Academic Journal of Life Sciences
ISSN(e): 2415-2137, ISSN(p): 2415-5217
Vol. 9, Issue. 2, pp: 14-18, 2024
URL: https://arpgweb.com/journal/journal/18
DOI: https://doi.org/10.32861/ajls.92.14.18

Academic Research Publishing Group

Original Research **Open Access**

Proximate Composition and Phytochemical Characterization of a Commercial and Two Wild Strains of *Ganoderma Lucidum* Collected From Tree Stumps in Benin City, Nigeria

Wisdom R. Poyeri
Department of Microbiology, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

Elijah I. Ohimain (Corresponding Author)
Department of Chemical Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria
Email: eohimain@gmail.com

Godwin Jackson
Amrita Vishwa Vidyapeetham, Amritanagar, Ettimadai, Coimbatore, Tamil Nadu 641112

Article History
Received: 8 October, 2024
Revised: 5 December, 2024
Accepted: 12 December, 2024
Published: 18 December, 2024

Copyright © 2024 ARPG & Author
This work is licensed under the Creative Commons Attribution International
CC BY: Creative Commons Attribution License 4.0

Abstract

Ganoderma lucidum is a fungus that is used for the treatment of diverse chronic conditions in many countries. This study used standard chemical laboratory methodologies to assess the proximate composition and phytochemicals present in a commercial strain (W1) and two wild strains (W2 and W3) of Ganoderma lucidum that were obtained from wood stumps in a tropical rainforest in Benin City, Nigeria. The results showed that the three *Ganoderma* strains were significantly different in their proximate composition ($P<0.05$). The proximate composition of W1 was 1.74±0.06 %, 18.40±0.04%, 13.56±0.03%, 2.29±0.04%, 3.88±0.02% and 60.15±0.13% for ash, moisture, crude protein, crude lipid, crude fibre and carbohydrate respectively. The commercial strain had lower levels of ash, crude fibre and carbohydrates but higher levels of moisture, crude protein and crude lipid relative to the wild strains ($P<0.05$). The concentrations of phytochemicals among the strains were significantly different except for steroids. Relative to the wild strains, higher levels of phenol and hydrogen cyanide, but lower levels of alkaloids, flavonoid, tannin, glycoside, terpenoid, phytate, oxalate and anthroquinones was detected in the commercial strains ($P<0.05$). The wild strains because of their higher concentrations of phytochemicals are expected to be more potent.

Keywords: Bioactive substances; Herbal medicine; Indigenous mushroom; Medicinal mushroom; Phytochemical screening; proximate constituent; Reishi mushroom.

1. Introduction

Ganoderma lucidum is a macrofungus belonging to the Division Basidiomycota. This mushroom has been cultivated and used for the management of chronic conditions in the Orients for centuries [1]. Diverse species of *Ganoderma* mushrooms, which are typically harvested from the wild, have also been used in traditional African medicine [2]. Aqueous, ethanol, methanol, ether and other extracts from the fruiting bodies or spores of the fungus have been shown to possess several bioactive properties. For instances, extracts of the fungus have exhibited anti-inflammatory, antioxidant, and anti-ageing properties [3, 4]. The mushroom has exhibited antimicrobial properties including antifungal, antibacterial, and antiviral [5, 6]. The mushroom has been used for the treatment of diverse forms of cancers including breast and prostate cancers [7]. The mushroom has also been shown to possess antihypertensive, antidiabetic, cardioprotective and anti-asthmatic, anti-obesity properties, which guide against cardio-vascular diseases [8]. Besides, the mushroom has demonstrated protective ability on major organs including liver, neurons, kidney and heart [9]. Hence, in Chinese literature, the fungus is commonly referred to as the mushroom of immortality [1].

