analyzed by kjeldal method as described by American Organization for Analytical Chemistry (1998).

2.5 Phytochemical analysis

Each of the different organic solvent soluble fractions of the extracts (Methanol and N-butanol) was weighed (2g) and dissolve in 5mls distilled water while Ethyl acetate soluble fraction was dissolved by a 1 ml of ethyl acetate and made up to volume by adding 4mls of distilled water. These were analyzed for phytochemicals using methods described by Allen (1974) and Harbone (1976). The different phytochemical constituents tested for include: Tannins, Saponins, Anthraquinones, Cardiac glycosides, Flavonoids, Steroids, Alkaloids, Terpenoids, Reducing sugars, Soluble starch, Carbohydrate, Phlebotannins and Volatile oils.

2.6 Elemental analysis

The crude powder (2g), before subjecting it to various organic solvents, was ashed in an oven at 60^{0}C for three hours, 0.5g of the cooled ash was digested by heating for two hours with a mixture of 10mls Hydrochloric acid (HCl) Nitric

acid (HNO_3) and hydrogen tetra oxochloric acid ($HCLO_4$). The digested mixture was evaporated down to 5mls using rotator evaporator; it was then made up to 10mls with 2M HNO_3, and to which was added 30mls of distilled water and kept in a 100mls beaker. Reagent blank samples were also prepared , these sample were analyzed for: Ca, Fe, Zn, Cu, Co, Mn, Mg, Pb, Mo, Se, Cr, As, Ni, C, Si, Al using Winlad 32 software flame atomic absorption spectrophotometer (AAS), while Na, K P, and F, were determined by flame emission spectrophotometry (AOAC, 1998).

2.7 Statistical analysis

Results from findings were presented in simple concentrations based on percentages.

3. Results

A bitter tasting, chocolate coloured, sticky extract with a pH of 6.9 and which dissolves in water to give a greenish-yellow colour was realized from the methanol fraction of the mushroom, it was weighed, and the yield was calculated to be 79.5g. (5.3%), Ethyl acetate fraction had a pH of 6.9 too and yielded a black colored bitter tasting extracts that was immiscible in water, but miscible in ethyl acetate, it was weighed and gave 12.86g (0.91%), and N-butanol fraction yielded water soluble, bitter tasting, chocolate colored extract with a pH of 7.1, and weighed 12.76g (0.91%).

Analysis for proximate chemical composition in percentages, from 0.1g of the crude powder of the mushroom was observed (Table I.) to contain: 10.54% moisture, 5.93% total ash, 17.55% crude protein, 2.60% crude fat, 30.25% crude fiber, 33.13% carbohydrates and 23.52% nitrogen. Phytochemical analysis from dried mushroom powder was done before and after fractionation with different organic solvents. The powder, before fractionation showed the presence of Alkaloids, Flavonoids, Carbohydrates, Tannins, Cardiac glycosides and Saponins. (Table II). However, the phytochemical constituents from various organic solvents after fractionation with Methanol, Ethyl acetate and N-butanol, (Table III), showed difference and variations from that in Table II. Results for elemental composition of *Ganoderma lucidum* is presented in Table IV, this results showed high concentration of calcium (322.6mg/kg), potassium (317.1mg/kg), phosphorus (197.1mg/kg) and sodium (192.5mg/kg). Moderate presence of carbon (68.2mg/kg), iron (44.6mg/kg) and zinc (14.65mg/kg) were observed. However, other elements such as magnesium (8.7mg/kg), silicon (4.10mg/kg), arsenite (1.23mg/kg) and manganese (1.03mg/kg) were found in moderately slight concentrations.

Elements such as copper (0.843mg/kg), aluminum (0.20), chromium, (0.140mg/kg), lead, (0.106), molybdenum nickel (0.095mg/kg), (0.090mg/kg), cobalt (0.026mg/kg) and fluorine (0.0039mg/kg), were found in this study, to be in trace quantity, but selenium (0.00mg/kg) was totally absent.

