British Journal of Pharmaceutical Research
6(1): 61-67, 2015, Article no.BJPR.2015.049
ISSN: 2231-2919

SCIENCEDOMAIN *international*
www.sciencedomain.org

In vitro Antioxidant Activity and Total Polyphenols Content of Wild Edible Polypore Mushrooms – *Bondazewia berkeleyi* and *Ganoderma lucidum*

Emmanuel E. Essien[1*], Blessing I. Udoh[1] and Nimmong-uwem S. Peter[1]

[1]Department of Chemistry, University of Uyo, Akwa Ibom State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author EEE designed the study, wrote the protocol, and the first draft of the manuscript. Authors BIU and NSP conducted experimental work, managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2015/16196
Editor(s):
(1) Abdelwahab Omri, Department of Chemistry and Biochemistry AND Departments of Biomolecular Sciences, Laurentian University, Canada.
Reviewers:
(1) A.O. Ogbe, University of Abuja, Nigeria.
(2) Anonymous, Malaysia.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=982&id=14&aid=8117

Original Research Article

Received 14th January 2015
Accepted 30th January 2015
Published 11th February 2015

ABSTRACT

Aim: To determine the total phenols content and antioxidant activity of *B. berkeleyi* and *G. lucidum* methanol extracts.
Study Design: *In vitro* evaluation of antioxidant assays and quantitative determination of total phenolics and flavonoids content of *B. berkeleyi* and *G. lucidum* mushroom extracts.
Place and Duration of Study: Department of Chemistry, University of Uyo, Nigeria (August – November, 2014).
Methodology: The total phenolics content was estimated using Folin-Ciocalteu method and total flavonoid content was determined using aluminum chloride. *In vitro* antioxidant activities of extracts were studied in different systems including 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay and metal chelating activity along with standards.
Results: Total phenols content in *B. berkeleyi* and *G. lucidum* was 32.99 and 42.7 mg GAE/g and flavonoids amounted to 22.4 and 35.23 mg QE/g respectively. The extracts of *B. berkeleyi* and *G. lucidum* exhibited significant DPPH radical scavenging activity with IC_{50} values 22.9 µg/ml and 14.6 µg/ml; metal chelating capacity of 67.4% and 82.6% at 500 µg/mL respectively.

**Corresponding author: Email: emmaflowus1@yahoo.co.uk;*

Conclusion: The results of this study substantiates the use of the extract of *G. lucidum* powder as an antioxidant agent and serves as a pointer for further exploitation of *B. berkeleyi* mushroom for its antioxidant compounds and other potential biological activity.

Keywords: Macrofungi polypore; Bondazewia berkeleyi; Ganoderma lucidum; antioxidant activity.

1. INTRODUCTION

Myconutraceuticals are neutraceutical substances derived from fungi. Natural products have been the source of most of the active ingredients of medicines [1]. Macrofungi have long been used as valuable food source and as traditional medicines around the world [2]; they are known to produce large and diverse variety of secondary metabolites [3]. These secondary metabolites have health promoting properties such as antioxidant, antimicrobial, anticancer, cholesterol lowering and immunostimulatory effects [4-6]. Antioxidant compounds have important ability to trap free radicals and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Naturally occurring antioxidants can be found in whole grains, fruits, vegetables, teas, spices and herbs. Mushrooms have also been reported as organisms with antioxidant activity which is correlated with their phenolic and polysaccharide compounds [7,8].

The fungus *B. berkeleyi* (Fr.) Bond. et singer (synonymous: *Polyporus berkeleyi*) of the family Bondarzewiaceae (*Basidiomycota*) grows at the base or roots of Abies and other conifers of the family Fagaceae. It is edible when young and gets tough and unappetizing with age [3]. Bernd et al. [9] reported a cytotoxic metabolite, montadial A, isolated from the polypore *B. montana*. Reishi mushroom, *G. lucidum* (Fr.) Krast (*Polyporaceae*) is used for the treatment of bronchitis, hepatitis, hypertension, tumorigenic diseases and immunological disorders [10] and as remedy for the promotion of health and longevity [11]. A number of polysaccharides have been isolated, characterized and assayed for antiturmor, antimetastatic and immunological activity [12,13]. *Ganoderma lucidum*, is utilized as dried whole, powder or capsule and as tablets for promoting health in humans [14] and in some countries like China and Japan it is cultivated and utilized as source of feed supplement [15,16]. However, the pharmacological efficacy and bioactive components in *Ganoderma* from different origins or hosts are significantly different [17,18] and the reasons leading to this phenomenon are still unclear. There are many synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which are commonly used, but they are reported to have side effects and are carcinogenic [19]. Therefore, there is an increased interest in the use of natural antioxidants due to their presumed safety, nutritional and therapeutic value [20]. This may explain the interest in examining plants and mushroom extracts as sources of cheaper and effective antioxidants and the growing interest in nutraceuticals. In addition, there seems to be no report hitherto on the antioxidant activity of *B. berkeleyi* and a paucity of information on the antioxidant potential of *G. lucidum* collected in Nigeria. Therefore, in continuation of our work on the nutraceutical potential of mushroom polypores [21], this study seeks to assess the total polyphenols content and antioxidant activity of *B. berkeleyi* and *G. lucidum*.