The medicinal uses of Ganoderma mushroom is linked to its proximate composition and the presence of phytochemicals especially bioactive substances such as triterpenes and polysaccharides [3, 4]. The fungus has been reported to contain over 400 different phytochemicals including alkaloids, tannins, flavonoids, terpenoids, steroids, sterols nucleotides, phenols, glycoproteins, proteins, fatty acids, and trace elements [10]. In Nigeria, Ganoderma lucidum harvested from the wild have been tested for their phytochemical and proximate composition, antimicrobial, antioxidant and hypoglycemic properties [11] of wild Ganoderma mushrooms obtained from Nigeria. Hence, this study aimed to determine the proximate composition and phytochemical properties of a commercial strain and two wild strains of *Ganoderma* mushroom harvested from tree stump in a rainforest in Benin City, Nigeria.

2. Materials and Methods

2.1. Source of the Mushroom

Three mushroom stains sourced from different locations in Nigeria was used for the study. A commercial strain (W1) of the mushroom was obtained from Rohi Biotechnologies Ltd in Port Harcourt, River State, Nigeria, while two wild strains of Ganoderma mushrooms (W2 and W3) were collected from the stump of dead trees in a rainforest ecosystem, Benin City, Nigeria. The three strains were identified to be *Ganoderma lucidum* using morphological and molecular methods [1].

2.2. Sample preparation

The fruiting bodies of the mushroom were dried, surface cleaned and milled using Hammer Mill before analysis.

2.3. Proximate Analysis

Proximate composition of the fungi was determined using standard methods of AOAC [12]. Moisture content was determined by gravimetric method, which involved estimating water loss when dried at a temperature of 105 °C in a laboratory oven for 4-6 hours until a constant weight was obtained. Carbohydrate content was determined spectrophotometrically using Anthrone reagent at 630nm wavelength (Shimadzu UV/VIS spectrophotometer model 160A, Kyoto, Japan. Crude protein was determined by estimating organic nitrogen using the Kjeldahl method. Crude fat and crude fibre were determined by diethyl ether extraction using Soxhlet apparatus.

2.4. Phytochemical Analysis

The samples were first screened qualitatively for the presence of phytochemicals before detailed quantitative analysis. Phytochemical analysis was determined using methods described by Harbone [13]. Alkaloid was determined using ammonium hydroxide method. Tannins content was determined colorimetrically (Shimadzu UV/VIS spectrophotometer model 160A, Kyoto, Japan) at 760nm using Folin–Denis reagent. Total phenol was determined by spectrophotometric method using spectrophotometer (Shimadzu UV/VIS spectrophotometer model 160A, Kyoto, Japan) at 550 nm. Flavonoid, terpenoids and saponin were determined by extraction followed by gravimetric method. Phytate was determined by titrating with standard iron (III) chloride solution using ammonium thiocyanate as indicator. Oxalate was determined by titrating against 0.05M $KMnO_4$ solution. Anthraquinone content was determine spectrophotometrically at a wavelength of 450nm (Shimadzu UV/VIS spectrophotometer model 160A, Kyoto, Japan). Glycosides was determined spectrophotometrically using Baljet's reagent by measuring absorbance at 495nm using Shimadzu UV/VIS spectrophotometer model 160A, Kyoto, Japan.

Steroid was determined by petroleum ether extraction using Soxhlet apparatus while hydrogen cyanide was determined using alkaline picrate filter paper apparatus.

2.5. Statistical Analysis

Result were presented as the mean ± standard deviation. Analysis of variance was carried out followed by multiple comparison with Tukey Test using Minitab software version 21. Difference between treatment groups were considered statistically significant at $P < 0.05$.