4. Discussion

The result from proximate analysis of *G.lucidum* (Table I) showed high content of protein (17.6%) which is higher than reported by Ogbe *et al.,* (2008), Carbohydrates (33.13%) and low levels of fat (2.60%). Their presence in this finding agrees with that of Ogbe *et al.,* (2008), which suggest the high nutritional values of this mushroom. Wasser, (2005) reported that more than 100 polysaccharides are found in *G. lucidum* and these polysaccharides are considered to contribute to the bioactivity of the mushroom. The polysaccharide is also known to potentiate immune function by binding to leukocyte surfaces or serum specific proteins leading to activation of macrophages, T-helper cell, Natural killer (NK) cells and other effector cells, and this can be the basis for its anti inflammatory effect, The high protein content suggest its importance in cellular function and tissue regeneration. It has been reported that protein isolate (LZ-8) from this mushroom can suppress bovine serum albumin induced anaphylaxis and an important means of managing histamine mediated allergic responses (Mueller *et al.,* 2000, Muller *et al.,* 2006; Stavinoha, 2011; Kawagishi *et al.,* 1997; Powell, 2006)

Low fats content (2.60%), as reported in other plants (Ogbe *et al.,* 2012) shows the health benefits of this mushroom and stressing its nutritional value, it is reported that extract from this mushroom has cholesterol lowering properties in Hamsters and Minipigs (Berger *et al.,* 2004) hence , its antihypertensive potentials in humans (Mizuno, 2011).

The Nitrogenous component consists of 23.52% (Table I), and this provide essential requirement for nucleotides and nucleosides formation in the body, these amino acids, are important components of DNA and RNA that are useful in cellular function and cell differentiation, thus, its mitogenic capacity. (Wasser, 2005)

The high fiber (32.13%), low moisture (10.54%) and 5.93% Total ash contents implies its digestibility and high absorption rate to provide energy requirements for cellular and gastrointestinal functions.

Preliminary phytochemical screening of *G.lucidum* unprocessed powder, reveals the presence of phytochemicals which are absent in the fractionated extracts, this is a possible explanation to metabolites formation in the body when extract of this mushroom is ingested, and the formed metabolites may be responsible for the bioactive properties of the mushroom.

The presence of alkaloids in the mushroom powder explains its anti-bacterial activity, since this phytochemical is reported to have anti-bacterial activity (Idowu *et al.,* 2003). However, this property may be lost during fractionation with Methanol, Ethyl acetate and N-butanol which showed absence of this phytochemical.

The polyphenol, Flavonoids, found in weak concentration in this study are known to be source of plant based antioxidants which can protect the nerves, heart, liver and other organs and tissues. This anti-oxidant property (Saikumar *et al.,* 2010) may be responsible for reduction of hepatic damage (Lakshmi *et al.,* 2006)

Carbohydrates or high fiber fraction of diets which are broadly classified as polysaccharides or indigestible carbohydrates are known to inhibit colonization of pathogenic microbial flora in the intestines, hence, the elimination of these pathogens from the gut system accompanied by improved immunity. (*Guo et al.,*2003).The high concentration of carbohydrates 33.13%, and fiber constituents (30.25%) were both lower than findings by Ogbe *et al.,* (2008), but can contribute significantly to anti-microbial activity of the mushroom, and the slight presence of these composites in the fractionated components, can compromise the anti-microbial properties of the mushroom in the methanolic fraction, despite high presence of soluble starch, when compared to anti-microbial activity of Ethyl acetate and N-butanol fractions, though they both showed slight presence of carbohydrates as reducing sugars

The presence of tannins in both the powdered and extract fractions of methanol and n-butanol which can complex with the metal ions and macromolecules such as proteins and carbohydrates (Dei *et al.,* 2007) obtained in the powdered sample can be utilized in weight reduction management.

Saponins, as secondary metabolites can be found as hydrophilic glycoside moiety combined with a lipophyllic triterpene derivative to form a therapeutically cardio-active agent in form of steroid-saponins and triterpenoid saponins (Dei *et al.,* 2007), when found in high concentration they can cause hypercholesterolemia by binding to cholesterol, thereby making it unavailable for absorption (Soetan and Oyewole, 2009). Saponins, in free forms are also reported to have hemolytic activity (Khalil and Eladawy, 1994), the bitter taste of this extract can be attributed to the Saponin-triterpenoid complex, however, Saponin-glycosides in this study, is found in moderate presence in the fresh powder of *G.lucidum* and with weak presence in all the organic solvent fractions, therefore, their surfactant activity can be used to enhance penetration of macromolecules such as proteins through cell membrane and can also be used as adjuvant in vaccines. (Wikipedia, 2012). Oral administration of Saponins leads to hydrolysis of glycosides from Terpenoids, hence reducing toxicity associated with intact glycoside molecule. (Wikipedia, 2012), Saponins are also reported to have anti-inflammatory, expectorant and immune stimulating effects (Ray Sahelian, 2012),

Cardiac glycosides reported in were found in moderate concentration in this study, this phytochemical has therapeutic use in cardiac failure, when administered, it acts by increasing intracellular calcium concentration, thus increasing cardiac output through increase in the force of contraction. The moderate presence of this phytochemical in this finding can suggest the cardio tonic activity of the mushroom extracts.