2. MATERIALS AND METHODS

2.1 Samples Collection and Extraction

The fresh sporocarps of mature mushroom species (*B. berkeleyi* and *G. lucidum*) were collected from the forest in the rainy season (July to August) in Uyo and Ikono Local Government Areas of Akwa Ibom State. Specimen identifications and authentication were done by a mycologist, Dr. Joseph Essien and the voucher specimens were deposited in the School of Pharmacy herbarium, University of Uyo, Nigeria. The macroscopic descriptions, including size, shape, color, texture, and odor, were noted. The color of the carpophore, shape of the cap and stipe, color of the flesh and latex, and its smell and habitat were also noted. The mushroom samples were packed in opaque plastic bags and transferred to the analytical laboratory. The samples were carefully cleaned manually to remove any extraneous materials, cut, sun-dried and oven-dried (Gallenkamp, DV 333) at 45°C for 40 h to constant weight. Dried samples were pulverized using an agate homogenizer, and stored in pre-cleaned polyethylene bottles, prior to analyses. All reagents were of analytical grade, except otherwise stated. The ground samples were extracted with methanol using a

Soxhlet apparatus. The extract was concentrated under vacuum using a rotator evaporator.

2.2 Determination of Total Phenolics

The amount of total phenols in the methanol extracts *B. berkeleyi* and *G. lucidum* was determined by Folin-Ciocalteu's reagent method [22]. The extracts (0.5 ml) and Folin-Ciocalteu's reagent (0.5 N, 0.1 ml) were mixed and the mixture was incubated at room temperature for 15 min. Then saturated sodium carbonate solution (2.5 ml) was added and further incubated for 30 min at room temperature. The absorbance was measured at 760 nm. Gallic acid was used as a positive control. Total phenol values are expressed in terms of gallic acid equivalent (mg/g of extract).

2.3 Determination of Flavonoids Content

The flavonoids content in the mushroom extracts was determined by aluminium chloride colorimetric method [23]. The reaction mixture (3 ml) consisted of sample (1 ml, 1 mg/ml), aluminium chloride (1.2%, 0.5 ml) and potassium acetate (120 mM, 0.5 ml) was incubated at room temperature for 30 min. The absorbance of all samples was measured at 415 nm. Quercetin was used as positive control. The flavonoid content is expressed in terms of quercetin equivalent (mg/g of extract).

2.4 DPPH Radical Scavenging Activity

DPPH radical scavenging activity of each extract was determined according to the method of Blois [24]. DPPH (0.1 mM) in methanol was prepared and the solution (1 ml) was mixed with crude extracts (1.0 ml) prepared in methanol at different concentrations (20, 40, 60, 80, and 100 µg/ mL). The mixture was shaken and kept for 30 mins at room temperature. The decrease of solution absorbance due to proton donating activity of components of each extract was determined at 517 nm. BHA was used as the positive control. The DPPH radical scavenging activity was calculated using the following formula:

$$\%\ \text{inhibition} = \frac{\mathrm{A_{control}} - \mathrm{A_{sample}}}{\mathrm{A_{control}}} \mathrm{X} \quad 100$$

2.5 Metal Chelating Activity

The method of Dinis et al. [25] was used. Crude extract (0.5 g) was mixed with $FeCl_2$ (2 mM, 0.05 ml) and Ferrozine (5 mM, 0.4 ml). The total volume was diluted with methanol (2 ml). The mixture was shaken vigorously and left standing at room temperature for 10 mins. After the mixture had reached equilibrium, the absorbance of the solution was measured at 562 nm in a spectrophotometer. The percentage inhibition of ferrozine Fe^{2+} complex was calculated using the formula:

$$\%\ \text{inhibition of ferrozine} - \mathrm{Fe2+} = \frac{\mathrm{A_{control}} - \mathrm{A_{sample}}}{\mathrm{A_{control}}} \mathrm{X}\ 100$$

