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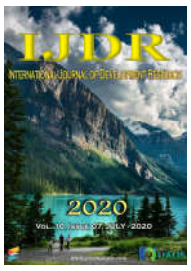

RESEARCH ARTICLE OPEN ACCESS

ANTIOXIDANT ACTIVITIES AND ACETYLCHOLINE ESTERASE INHIBITION OF NINE WILD MUSHROOMS FROM BURKINA FASO

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ABSTRACT

Mushrooms are used as traditional food and to a lesser extent, as medicine compared to plants. In this study the biological properties of nine Burkinabé mushroom species (*Chlorophyllum* aff. *molybdites*, *Phlebopus sudanicus*, *Lentinus squarrosulus*, L*endzites elegans*, *Psatyrella tuberculata*, *Schizophylum commune*, *Ganoderma lucidum*, *Itajaya rosea* and *Laetiporus baudhanii)* were evaluated, in order to contribute to the overall characterisation of these non-wood forest products. The total phenolic and flavonoid contents were analysed spectrophotometrically; antioxidant activities were evaluated using the DPPH and FRAP methods and also AChE inhibition was performed. Mushroom species analysed contain powerful antioxidant such as phenol compounds. Among them, *Laetiporus baudhanii* which has the highest total phenolic content (48.37mg⁄ml) was found, not only to possess the best antioxidant activities through radical reduction power (87.07 AA mg⁄ml) and radical scavenging capacity (IC_{50}: 0.003 mg/mL), and but also to have highlighted the best AChE inhibition (29.44%). So, we conclude that *L. baudhanii* is a potential source of antioxidant compounds and AChE inhibitors. However, further investigations are needed to elucidate his valuable therapeutic use.

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INTRODUCTION

Non-wood forest products play an important place in rural West African food habits. They provide food, medicine and income for local populations. Nowadays, there is an increase interest for researchers to work on forest products. Thus, several studies on plants related to their inventory, chemical composition and biological activities have been conducted throughout the world. Mushrooms are valuable healthy food, with high content in vegetable proteins, iron, zinc, chitin, fiber, vitamins and minerals but with low calories content. They are often considered as an ideal and healthy food for people with high blood cholesterol and hypertension (Manzi *et al.*, 1999). Compared to vegetables, mushrooms proved to be a good source of several minerals such as K, P, Zn, and Cu. In addition to their nutritional value, they contain some medicinal properties. Mushrooms also have a long history of use in Traditional Chinese Medicine. They have been reported to contain a wide variety of free radical scavenging molecules, such as polysaccharides and polyphenols (Cui *et al.,* 2005). Several mushrooms species contain a wide variety of free radicals or reactive oxygen species scavengers which have made mushrooms attractive as nutritionally beneficial foods and a source for drugs development (Guerra-Dore *et al.*, 2007). Nutritional investigations on edible and therapeutic mushrooms species are rare for the most important species in Africa. Degreef and al., (1997) analysed the nutritional and ecological value of some edible mushrooms of the Zambezian woodland area. Parent and Thoen (1977) studied the food value of edible mushrooms from the Upper-Shaga Region. A number of studies have reported the content of some chemical nutrients such as proteins, lipids, vitamins and mineral traces in edible mushrooms from Africa. Production and consumption of mushroom have increased throughout the world. *Pleurotus ostreatus* is the second most cultivated edible mushroom worldwide after *Agaricus bisporus.* It has economic and ecological values as well as medicinal properties (Sánchez, 2010). In Western Africa, Mushrooms cultivation is effective in Benin, Togo and Ghana. The nutritional properties and biological active components of fungi have received more attention by researchers in Asia compared to Africa. In Asia Mushrooms belonging to the genus *Ganoderma* are well known in traditional medicine and some researchers laid much emphasis on their chemical constituents (Ziegenbein *et al.*, 2006). *Ganoderma lucidum*, *Phellinus rimosus*, *Pleurotus*