The aromatic organic compound, Anthraquinone, found in free state and in trace quantity adds to the medicinal value of this mushroom extract, derivatives of Anthraquinones have laxative (Dantron, Emodin), anti-malarial (Rufigall) and anti-neoplastic use (Mitoxanthene, pixantron and the anthracyclines) (Wikipedia, 2012), however, this phytochemical was not detected in all the fractionated portions of the mushroom, this can be a probable explanation of the formation of other metabolites, not tested in this study, (derivatives in the body systems) when consumed and the medicinal values it has in related disease.

Phlebotannins are not found in this study from both the fractionated portion of the mushroom and the fresh powdered part, as was reported by Ogbe *et al.,* (2008). This can be explained on the effect of seasonal variation (Kamiya *et al.,* 2010) this phytochemical is reported to exert its effect through its derivative-phloroglucinol which has non -specific effect in the treatment of gallstone, spasmodic pain and related gastrointestinal disorders (Chassany *et al.,* 2007).

Volatile oils (essential oils) found in moderate concentrations in this study, are reported to be medically important in respiratory disinfection, decongestion and expectoration, this provide the basis for its claimed expectorant and anti-tussive effects (HealthyGanoderma.com 2011). The anti-bacterial and anti-viral properties of this essential oil from *G.lucidum* has also been reported by Carson *et al., (*2006) and Edris *et al.,* (2007) in *in vitro* studies

High concentration of steroids was also observed in this study, this can explain the claims of analgesic effects of the mushroom. This finding agrees with that of Ko *et al.,* (2008) who reported that steroids found from *G.lucidum* includes 0.3-0.4% which has anti-inflammatory activity. These steroids are also precursors of ganoderic acid and protease inhibitors (Wikipedia, 2012).

Triterpenoids are the bitter tasting phytochemical which gives the extract its bitter taste, the terpenoids are said to form complexes with steroids (sterols) to provide the said anti-inflammatory effects of this wild mushroom and equally, its anti-bacterial property (Wikipedia, 2012).

Appreciable concentrations of various elements was revealed from the elemental analysis of the *G.lucidum* powder; Calcium has 322.6mg/kg, potassium 317.1mg/kg, phosphorus 197.1mg/kg sodium 193mg/kg, these elements are found in high concentration while carbon 68.2mg/kg, iron 44.6mg/kg, zinc 14.65mg/kg and magnesium 8.7mg/kg, are found to be in moderate concentration when compared to other elements. The above observed elements have physiological importance and maintenance of cellular enzymatic functions; these elements are required for normal growth, muscular activity and skeletal muscle development, especially calcium (Ogbe *et al.*, 2012), blood viscosity; Calcium, manganese and cobalt, oxygen transport and cellular activity are enhanced by elements such as copper and iron. Manganese is found with lecithin and needed as a co-factor for some enzymes, especially in the synthesis of fatty acids and cholesterol, it is also involved in chemical reaction the body and assists in intestinal nutrients absorption; it is also an important co-factor in energy production through the ATPase channel and by supporting immune system (Muhammad *et al.,* 2011). Manganese complex with Vitamin K to enhance blood clotting factor and with Vitamin B complex to reduce effect of stress (Muhammad *et al.,* 2011), thus providing a strong anti-oxidant effect as claimed (HealthyGanoderma.com 2012). Sodium and potassium are required for the maintenance of fluid balance, while potassium and calcium are important in stimulating action potential across nerve endings, and also to enhance heart contractile rate. Iron is highly required physiologically for heme formation and to enhance oxygen carrying capacity of red blood cells. Zinc is an important requirement in protein synthesis, normal body development and recovery from illnesses. it is a co-factor in the function of the enzyme carbonic anhydrase required for carbon dioxide transport and as part of peptidases needed for protein digestion (Muhammad *et al.,* 2011), it is also a necessary part of DNA, for cell division and synthesis hence its importance in wound healing (innvista.com/health/elements.htm, 2012).