3. RESULTS AND DISCUSSION

The results of total phenolics content and flavonoids are given in Table 1. *Ganoderma lucidum* methanol extract was found to contain higher amounts of polyphenols and flavonoids compared with *B. berkeleyi* extract. The estimated polyphenols content for *G. lucidum* extract in this study is similar to total phenolics (71 µg GAE/mg) and flavonoids (45 µg QE/mg) content of the Indian sample, *G. applanatum* reported by Nagara et al. [26]. However, relative low concentration of total phenols (9.245 mg CE/g) and flavonoids (2.14 mg QE/g) were found in *G. lucidum* sample from India [27]. Estimation of phenolic content is important because there are many reports that phenols and antioxidant activity are directly correlated [7,8]. Invariably, total phenolics content can be an indicator of the antioxidant capacity of the extract [28]. In addition, natural polyphenols have chain-breaking antioxidant activities and are believed to prevent many degenerative diseases, including cancer and atherosclerosis [29].

Table 1. Total phenolics and flavonoid contents of mushroom fruiting bodies

Mushroom extract	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/g)
B. berkeleyi	32.99	22.40
G. lucidum	42.70	35.23

DPPH radical was used as a stable free radical to determine antioxidant activity. Antioxidants, on interaction with DPPH transfer an electron (hydrogen atom) to DPPH, neutralizing its free radical character [30]. Fig. 1 illustrates the concentration of DPPH radical due to the scavenging ability of extracts of *B. berkeleyi* and *G. lucidum*. Standard BHA was used as a reference. The percentage inhibition of the free radical was dose dependent. Increase in concentration gave corresponding increased % inhibition. In a concentration of 500 µg/ml of extracts and standard BHA, the descending order of DPPH radical scavenging is as follows: BHA (89.28%) > *G. lucidum* extract (86.28%) > *B. berkeleyi* extract (74.66%).

The DPPH radical scavenging capacity (IC_{50}) of the extracts is presented in Table 2. This is the concentration that decreases the initial DPPH radical concentration by 50% in each extract. The effectiveness of antioxidant properties is inversely correlated with IC_{50} values. *Ganoderma lucidum* methanol extract showed more potent antioxidant activity (IC_{50}, 14.6 µg/ml) than *B. berkeleyi* (IC_{50}, 22.91µg/ml). However, BHA exhibited higher DPPH scavenging effect (IC_{50}, 4.82 µg/ml) than both extracts in the study. Rawat et al. [27] demonstrated that in DPPH assay, IC_{50} value for methanolic extract of wild *G. lucidum* collected in India was 1.162 mg/ml and 2, 2'-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid] (ABTS) assay, 0.555 mg/ml. *Ganoderma lucidum* extract in this study displayed better DPPH activity than this Indian sample.

Chelating activity of the mushroom extracts were also tested in a metal chelating assay. All the extracts showed metal chelating capacity in a concentration-dependent manner; *B. berkeleyi* (67.4%) and *G. lucidum* (82.6%) at 500 µg/ml whereas the standard ethylenediaminetetraacetic acid (EDTA) exhibited higher activity (Fig. 2). Nagaraj et al. [26] reported good metal chelating activity for methanol extract, followed by chloroform, aqueous and petroleum ether extracts of *G. applanatum* with IC_{50} values, 50.84 µg/ml, 54.74 µg/ml, 58.13 µg/ml and 69.12 µg/ml respectively. Iron is an essential mineral for normal physiological activity of the human body, but excess can cause cellular damage and injury. The ferrous ions are the most effective pro oxidants in food systems, the good chelating effect would be beneficial and removal of free ion from circulation could be a promising approach to prevent oxidative stress induced disease [31]. Ferrous ion reacts with ferrozine forms violet color Ferrozoin-Fe2+ complex. Chelating compounds present in the extract prevent the formation of Ferrozoine-Fe^{2+} complex, which leads to decrease in the intensity of violet color [32].

