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PHYTOCHEMICAL ANALYSIS OF CULTIVATED MEDICINAL MUSHROOM- *GANODERMA SP.*

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ABSTRACT

The aim of this study was to evaluate the phytochemical contents of indigenous cultivated *Ganoderma sp*. (Curt.) P. Karst. in comparison to other mushrooms found in literature. The cultivated indigenous medicinal mushroom (*Ganoderma sp.)* can be available all-year round, as compared with the wild or imported/foreign *Ganoderma sp*. which are scarce, seasonal and expensive. Phytochemical analysis was carried out using Methanol, Ethanol, Dichloromethane and Aqueous extracts. Preliminary tests indicated the presence of secondary metabolites (alkaloids, tannins, phenols, terpenoids, flavonoids, steroids) and high carbohydrate contents in the cultivated *Ganoderma* fruiting bodies. Results of the quantitative analysis showed the highest value of phenol in aqueous extract, with the least value in Dichloromethane extract for flavonoids. The results obtained from this study reveals that the cultivated indigenous medicinal mushroom, *Ganoderma sp*. is a potential source of secondary metabolites which are beneficial as nutraceuticals in industries and pharmaceuticals.

Keywords : *Ganoderma sp.*; extracts; phytochemicals; cultivated; methanol

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INTRODUCTION

Herbal medicines and supplements have become trendy due to popular belief that green medicine is safe, easily available and with less side-effects (Savithramma *et al*., 2011). The use of herbal medicine/supplements has been attributed to about 80% of the world's population, especially for millions of people in developing countries, who rely on them for relief from ailments and diseases (WHO, 2000).

Medicinal mushrooms from Asia have been reported to display a range of biological activities which are mainly attributed to antioxidants as well as theurapeutic values (Yue *et al*., 2012). These biological activities are enhanced by phytochemicals which are secondary metabolites including alkaloids, flavonoids, triterpenoids, polysaccharides, tannins, sterols, phenols, gums, glycosides and coumarins (Royse, 2005). These are biocatalysts synthesised during metabolism of mushrooms and are potential source of drugs. Investigation on these phytochemicals has been associated with antioxidant agents which are able to reduce the damages caused in tissue during physiological processes as well as being capable of lowering cholesterol and blood pressure levels, along with many other health-related benefits (Fakoya *et al*., 2013). Ttriterpenoids isolated from *G. lucidum* has shown beneficial effects on the frostbite healing process by increasing the wound healing area and improving the degree of pathological change in skin tissue of rats with frostbite (Shen *et al*., 2016).

Medicinal mushrooms have been in use as prophylactic agents in many health-related ailments such as immuno-modulatory, hypertension, asthma, antitumor activity, arthritis, cardiovascular effects, arteriosclerosis, respiratory, bronchitis, gastric ulcer, cancer and anti-inflammatory (Bishop *et al*., 2015). Mushrooms are also shown to be highly nutritive with high content of carbohydrate, proteins, vitamins, minerals, fibers and low/no calories and cholesterol (Thatoi and Singdevsachan, (2014).

Ganoderma sp. (Curt.) P. Karst. which possesses a wide range of biological activities due to its nutritional, antioxidant and therapeutic values (Cör *et al*., 2018), belongs to the Basidiomycetes and has been used in traditional Chinese medicinal herbs (TCM) for thousands of years in the treatment of several diseases and is referred to as "Mushroom of life enhancer and God of herbs" (Al-Obaidi *et al*., 2016).

Most people are scared of consuming mushrooms from the wild, as they are not able to distinguish between the edible and the poisonous species because of the morphological similarities. There is, therefore, the need to cultivate edible and medicinal mushrooms for culinary uses as well as for their nutritional and health benefits. Although few studies on phytochemistry of *Ganoderma* mushroom has been carried out on wild species in Nigeria, this research was aimed at determining the qualitative and quantitative phytochemical components in methanol, ethanol, dichloromethane and hot water extracts of cultivated *Ganoderma sp*. in order to harness and produce plant secondary metabolites for medicinal and industrial purposes.