3. Results and Discussion

The results of the proximate analysis of the three Ganoderma strains is presented in Table 1. The moisture content was significantly different among the strains, with W1 having the highest value of 18.40±0.04%, followed by W3 having 16.17±0.04% while W2 had the least value of 15.62±0.02 % (P<0.0001). The commercial strain, W1 had the highest crude protein content of 13.56±0.03 %, followed by W3 (10.74±0.04 %), with W2 (10.16±0.04 %) being the least (P<0.0001). The carbohydrate content of the strains was 60.15±0.13%, 66.25±0.05% and 64.63±0.06% for W1, W2 and W3 respectively, which were significantly different (P<0.0001). Crude Lipid was 2.29±0.04% in W1, 1.30±0.01% in W2 and 1.46±0.04% in W3 (P<0.0001). Crude fibre was also significantly different (P<0.0001) among the strains, being 3.88±0.02% in W1, 4.15±0.04% in W2 and 4.93±0.05% in W3. The ash content was however lowest in the commercial strain W1 and highest in W2 (P=0.001).

Table-1. Proximate composition of commercial and wild strains of *Ganoderma lucidum*

Proximate Composition Parameters	Commercial strain W1	Wild strain W2	Wild strain W3	p-value
Ash (%)	1.74±0.06 ^{c}	2.54±0.03 ^{b}	2.10±0.02 ^{a}	0.001
Moisture Content (%)	18.40±0.04 ^{c}	15.62±0.02 ^{b}	16.17±0.04 ^{a}	<0.0001
Crude Protein (%)	13.56±0.03 ^{c}	10.16±0.04 ^{b}	10.74±0.04 ^{a}	<0.0001
Crude Lipid (%)	2.29±0.04 ^{c}	1.30±0.01 ^{b}	1.46±0.04 ^{a}	<0.0001
Crude Fibre (%)	3.88±0.02 ^{c}	4.15±0.04 ^{b}	4.93±0.05 ^{a}	<0.0001
Carbohydrate (%)	60.15±0.13 ^{c}	66.25±0.05 ^{b}	64.63±0.06 ^{a}	<0.0001

Data is presented as mean ± standard deviation (n=3). Across the row, different alphabet represent means that are significantly different (P<0.05)

Shamaki, *et al.* [14], analyzed the fruiting bodies of *Ganoderma lucidum* obtained from the wild in Nigeria and found protein 17.55%, carbohydrates 33.13%, crude fats 2.60%, crude fibre 30.25%, total ash 5.93% and moisture content 10.54%. The results were in agreement with our findings for most parameters except for fibre and carbohydrates. Peng, *et al.* [15], reported the proximate constituent of *G. lucidum* from China; 9.56 ± 0.43% moisture content, 14.0 ± 0.36 crude protein, 2.02 ± 0.07% fat, 1.20 ± 0.11% ash and 64.2 ± 2.30% fibre. Garuba, *et al.* [16] carried out proximate analysis of *Ganoderma lucidum* collected from the wild in Ilorin, North Central Nigeria and found 44.95% carbohydrate, 15.75% protein, 14.81% crude fibre, 12.99% moisture and 4.00% ash. Singh, *et al.* [17] reported the proximate composition of various species of *Ganoderma* mushroom including *G. lucidum* collected from forests in Uttarakhand, India and found varying levels of constituents with carbohydrates ranging from 75.5–80.3%, proteins 9.29–12.4%, ash 6.14–8.32%, fibre 4.92–8.07% and fats 1.62–2.87%. Wu, *et al.* [18], did a recent comprehensive review of the proximate composition of *G. lucidum* and similarly reported ranging values of fibre 59%–65%, carbohydrate 21.83%–27.78%, protein 7%–8%, fat 1.1%–8.3% and ash 0.72%–1.77%.

Phytochemical screening of the fruiting bodies of the fungi shows the presence of various phytochemicals (Table 2). The results of the quantitative analysis of the phytochemical content of the fungi is presented in Table 3. Alkaloids was significantly different (P=0.002) among the strains, it was 0.571±0.010% in W1, 0.621±0.003% in W2 and 0.656±0.002% in W3. Tannin was least (P=0.027) in the commercial strain W1 (0.124±0.006%) followed by wild strain W2 (0.141±0.007%) and W3 (0.154±0.001%). Flavonoid was highest (P=0.005) in the wild strains (W2 = 1.835±0.078 %, W3= 1.830±0.014%) compared to the commercial strain W1 (1.425±0.007 %). Saponin (%) was 0.044±0.004% in W1, 0.040±0.004% in W2 and 0.435±0.035% in W3 (P=0.000). Steroid was not significantly different (P= 0.356) among the strains, it was 0.015±0.003 % in W1, 0.019±0.002% in W2 and 0.017±0.003% in W3. Phenol was significantly different (P<0.0001) among the strainswith the highest concentration occurring in the commercial strains W1, 0.271±0.004% while W2 and W3 had concentrations of 0.183±0.001% and 0.199±0.004% respectively.