Other elements found in trace quantities include, lead 0.11mg/kg, nickel 0.095mg/kg, fluorine 0.0039mg/kg, molybdenum 0.090mg/kg, arsenite 1.23mg/kg, chromium 0.14mg/kg, alluminum 0.020mg/kg, silicon 4.10mg/kg. Some of these elements, especially lead and fluorine are toxic, and are found in this study to be in negligible quantity implying that the mushroom extract may be less or non toxic for these elements. However, chromium is involved in insulin regulation and sugar metabolism, and this can explain the claim of treatment of insulin dependent diabetes. It is observed that Selenium, an important requirement in skeletal muscle function and coloration is absent.

4.1 Conclusion

Results of this study showed that extract from *Ganoderma lucidum* harvested from Lafia, Nassarawa State, Nigeria demonstrate appreciable quantities of Carbohydrates, Crude protein, Crude fats (Lipids), and essential mineral element required by the body for normal function of organs and tissues, it also showed that fractionation of this mushroom extract can alter the presence or absence of a phytochemical, and this can affect some of the claimed

effects of the mushroom in disease conditions. Hence the need to evaluate the toxicity and anti-microbial activity of both the crude and organic solvent fraction of this extract.

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Table I: Proximate chemical composition of crude *G.lucidum* powder.

Parameter	Contents (%)
Moisture	10.54
Total ash	5.93
Crude protein	17.55
Crude fat	2.60
Crude fiber	30.25
Carbohydrates	33.13
Nitrogen	23.52

% = percent.

Table II: Qualitative Phytochemical compositions of crude extract *G. lucidum* powder.

Phytochemicals	Tests	Observations
Alkaloids	Dragendorff's	+
	Meyer's	+
Flavonoids	Shinoda's	–
	Ferric chloride	–
	Lead acetate	+
	Sodium hydroxide	–
Carbohydrates	Molisch's	+
	Monosaccharide's	–
Reducing sugars	Fehling's	+
	Combined reducing sugars	+
	Ketone's	–
	Pentose	–
Tannins	Ferric chloride's	–
	Lead acetate	+
	Hydrochloric acid	–
Phlebotannins	Phlebotannins	–
Cardiac glycosides	Salkowski's	+
	Lieberman's	–
	Total glycoside's	+
Terpenoids	Terpenoids	–
Anthraquinones	Free Anthraquinones	+
	Combined anthraquinone	–
Saponins glycosides	Frothing's	+
	Fehling's	+
Volatile oils	Fehling's	+
Steroids	Salkowski's	+

+ Present. - Absent.

Table III: Qualitative phytochemical composition of *G.lucidum* from different organic Solvent soluble fraction

Phytochemical.	Tests.	Observations from different organic solvent soluble fractions		
		Methanol.	Ethyl acetate.	N-butanol.
Alkaloids	Dragendorff's reagent	+	+	+
	Meyer's reagent	+	+	+
Flavonoids	Shinoda's	+	+	+
	Ferric chloride	+	+	+
	Lead acetate	+	+	+
	Sodium hydroxide	+	+	+
Carbohydrates	Molisch's	+	+	+
	Bartoed (monosaccharide)	+	+	+
	Fehling's	+	+	+
	Combined reducing sugars	+	+	+
	Ketones	+	+	+
	Pentose	+	+	+
	Soluble starch	+	+	+
Tannins	Ferric chloride	+	+	+
	Lead acetate	+	+	+
	Hydrochloric acid	+	+	+
Phlebotannins	Phlebotannins	+	+	+
Cardiac glycosides	Salkowski's	+	+	+
	Lieberman-Bucharnard's	+	+	+
Anthraquinone	Free anthraquinones (Berntragent's)	+	+	+
	Combined anthraquinones	+	+	+
Saponin glycosides	Frothing	+	+	+
	Fehling's solution	+	+	+
Terpenoids	Terpenoids	+	+	+

\+ Present - Absent

Table IV: Elemental composition of crude extract of *G.lucidum* powder.

Elements	Content (mg/kg)	WHO Standards, (µ/g)
Sodium	192.5	400-500
Calcium	322.6	3,600-80,000
Magnesium	8.7	100-200
Iron	44.6	50-5,000
Potassium	317.1	10-100
Lead	0.106	5-30
Copper	0.843	100-300
Zinc	14.65	150-20,000
Manganese	1.03	100-20,000
Chromium	0.140	300-14,000
Phosphate	197.1	5-300
Arsenite	1.230	100-200
Molybdenum	0.090	300-5000
Nickel	0.095	200-5000
Carbon	68.2	360-800
Fluorine	0.0039	300-5000
Silicon	4.10	400-10,000
Aluminum	0.20	200-30,000
Cobalt	0.026	100
Selenium	0.00	10-2,000

1pmm=1mg/kg