MATERIALS AND METHODS

Sample collection

Ganoderma sp. was collected from wood stumps of *Delonix regia* (flamboyant tree) at Igikhinwin village along Benin-Akure road; identified and grown from the pure culture to the harvesting stage (Ihayere *et al*. (2017) at the African Centre for Mushroom Research & Technology Innovations [ACMRTI] in Faculty of Life Science, University of Benin, Benin City. Medium for pure culture was PDA (Potato Dextrose Agar) and source of substrate medium was saw dust from *Brachystriga nigerica* (Ihayere *et al*., 2017). Fruiting bodies used for this study were harvested at full maturity, 10 days after appearance of primodium. The fruiting bodies were properly washed with distilled water, air-dried and pulverised. The powdered samples were stored in air-tight glass containers.

Preparation of crude extract

Ten grams of mushroom powder (*in-vivo* harvested fruit bodies) was subjected to Soxhlet extraction for 8 hours using 100 ml each of the following solvents: hot water (70^0 C), methanol, ethanol and dichloromethane. (Quereshi *et al*., 2010). These extracts were subjected to qualitative and quantitative phytochemical determination using standard procedures as follows:

For qualitative phytochemical analysis of Carbohydrates, Flavonoids, Triterpenoids, Phenols and Sterols, and Tannins and Alkaloids, the procedures by Harborne (1998), Harborne (1973), Ayoola *et al*. (2008), Sofowora (1993), and Trease and Evans (1989) were followed, respectively. The quantitative analysis /determination for the various phytochemicals were carried out as outlined below:

i. Alkaloids

Five grams of each *Ganoderma* extract was dispersed separately in 10% acetic acid to form a ratio of 1:10 (10%). Each mixture was allowed to stand for 4h at 28ºC, filtered with Whatman No 42 grade of filter paper to one-quarter of its original volume by evapouration and treated with drop-wise addition of concentrated aqueous NH_4OH until the alkaloid was precipitated. The alkaloid precipitated was received in a weighed filter paper, washed with 1% ammonia solution and dried in the oven at 80 ^{0}C (Harborne, 1973).

ii Total Tannins Content (TTC) Tannins were determined by the method of Peri and Pompei (1971). One (1) ml of each extract (hot water, methanol, ethanol and Dichloromethane) of concentration (1mg/ml) was taken in a test tube and 1ml of distilled water was added while another of distilled water served as the blank. To each of the test tubes, 0.5 ml of Folin's phenol reagent (1:2) was added followed by 5 ml of 35% sodium carbonate and kept at room temperature for 5 min. Blue colour was formed and the colour intensity was read at 640 nm. The tannin content was expressed in % mg of GAE /g of extract.

iii Total phenols

Total phenolic content was determined by the Folin–Ciocalteau reagent method according to Ainsworth and Gillespie (2007). To 200 ml of each extract (hot water, methanol, ethanol and Dichloromethane), 1ml of 0.2 N Folin–Ciocalteau reagent and 0.8 ml of 7.5% sodium carbonate solution were added, mixed well and allowed to

Stand for 30 min at room temperature. Absorption at 765 nm was read using a Shimadzu 300 UV spectrophotometer. Quantification was based on the standard curve generated with 100– 400 mg/l of garlic acid. The total phenolic content was expressed as garlic acid equivalent (GAE) per 100 g of dry weight (dw).

iv Triterpenoids

After filtration, each extract was further extracted with 10 ml of petroleum ether using separating funnel, into pre-weighed glass vials and left completely dry (evapourated). Total terpenoids contents was measured according to Indumathi *et al.* (2014).