florida and *Pleurotus pulmonaris* (Ajith and Janardhanan, 2007), *Thelephora ganbajun*, *Thelephora aurantiotincta*, *Boletopsis grisea* (Liu et al., 2004) and other mushrooms have been reported to have significant antioxidant activities. *Ganoderma lucidum* in particular has been used in Chinese medicine as a tonic for promoting health, perpetual youth, vitality, and longevity (Chang and Mshigeni, 2000). As reported by, Ye et al., 2001, De Silva et al., 2012, *Ganoderma lucidum* contains intrinsic immunomodulating and anti-tumor properties. In Africa, research on macromycetes species has been reduced to the listing of species and their ethnomycological knowledge and followed by some notes on the taxonomic descriptions. In this sense, few studies have been conducted in Nigeria (Zoberi, 1979, Adewusi et al., 1993), Tanzania (Saarimäki *et al*., 1994; Härkönen *et al*., 2003), Burundi (Buyck, 1994), Ghana (Holden, 1970) and Kenya (Pegler, 1972). As for Burkina Faso, it is known that edible species are disappearing because of the lack of rain (Guissou et al., 2008). The consequence is that, ethnomycological knowledge is decreasing from generations to generations. In Burkina Faso, numerous biological investigations have been made on medicinal plants. Various parts of these plants such as fruit, leaves, and roots have been studied (Kiendrebeogo et al., 2005; Karou et al., 2005, Meda et al., 2005; Lamien-Meda et al., 2008; Bakasso et al., 2008; Meda et al., 2010, Konaté *et al.*, 2010; Ouédraogo et al., 2011; Nana et al., 2012). However, pharmacological investigations on mushrooms are inexistent. According to the literature, studies on mushroom constituents in Burkina Faso are rare and are related to inventory of species. Burkinabe people do not have more information on edible and medicinal value of mushrooms. Research on mushrooms by inventories is intended to promote the preservation of these species which are not well known by populations. The aim of this study is to contribute to the characterization of Burkina Faso mushrooms species in order to promote them through the evaluation of the total phenolic, flavonoid contents and the biological activities.

MATERIALS AND METHODS

This study was carried at Laboratoire de Biochimie et Chimie Appliquées, UFR/SVT, University Joseph KI-ZERBO, Burkina Faso. Voucher specimens were deposited in the University Joseph KI-ZERBO herbarium (OUA)

Mushroom material: All the species were collected in Burkina Faso. *Ganoderma lucidum*, *Itajaya rosea* and *Lentinus squarrosulus* were collected in the Ouagadougou region; *Chlorophyllum* aff. *molybdites*, P*hlebopus sudanicus,* L*endzites elegans* and *Psatyrella tuberculata* in Mare aux hippopotames de Bala, *Laetiporus baudhanii* and L*endzites elegans* in the Forêt classée de Niangoloko. The species were identified by Dr Guissou K.M.L., a mycologist from the University Norbert ZONGO, Burkina Faso. *Chlorophyllum* cf *molybdites*, P*hlebopus sudanicus* and *Psatyrella tuberculata* are recorded to be edible mushrooms in Burkina Faso (Guissou *et al.*; 2008).

METHODS

Preparation of mushroom extracts: The dried mushrooms were reduced to fine powder using a grinder. The powder was extracted in acetone 80% by maceration at room temperature during 48h with the proportion 1/10 (m/v). The extracts were subsequently filtered and concentrated to dryness at 40ºC under vacuum.

Total phenols content: Total phenols were determined using Folin-Ciocalteu method as described by Singleton et al. (1999). Aliquots (125 µl) of solution of extracts in methanol (10 mg/ml) were mixed with 625 µl Folin-Ciocalteu reagent (0.2 N). After 5 min, 500 µl of aqueous Na_2CO_3 (75 g/l) were added and the mixture was vortexed. After 2h of incubation in the dark at room temperature, the absorbances were measured at 760 nm against a blank (0.5 ml Folin-Ciocalteu reagent + 1 ml Na_2CO_3) on a UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England). The experiments were carried out in triplicate. A standard calibration curve was plotted using gallic acid (0-200 mg/l). The results were expressed as mg of gallic acid equivalents (GAE)/g of extract.