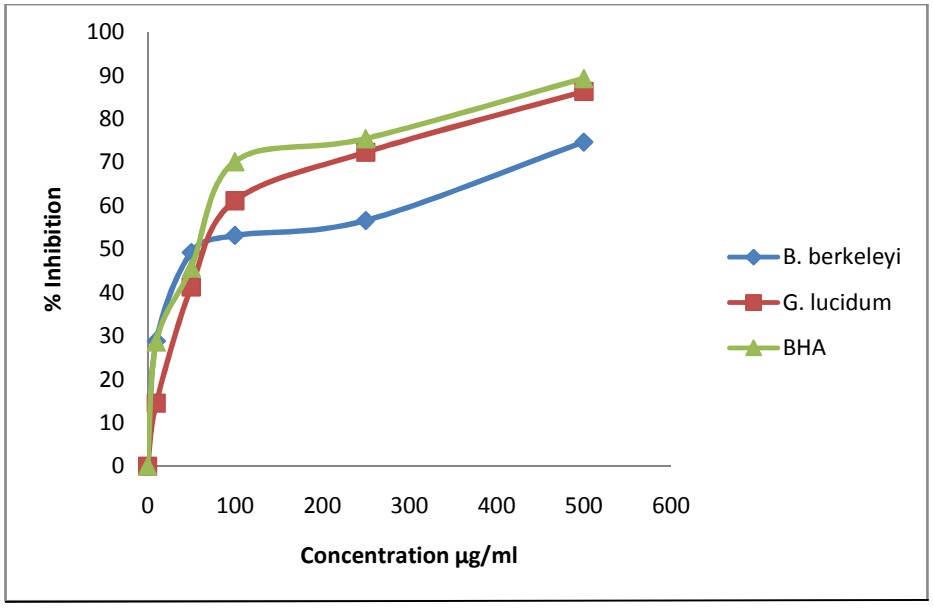

Fig. 1. Percentage DPPH scavenging activity of *B. berkeleyi* and *G. lucidum* extracts, and standard BHA

Table 2. IC_{50} (µg/ mL) of *B. berkeleyi* and *G. lucidum* extracts

Activity	*B. berkeleyi*	*G. lucidum*	BHA
DPPH	22.91	14.60	4.82

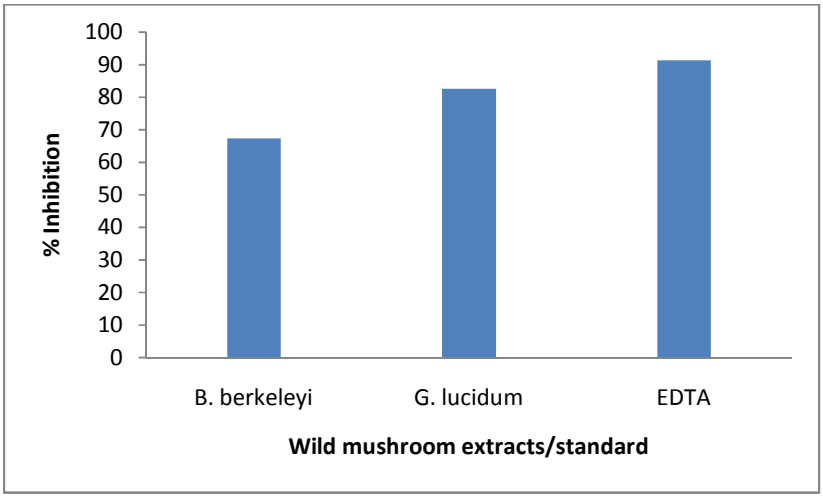

Fig. 2. Metal chelating activity of *B. berkeleyi* and *G. lucidum* extracts, and standard EDTA

4. CONCLUSION

This study demonstrates that *B. berkeleyi* and *G. lucidum* extracts possess potent anti-oxidative properties by showing DPPH radical scavenging and metal chelating activities. This potent antioxidant activity may be attributed to its high phenolics and flavonoids content. The methanol extract can be considered as a valuable source of antioxidant products as the obtained values compared with the standard compounds. Furthermore, the results of this study substantiates the use of the extract of *G. lucidum* powder as an antioxidant agent and serves as a pointer for further exploitation of *B. berkeleyi* mushroom for its antioxidant compounds and other potential biological activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Harvey AL. Natural products in drug discovery. Drug Discov Today. 2008; 13(19/20):894 901.
2. Wasser SP. Medicinal mushrooms as a source of antitumour and immunosti-mulating polysaccharides. Appl Microbiol Biotech. 2002;60:258-274.
3. Liu JK. Secondary metabolites from higher fungi in China and their biological activity. Drug Discov Ther. 2007;1(2):94-103.
4. Anderson JB, Stasovski E. Molecular phylogeny of northern hemisphere species of *Armillaria.* Mycologia. 1992;84:505-516.
5. Mizuno T. The extraction and development of antitumor active polysaccharides from medicinal mushrooms in Japan- review. Inter J Med Mushr. 1999;1:9-30.
6. Mau JL, Chang CN, Huang SJ, Chen CC. Antioxidant properties of methanolic extracts from *Grifola frondosa*, *Morchella esculenta* and *Termitomyces albuminosus* mycelia. Food Chem. 2004;87:111-118.
7. Mau JL, Tsai SY, Tseng YH, Huang SJ. Antioxidant properties of hot water extracts from *Ganoderma tsugae* Murrill. LWT Food Sci Technol. 2005;38:589-597.