v. Flavonoids

Total flavonoid content was measured using the aluminium chloride colorimetric assay. The reaction mixture consisting of 1 ml of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask which was treated with 0.30 ml of 5 % sodium nitrite and 0.3 ml of 10 % aluminium chloride each for 5 minutes. This was followed by 2 ml of 1M Sodium hydroxide and diluted to 10 ml with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) was prepared and the absorbance for test and standard solutions was determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed in mg of QE/g of extract (Har and Ismail, 2012).

vi. Sterols

To test for sterols, a standard sterol solution and Liberman- Burchard solution was prepared. Standard sterol solution was prepared by thoroughly shaking 10 mg (0.01g) of standard cholesterol in 10 ml chloroform. .
Liberman-Burchard reagent was also prepared by dissolving 0.5 ml of sulphuric acid (H_2SO_4) in 10 ml of acetic anhydride, covered and kept in the freezer.

Standard sterol solution ranging from 0.5, 1, 1.5, 2 and 2.5 ml was pipetted into five test tubes leaving test tube 6 blank, then two (2) ml of Liberman-Burchard reagent was added to all the six test tubes and final volume was made equal in all the test tubes by adding chloroform to 7 ml. All the tubes were covered with black carbon paper and kept in the dark for 15 mins. The spectrophotometer was set to zero by the 6^{th} test tube (blank) at 640 nm.The absorbance of all the six test tubes was taken on a UV- Vis Spectrophotometer and a graph plotted to be used as standard. To 3 ml of the *Ganoderma* extract, 2 ml of Liberman-Burchard reagent was added and made up to 7 ml mark with chloroform and the absorbance determined on the spectrophotometer comparing with the standard (Sabir *et al*., 2003).

vii. Polysaccharides

From an aliquot of each extract, 2 ml was pipetted into a 50 ml quick fit stoppered boiling test tube. Two (2) ml was pipetted into another test tube as blank followed by 8 ml of ferrocyanide reagent. Standard working solutions of raffinose of range 10-50 µg/ml were treated with 8 ml ferrocyanide reagent as above. The absorbances of the mixtures and raffinose working standard were read on a UV spectronic 21D spectrophotometer at a wavelength of 380 nm against the blank (Englyst *et al*., 1994; Chin *et al*., 2011). Results were expressed as the % w/w of each test. For each phytochemical quantification, samples were analysed in triplicates (n=3) and the results were expressed in percent (%). Phytochemical analysis was carried out at Nigerian Institute of Science Laboratory Technology (NISLT), Ibadan

Statistical Analysis

The SPSS program (version 16.0 SPSS Inc., Chicago, IL, USA) was used for the analysis of variance (ANOVA) test and means were separated using the new Duncan's Multiple Range Test (DMRT).

RESULTS

Table 1: Quantitative estimates of cultivated *Ganoderma sp.* fruiting bodies in aqueous and organic solvent extracts

Bioactive compound	Quantity (%) in Extracts:			
	Aqueous	Ethanol	Methanol	Dichloromethane
Alkaloids	0.54 ± 0.05^{a}	0.26 ± 0.03^{a}	0.44 ± 0.02^{ab}	0.49 ± 0.40^{b}
Tannins	4.60 ± 0.32^{e}	2.03 ± 0.20^{d}	4.53 ± 0.4^{e}	1.10 ± 0.30^{d}
Phenols	5.90 ± 0.27^{f}	2.73 ± 0.30^{e}	5.90 ± 0.43^{f}	1.07 ± 0.15^{d}
Triterpenoids	2.05 ± 0.04^{c}	0.48 ± 0.06^{c}	1.82 ± 0.08^{d}	1.10 ± 0.07^{d}
Flavonoids	0.65 ± 0.05^{b}	0.30 ± 0.06^{ab}	0.52 ± 0.02^{c}	0.05 ± 0.01^{a}
Sterols	0.49 ± 0.04^{a}	0.22 ± 0.04^{a}	0.36 ± 0.02^{a}	0.57 ± 0.04^{bc}
Polysaccharide	3.73 ± 0.30^{d}	2.20 ± 0.25^{de}	5.87 ± 0.18^{f}	1.90 ± 0.26^{e}