Flavonoid contents: The total flavonoids were estimated according to the Dowd method as adapted by Arvouet-Grant et al. (1994). 0.5 ml of methanolic AlCl3 (2%, w/v), then were mixed with 0.5 ml of methanolic extract solution (0.1 mg/ml). After 10 min, absorbances were measured at 415 nm against a blank (mixture of 0.5 ml methanolic extract solution and 0.5 ml methanol) and compared to quercetin calibration curve (0-200 mg/L). Data were obtained by means of the three determinations. The amounts of flavonoids in plant extracts were expressed as mg of quercetin equivalents (QE)/g of extract.

Antioxidant activity determination

DPPH radical method: Radical scavenging activity of plant extracts against stable DPPH (2, 2'-diphenyl-1-picrylhydrazyl, Fluka) was determined spectrophotometrically at 517 nm as described by Vélazquez et al. (2003). Extract solutions were prepared by dissolving 10 mg of dry extract in 10 ml of methanol. The samples were homogenized in an ultrasonic bath. 0.5 ml of aliquots which were prepared at different concentrations from each sample was mixed with 1 ml of methanolic DPPH solution (20 mg/ml). After 15 min in the dark at room temperature, the decrease in absorption was measured. The blank sample was constituted by the same amount of methanol and DPPH solution. All experiments were performed in triplicate. Radical scavenging activity was calculated using the following formula:

Inhibition (%) = (1-B/A)*100

$\mathbf{A_0}$: absorption of blank sample; **A**: absorption of tested extract solution

Amount of extracts in samples and DPPH radical scavenging activity curve was plotted. The concentration which was responsible of half scavenging activity IC50 (concentration causing 50% inhibition) value of each extract was determined graphically and expressed as mg/ml.

Iron (III) to iron (II) reduction activity (FRAP): FRAP assay was performed according to the method of Hinnebourg et al. (2006). Briefly, 0.5 mL of each extract (1 mg/mL) was mixed with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of aqueous potassium hexacyanoferrate [K_3Fe $(CN)_6$] solution (1%). After 30 min incubation at 50°C, 1.25 mL of trichloroacetic acid (10%) was added and the mixture was centrifuged at 2000 × g for 10 min. Then, the upper layer

solution (0.625 mL) was mixed with distilled water (0.625 mL) and a freshly prepared $FeCl_3$ solution (0.125 mL, 0.1%). Absorbencies were read at 700 nm and Ascorbic acid was used to produce the calibration curve. The iron (III) reducing activity determination was performed in triplicate and expressed in mg Ascorbic Acid Equivalent per mL of extract.

Acetylcholine esterase inhibition: AChE inhibition was conducted according to the protocol described by Lopez et al. (2002) with some modifications. Briefly, the assay mixture consisted of 200 µL of tris-HCl 50 mM pH 8, 0.1% BSA buffer, 100 µL of extract solutions (final concentration 100 µg/mL) and 100 µL of AChE (0.22 U/mL). The mixture was incubated at room temperature for 2 min before adding 500 µL de DTNB (3 mM) and 100 µL of substrate (ATCI 15 mM). The developing yellow color was measured at 405 after 4 min. Galantamine was used as positive control. AChE inhibitory activity was expressed as percent inhibition of AChE, calculated as:

$$\text{AChE inhibition (\%)} = (1\text{-}B/A)*100$$

Where A is the change in absorbance of the assay without the plant extract and B is the change in absorbance of the assay with the plant extract.

Statistical analysis: Data are expressed as mean ± SD (n = 6). Significant differences are determined by using one way anova of variance with Newman-Keuls multi-comparative post test.