Table-2. Qualitative assessment of phytochemicals in the tested *Ganoderma lucidum* strains

SAMPLE	Commercial *Ganoderma lucidum* W1	Local *Ganoderma lucidum* W2	Local *Ganoderma lucidum* W3
Alkaloid (%)	+	+	+
Saponin (%)	+	+	+
Steroid (%)	+	++	+
Flavonoid (%)	+	+++	++
Tannin (%)	+	+	+
Phenol (%)	++	+	+
Glycoside (%)	++	+++	+++
Terpenoid (%)	++	++	++
Phytate (%)	+	+	+
Oxalate (%)	+	+	+
Hydrogen cyanide (mg/100g)	+++	+++	+++
Anthroquinones (%)	+	+	+

Table-3. Phytochemical Characteristics of commercial and wild strains of *Ganoderma lucidum*

Physiochemical Parameters	Commercial strain W1	Wild strain W2	Wild strain W3	p-value
Alkaloids (%)	0.571±0.010^{c}	0.621±0.003^{b}	0.656±0.002^{a}	0.002
Saponin (%)	0.044±0.004^{b}	0.040±0.004^{b}	0.435±0.035^{a}	0.000
Steroid (%)	0.015±0.003^{a}	0.019±0.002^{a}	0.017±0.003^{a}	0.356
Flavonoid (%)	1.425±0.007^{b}	1.835±0.078^{a}	1.830±0.014^{a}	0.005
Tannin (%)	0.124±0.006^{b}	0.141±0.007^{ab}	0.154±0.001^{a}	0.027
Phenol (%)	0.271±0.004^{c}	0.183±0.001^{b}	0.199±0.004^{a}	<0.0001
Glycoside (%)	4.139±0.029^{c}	6.421±0.001^{b}	5.874±0.001^{a}	<0.0001
Terpenoid (%)	2.542±0.001^{c}	2.954±0.002^{b}	2.818±0.002^{a}	<0.0001
Phytate (%)	0.393±0.001^{c}	0.466±0.003^{b}	0.425±0.006^{a}	0.001
Oxalate (%)	0.526±0.002^{c}	0.571±0.001^{b}	0.584±0.001^{a}	<0.0001
Hydrogen Cyanide (mg/100g)	7.183±0.001^{c}	6.240±0.001^{b}	6.761±0.002^{a}	<0.0001
Anthroquinones (%)	1.050±0.011^{c}	1.682±0.004^{b}	1.807±0.006^{a}	<0.0001

Data is presented as mean ± standard deviation (n=3). Across the row, different superscript alphabet represent means that are significantly different (P<0.05).

Glycoside was significantly different (P<0.0001) among the strains, with the least concentration of 4.139±0.029% recorded in W1, while W2 was 6.421±0.001% and W3 was 5.874±0.001%. Terpenoid exhibited a similar pattern, with the least concentration of 2.542±0.001% in W1, while W2 was 2.954±0.002% and W3 was 2.818±0.002% (P<0.0001). The concentration of phytate was also lowest (P=0.001) in W1 being 0.393±0.001%, whereas, it was 0.466±0.003% in W2 and 0.425±0.006% in W3. The least concentration (P<0.0001) of oxalate was recorded in W1, it was 0.526±0.002%, whereas it was 0.571±0.001% and 0.584±0.001% in W2 and W3 respectively. The concentration of hydrogen cyanide was significantly higher (<0.0001) in the commercial strain

compared to the wild strains. The concentrations of HCN were 7.183±0.001 mg/100g, 6.240±0.001 mg/100g and 6.761±0.002 mg/100g for W1, W2 and W3 respectively. The least level of anthroquinones of 1.050±0.011% was recorded in W1, while W2 was 1.682±0.004% and W3 was 1.807±0.006% (P=<0.0001).