8. Dubost NJ, Ou B, Beelman RB. Quantification of polyphenols and ergothioneine in cultivated mushroom and correlation to total antioxidant capacity. Food Chem. 2007;105:727-735.
9. Bernd S, Norbert A, Steglich W, Anke T. Montadial A. A cytotoxic metabolite from *Bondarzewia montana*. J Nat Prod. 1999;62:1425-1426.
10. Cong Z, Lin ZB. The pharmacological study of Lingzhi (*Ganoderma lucidum*) and the research of therapeutical principle of "Fuzheng guben" in traditional chinese medicine. J Beijing Med Coll. 1981;13:6-10.
11. Lin ZB, Zhang HN. Anti-tumor and immunoregulatory activities of *Ganoderma lucidum* and its possible mechanisms. Acta Pharmacol Sin. 2004;25:1387-1395.
12. Boh B, Berovic M, Zhang J, Zhi-Bin L. *Ganoderma lucidum* and its pharmaceutically active compounds. Biotechnol Annu Rev. 2007;13:265-301.
13. Zhang J, Tang Q, Zhou C, Jia W, Da Silva L. GLIS, a bioactive proteoglycan fraction from *Ganoderma lucidum* displays anti-tumour activity by increasing both humoral and cellular immune response. Life Sci. 2010;87:628-637.
14. Oei P. Mushroom cultivation. In: Appropriate technology for the mushroom growers; CTA, 3rd Ed. Backhuys Publishers Leiden. The Netherlands. 2003;1-7.
15. Wachtel-Galor S, Tomlinson B, Benzie IF. *Ganoderma lucidum* (Lingzhi), a Chinese medicinal mushroom: Biomarker responses in a controlled human supplementation study. British J Nutr. 2004;91(2):263-269.
16. Ogbe AO, Obeka AD. Proximate, mineral and anti-nutrient composition of wild *Ganoderma lucidum*. Implication on its utilization in poultry production. Iranian J Appl Ani Sci. 2013;3(1):161-166.
17. Hu JW, Fan QS, Xiao XN. Study on the functional components in grass-cultivated *Ganoderma lucidum* and tree cultivated *Ganoderma lucidum*. Nat Prod Res Dev. 2006;18:458-460.
18. Dai J, Chen SW, Zhu S, Li GX. Analysis and comparison of the molecular mass distribution of polysaccharides from different *Ganoderma lucidum* spore powder samples. Food Mach. 2010;26:12-14.
19. Namiki M. Antioxidants/antimutagens in foods. CRC Crit Rev Food Sci Nutr. 1990;29:273-300.
20. Ajila CM, Naidu KA, Bhat UJS, Rao P. Bioactive compounds and antioxidant potential of mango peel extract. Food Chem. 2007;105:982-988.
21. Essien EE, Udoh II, Peter NS. Proximate composition and anti-nutritive factors in some wild edible medicinal macrofungi. J Nat Prod Plant Resour. 2013;3(6):29-33.
22. Mc Donald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem. 2001;73:73-84.
23. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10:178-182.
24. Blois MS. Antioxidants determination by the use of a stable free radical. Nature. 1958;46:1199-1200.
25. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolics derivates (acetoaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophy. 1994;315:161-169.
26. Nagaraj K, Mallikarjun N, Naika R, Venugopal TM. Antioxdative activities of wild macro fungi *Ganoderma applanatum* (Pers.) Pat. Asian J Pharm Clin Res. 2014;7(Suppl 2):166-171.
27. Rawat A, Mohsin M, Negi PS, Sah AN, Singh S. Evaluation of polyphenolic contents and antioxidant activity of wildly collected *Ganoderma lucidum* from central Himalayan hills of India. Asian J Plant Sci Res. 2013;3(3):85-90.
28. Chanda SV, Nagani KV. Antioxidant capacity of *Manilkara zapota* L. leaves extracts evaluated by four *in vitro* methods. Nature and Sci. 2010;8(10):260-266.
29. Roginsky V. Chain breaking antioxidant activity of natural polyphenols as determined during the chain oxidation of methyl linoleate in Triton X-100 micelles. Arch Biochem Biophys. 2003;414:261-270.
30. Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohoni DP. Comparative antioxidant activity of individual herbal components used in ayurvedic medicine. Phytochem. 2003;63:97-104.

31. Hippeli S, Elstner EF. Transition metal ion catalyzed oxygen activation during pathogenic process. FEBS Lett. 1999; 443:1-7.

32. Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of *Spondias pinnta*. BMC Complement Altern Med. 2008;63:1-10.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=982&id=14&aid=8117