RESULTS

Total phenolic and flavonoid content: Table 1 summarises the results of the phenolic and flavonoid contents. Results are presented as mean values of six replicates. Total phenolic ranged from 17.63 to 393.73 mg GAE /g. The highest phenolic content was found in the extract of *Laetiporus baudhanii* followed by *G. lucidum* (111.31 ± 0.28mg GAE/g) and S. Commune (49.05 ± 2.14 mg GAE /g). The lowest one was found in *Psatyrella tuberculata.* The other species compounds are > to 20 mg GAE /g. Average concentration of flavonoid ranged from 0.41 ± 0.01 to 181.68 ± 2.50 mg QE/g and the highest flavonoid amounts was also found in *Laetiporus baudhanii* extract, followed by *G. Lucidum* (16.29 ± 0.03 mg QE/g). There is a correlation between phenolic and flavonoid contents (y = 0.37x-20; R^2 = 0.69): the species which have highest phenolic content have also relative highest flavonoid content. The antioxidant activity of our samples is undeniable due to a major part of the total phenol contents. According to Dorman et al., (2003), there is no single, universal method capable of providing an accurate, comprehensive picture of antioxidant profile because several mechanisms underlying antioxidant activity have been proposed including termination of free radical mediated chain reaction, hydrogen donation, chelation of catalytic ions, and elimination of peroxides. Thus, two tests were used to determine the antioxidant capacities, the DPPH and the FRAP assays. The DPPH radical scavenging activity of phenolic compounds was expressed as IC50 value in micrograms per mL of extract. A low IC50 value represents a high antioxidant activity. Results are presented as mean values of six replicates in Table 2. IC50 values were ranged from 0. 003 ± 0.001 mg/mL to 0.703 ± 0.01 mg/mL. *L. badhaunii* exhibited significant radical scavenging activity with IC50 of 0.003 mg/mL, followed by *S. commune* (0,006 mg/mL) and *G.lucidum* (0,022 mg/mL). *L. squarrosulus* showed the lowest scavenging activity (0,703 mg/mL). The radical scavenging activity of the remaining mushroom species decrease in the following order: *L. baudhanii >S. commune > G. lucidum > L. lendzites > P. sudanicus >P. tuberculata >I. rosea > C. molybdites >L. squarrosulus*. FRAP values (Table 2) were ranged from 2.08 ± 0.06 mg AA/g to 87.07 ± 1.01 mg AA/g. The reduction power of the mushroom species decreases in the following order: *Laetiporus baudhanii > Schizophyllum commune > Ganoderma lucidum > Lendzites elegans > Chlorophyllum molybdites > Phlebopus sudanicus > Psatyrella tuberculata > Itajaha rosea > Lentinus squarrosulus.*

Total phenolic (y = 1.61x - 5,11; R^2 = 0.90) and total flavonoid (y = 3.66x + 6.01; R^2 = 0,9081) contents showed good correlations with FRAP. However, no correlation was elucidating with DPPH. The percentage of AChE inhibition for the nine mushroom species ranged between 5.73% and 29.45% (Fig 1). Once again, *Laetiporus baudhanii* got the highest AChE inhibition followed by *Ganoderma lucidum*. The lowest AChE inhibition (<10%) is found in *Lentinus squarrosulus, Schizophyllum commune* and *Phlebopus sudanicus.*

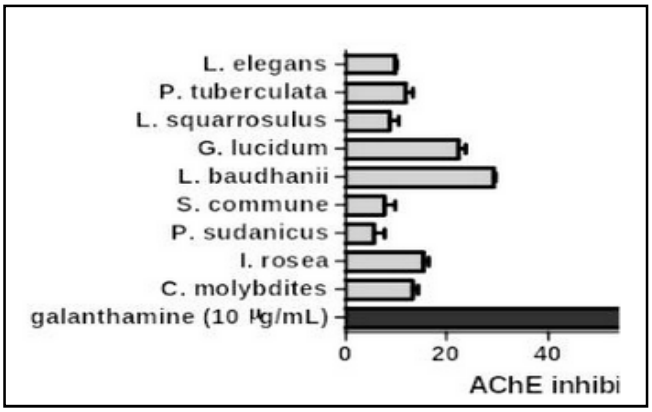

Figure 1. Acethylcholine esterase inihibition (%) by 100µg/mL of mushroom extracts. Values are mean`±`S.D. (n`=`6)