Ihayere and Okhuoya [19], used various extracts including ethanol, methanol, water and dichloromethane to analyze *Ganoderma* sp. collected from the forest in Edo State Nigeria and found the presence of phytochemicals in varying extents including alkaloids, tannins, phenols, terpenoids, flavonoids and steroids. The concentration of these parameters in the aqueous extracts of the fruiting bodies of the fungus was 0.54 ± 0.05%, 4.60 ± 0.32%, 5.90 ± 0.27%, 2.05 ± 0.04%, 0.65 ± 0.05% and 0.49 ±0.04% respectively. Shamaki, *et al*. [14], detected several phytochemicals from the methanol, ethyl acetate and n-butanol extracts of the fungus including tannins, flavonoids, saponins, cardiac glycosides, terpenoids and carbohydrates. The differences observed in the proximate composition and phytochemical content of the fungus across literature may be due to several factors such as the nature of mushroom substrate and habitat, the sampling location, the part of the mushroom analysed and the extent of drying before analysis.

4. Conclusion

The study involved the characterization of a commercial strain and two wild strains of *Ganoderma lucidum* that were harvested from a rainforest in Benin-City, Nigeria. Standard analytical procedures were followed. Proximate composition revealed the dominance of carbohydrates, protein, moisture, lipid, fibre and ash. The mushroom strains contained several phytochemicals including alkaloid, saponin, steroid, flavonoid, tannin, phenol, glycoside, terpenoid, phytate, oxalate, hydrogen cyanide and anthroquinones, with higher concentrations in the wild strains. Hence, the wild strains have the potential for higher potency.

Acknowledgement

This work was based on the Ph.D. research of the first author supervised by the second author. The second author wrote the initial draft, all authors reviewed and okayed the final manuscript. The authors wish to thank Rohi Biotechnologies Ltd, Port Harcourt for the production of the mushroom used for the study.

References

[1] Poyeri and Ohimain, E. I., 2024a. "Cultivation, molecular identification and productivity assessment of a commercial strain and two wild strains of ganoderma mushroom harvested from tree stumps in a rainforest at Ugbowo, Benin City, Nigeria." *J. Appl. Sci. Environ. Manage*, vol. 28, pp. 2207-2215.

[2] Okigbo, R. N. and Obanubi, S. I., 2020. "A study on the Ganoderma lucidum of Warri South Local Government Area." *Journal of Food Technology & Nutrition Sciences. SRC/JFTNS/103*,

[3] Wu, Han, F., Luan, S., Ai, R., Zhang, P., Li, H., and Chen, L., 2019. "Triterpenoids from ganoderma lucidum and their potential anti-inflammatory effects." *J. Agric. Food Chem.*, vol. 67, pp. 5147-5158.

[4] Su, H. G., Peng, X. R., and Shi, Q. Q., 2020. "Lanostane triterpenoids with anti-inflammatory activities from Ganoderma lucidum." *Phytochemistry*, vol. 173, p. 112256.

[5] Bharadwaj, S., Lee, K. E., and Dwivedi, V. D., 2019. "Discovery of ganoderma lucidum triterpenoids as potential inhibitors against dengue virus ns2b-ns3 protease." *Sci. Rep.*, vol. 9, p. 19059. Available: https://doi.org/10.1038/s41598-019-55723-5

[6] Ellan, K., Thayan, R., and Raman, J., 2019. "Anti-viral activity of culinary and medicinal mushroom extracts against dengue virus serotype 2: an in-vitro study." *BMC Complement Altern. Med.*, vol. 19, p. 260. Available: https://doi.org/10.1186/s12906-019-2629-y

[7] Wu, Na, K., Chen, D., Wang, Y., Pan, H., and Wang, X., 2018. "Effects of non-steroidal anti-inflammatory drug-activated gene-1 on ganoderma lucidum polysaccharides-induced apoptosis of human prostate cancer pc-3 cells." *Int. J. Oncol.*, vol. 53, pp. 2356-2368.