Values with similar letter (s) within the same column are not significantly different from each other ($p > 0.05$) using DMRT

The phytochemical screening of the harvested fruit bodies of *Ganoderma sp*. from this study showed the presence of the following bioactive compounds: alkaloids, tannins, phenols, triterpenoids, flavonoids, sterols and crude polysaccharide. The yields of these phytochemicals (bioactive compounds) from the various solvents are presented in Table 1. In the aqueous extraction, phenols yield (5.90±0.27%) was significantly the highest followed by tannins (4.60±0.32%). Alkaloids (0.54 ± 0.05%) and sterols (0.49 ±0.04%) showed no significant differences.

With ethanol extraction, phenols was significantly higher than the other phytochemicals. Sterols (0.22 ± 0.04%) had the lowest value. In the methanol extracts the yield of phenols was highest followed by polysaccharides and tannins (4.53 ± 0.4%). Dichloromethane extract yielded the highest polysaccharide followed by triterpenoids, tannins and phenols which showed no significant differences. The yield of flavonoids was the lowest.

DISCUSSION

Qualitative phytochemical screening indicated the presence of alkaloids, tannins, phenols, triterpenoids, flavonoids, steroids and crude polysaccharide in the cultivated *Ganoderma sp*. as earlier reported for the wild species (Ihayere *et al.*, 2010; Shah *et al*., 2014).

Results of phytochemical analysis of cultivated *Ganoderma sp*. compared favourably with other findings of wild and cultivated mushrooms. For phenolic content, Uddin *et al*. (2019), using cultivated *Ganoderma lucidum* mushroom ethanol extract reported 1.6±0.66% compared with the value of 2.73±0.30% observed in this study. A major class of phytochemicals, are Phenols and they are responsible for inhibiting the oxidative damage caused by free radicals generated inside the human body (Ferguson, 2001). Tannin value in this study was 4.60 ± 0.32% in aqueous extract which was higher than values of 2.29 ± 0.14% and 1.22± 0.01% reported by Rajoriya *et al*. (2015) and Wood *et al*., (2021), respectively.

Slynko *et al.*(2017), using aqueous extract of wild *G. lucidum* species, reported polysaccharide value of 4.44% in Altai, which can be compared to the result of the cultivated mushroom with 3.73 ± 0.30%. Flavonoid value in this study was highest in aqueous extract (0.65± 0.05%), which is similar to that reported by Wood *et al*. (2021) for wild *Ganoderma* (0.65± 0.05%). However, alkaloids value was different from that reported by Wood *et al.* (2021). In this study the value for sterols in Dichloromethane extract was 0.57± 0.04% which was comparable to the value reported by Wood *et al*. (2021) (1.14± 0.01%). Biologically active compounds in mushrooms may vary, and are affected by differences in substrate, cultivation and fruiting conditions, the developmental stage of the mushroom, and the age of the fresh mushroom sample (Mattila *et al*., 2001 ; Peksen and Yakupoglu, 2009). As a result of developments in cultivation techniques, which in turn affect the mineral contents in mushrooms, new nutritional data are needed (Mattila *et al*., 2001),

CONCLUSION

There is increasing demand for *Ganoderma* mushrooms in the food service, pharmaceutical, cosmetics and health product industries, as different active substances have been extracted from the fruiting bodies, mycelia, and spores of *G. lucidum.* Artificial cultivation has, therefore, become necessary as a major source of the mushroom, owing to the scarcity in the wild especially during the dry seasons. This study showed that the levels of secondary metabolites were low, suggesting that they could be useful as antibiotics and antioxidants. The secondary metabolites in cultivated *Ganoderma sp*. in Nigeria could positively correlate with *Ganoderma sp*. in literature and from the wild as they compare favourably. This study has demonstrated that the *Ganoderma* mushrooms have potentials to be used in the pharmaceutical industries.

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