DISCUSSION

Antioxidants are vital to any human being in preventing damage caused by free radicals to cell organelles, cell walls, and cell membranes. Results on total phenol of our species are in agreement with several works which showed the antioxidant activity of polyphenols on edible and medicine mushrooms. Total phenols are involved in several physiologic processes and play a protective role in degenerative diseases. Their contents have been isolated in lot of foods, vegetable and fruits. Polyphenols contents are the major antioxidant constituent of the fruit. The total phenol content of fourteen fruits for example varied from 308.83 to 5978.33 mg GAE/100mg of fruits and justified why Burkinabé people consume these fruits (Lamien-Meda et al., 2008). Medicinal mushrooms occurring in South India namely Ganoderma lucidum, Phellinus rimosus**,** Pleurotus florida and Pleurotus pulmonaris possessed profound antioxidant and antitumor activities (Thekkuttuparambil *et al.,* 2007). Several authors reported that wild mushrooms are best sources of antioxidants (Shi et al., 2002; Lakshmi et al., 2007; Jayakumar et al., 2007; Pala and Wani, 2011). All our studied mushroom species extracts contained phenols and most part of these phenols

could be flavonoids. The extracts possess higher total phenolic content compared to some previous studies in particular those of Kim et al. (2008) where the average total concentration of phenolic compounds was 174µg/g in edible mushrooms and 477µg/g in medicinal mushrooms and Ramesh et al., (2010). Parilla et al. (2007) have shown that the content from wild and commercial mushrooms from Mexico in 80% methanol extracts ranged from 45.6 mg CAE /100g FW to 308.3 mg CAE /100g FW. Total phenol from *Cantharellus* species according to Kumari et al., (2011) varied from 11.40 to 16.80 mg/g. Average concentration of flavonoid calculated by Kumari *et al.* (2011)

Table 1. Total phenolic and flavonoid contents in mushroom extracts

Samples	Extraction rate (%)	Total phenolic (mg GAE/g)	Total flavonoid (mg QE/g)
C. molybdites	4.13	17.63 ± 0.13^{a}	0.41 ± 0.01^{a}
I. rosea	9.71	23.11 ± 0.03^{a}	1.26 ± 0.02^{b}
P. sudanicus	7.10	$23.93 \pm 0,08^{a}$	0.92 ± 0.02^{ab}
S. commune	1.52	49.05 ± 2.14^{b}	1.56 ± 0.04^{b}
L. baudhanii	8.14	393.73 ± 0.55^{c}	181.68 ± 2.50^{c}
G. lucidum	5.88	111.31 ± 0.28^{d}	16.29 ± 0.03^{d}
L. squarrosulus	9.22	22.41 ± 0.10^{a}	0.92 ± 0.00^{ab}
P. tuberculata	2.28	8.41 ± 0.03^{e}	0.43 ± 0.05^{a}
L. elegans	3.29	27.41 ± 0.07^{f}	2.5 ± 0.02^{e}

Values are mean ± S.D. (**n** = 6). Various t letters in the same column indicate significant differences ($p < 0.05$).
Table 1: Total phenolic and flavonoid contents in mushroom extracts.

Table 2. Antioxidant activities in mushrooms

Mushroom species	DPPH free radical scavenging activity IC50 (mg/mL)	FRAP (mg AA/g)
C. molybdites	0.258 ± 0.01^{a}	3.70 ± 0.10^{a}
I. rosea	0.239 ± 0.00^{b}	2.08 ± 0.06^{b}
P. sudanicus	0.170 ± 0.01^{c}	3.09 ± 0.09^{ab}
S. commune	0.006 ± 0.001^{d}	31.98 ± 0.48^{c}
L. baudhanii	0.003 ± 0.001^{d}	87.07 ± 1.01^{d}
G. lucidum	0.022 ± 0.00^{e}	13.77 ± 0.55^{e}
L. squarrosulus	0.703 ± 0.01^{f}	1.73 ± 0.02^{b}
P. tuberculata	0.188 ± 0.00^{g}	2.86 ± 0.10^{a}
L. elegans	0.053 ± 0.00^{h}	8.57 ± 0.07^{f}
Positive controls		
Ascorbic acid (3 µg/ml)	$1.80 \pm 0,43^{i}$	n.d
Gallic acid (1.5 µg/ml)	0.61 ± 0.01^{j}	445.40 ± 1.70^{g}