[8] Diling, C. and Guo, Y., 2020. "Metabolic regulation of ganoderma lucidum extracts in high sugar and fat diet-induced obese mice by regulating the gut-brain axis." *Journal of Functional Foods*, vol. 65, pp. 1-22. Available: https://doi.org/10.1016/j.jff.2019.103639Getrightsandcontent

[9] Ren, Z., Wang, C., and Wang, T., 2019. "Ganoderma lucidum extract ameliorates mptp-induced parkinsonism and protects dopaminergic neurons from oxidative stress via regulating mitochondrial function, autophagy, and apoptosis." *Acta Pharmacol Sin*, vol. 40, pp. 441–450. Available: https://doi.org/10.1038/s41401-018-0077-8

[10] Martínez, M. M. M., Ling, T., Suarez, A. I. J., Ortiz, S. G., and Santiago, N. C. L., 2019. "Identification of biologically active ganoderma lucidum compounds and synthesis of improved derivatives that confer anticancer activities in vitro." *Front Pharmacol*, vol. 10, p. 115. Available: https://doi.org/10.3389/fphar.2019.00115

[11] Poyeri and Ohimain, E. I., 2024b. "Assessment of the anti-diabetic properties of ethanol extract of wild strains of Ganoderma lucidum mushroom using animal model." In *Proceedings of the 1st Annual Faculty of Science International Conference, Niger Delta University 2024. NDU Press, Wilberforce Island, Bayelsa State, Nigeria.* pp. 315-328.

[12] AOAC, 1990. *Official methods of analysis, association of official analytical chemists*. 15th ed. Washington, D.C., USA.

[13] Harbone, J. B., 1973. *Phytochemical methods*. London: Chapman and Hall Ltd.

[14] Shamaki, B. U., Sandabe, U. K., and Abdulrahman, F. I., 2012. "Proximate composition, phytochemical and elemental analysis of some organic solvent fraction of the wild mushroom, ganoderma lucidum." *Journal Natural Science Research*, vol. 2, pp. 24–35.

[15] Peng, G., Xiong, C., Zeng, X., Jin, Y., and Huang, W., 2024. "Exploring nutrient profiles, phytochemical composition, and the antiproliferative activity of ganoderma lucidum and ganoderma leucocontextum: A comprehensive comparative study." *Foods*, vol. 13, p. 614.

[16] Garuba, T., Olahan, G. S., Lateef, A. A., Alaya, R. O., Awolowo, M., and Sulyman, A., 2020. "Proximate composition and chemical profiles of Reishi mushroom (Ganoderma lucidum (Curt: Fr.) Karst)." *Journal of Scientific Research*, vol. 12, pp. 103-110.

[17] Singh, R., Kaur, N., and Shri, R., 2020. "Proximate composition and element contents of selected species of Ganoderma with reference to dietary intakes." *Environ Monit Assess*, vol. 192, p. 270. Available: https://doi.org/10.1007/s10661-020-08249-7

[18] Wu, Zhang, S., Peng, B., Tan, D., Wu, M., Wei, J., and Luo, H., 2024. "Ganoderma lucidum: a comprehensive review of phytochemistry, efficacy, safety and clinical study." *Food Science and Human Wellness*, vol. 13, pp. 568-596.

[19] Ihayere, C. A. and Okhuoya, J. A., 2022. "Phytochemical analysis of cultivated medicinal mushroom-Ganoderma sp." *Nigerian Journal of Botany*, vol. 35, pp. 11-18.