Values are mean ± S.D. (**n** = 6). Various letters in the same column show significant differences ($p < 0.05$). n.d: not determinate

from three different edible wild *Cantharellus* ranged from 1.34 to 1.92 mg/g. In a previous study, the average total flavonoids concentration was 49µg/g, in edible and medicinal mushrooms from Korea Kim *et al.* (2008). These amounts are very lower compared to the values of our samples. These differences could be explained by the type of extraction and the mushroom's species. Several studies analysing the total phenolic compounds and the antioxidant activities of wild and cultivated mushrooms have been published by lot of authors in the world (Lakshmi et al., 2005, 2007; Yang et al., 2002; Mau et al., 2001; Choi et al., 2006; Lo and Cheung, 2006). Total phenols have been shown to be the major antioxidant components in ethanolic extracts in a variety of culinary and medicinal mushrooms (Tsai et al., 2008; 2009). Preeti et al. (2012) highlighted the antioxidant mushrooms toward a review. They indicate the potential of mushrooms as panacea for several diseases and also reveal a novel potential to fight against tumor in man. In this study, the antioxidant activity of *L. baudhanii* was significantly higher than the other mushrooms and we observed a correlation between antioxidant activities and phenolic and flavonoid contents. Indeed, the DPPH scavenging activity has been shown to significantly correlate with total content of phenolic compounds in a variety of edible and medicinal mushrooms (Kim *et al.,* 2008, Kettawan et al., 2011). Also, Cheung and Cheung (2005) have demonstrated that the antioxidant activity of these two edible mushrooms (*Lentinus edodes* and *Volvariella volvacea*) have been shown to correlate with the phenolic content in different subfractions of the mushroom extracts. These results corroborate our results obtained in this study. The good correlation observed between total phenolic content and total flavonoid could means that flavonoid highly contributes to phenolic content and thus to the antioxidant activities of mushroom extracts. The antioxidant capacities of several flavonoids are much stronger than those of vitamins C and E (Procházková et al., 2011).

Flavonoids can prevent injury caused by free radicals by following mechanisms: direct scavenging of reactive oxygen species (ROS), activation of antioxidant enzymes, metal chelating activity, reduction of α-tocopheryl radicals, inhibition of oxidase, mitigation of oxidative stress caused by nitric oxide, increase in uric acid levels and increase in antioxidant properties of low molecular antioxidants (Procházková et al., 2011). So, our nine studied mushrooms, especially *L. baudhanii,* could be beneficial to the human health due to their flavonoid content. Previous pharmacological studies have shown that the consumption of medicinal mushrooms can be beneficial to human through their ability to cure various diseases (Ying et al., 1987; Hobbs, 1995; Francia et al., 2007, Ferreira et al., 2010). Medicinal mushrooms are also used in cancer therapies (Dilani et al., 2012). Several metabolites from plant extracts are known as good inhibitors of AChE, including phenolic compounds like flavonoids (Lopez et al., 2002; Lee et al., 2004). The AChE inhibitory effect of the mushroom extracts studied could be due to their flavonoid content. AChE inhibition is an alternative in the treatment of dementia, Alzheimer, and Parkinson diseases. So, these two mushrooms *Laetiporus baudhanii* and *Ganoderma lucidum* could be potential resources for isolation of AChE inhibitors which could be useful to the treatment of dementia, Alzheimer, and Parkinson diseases.

Conclusion

All the species in this study were found to possess significant antioxidant activities, with higher amount of total phenolic, flavonoid. They also, show good AChE inhibition. Among the nine mushrooms studied, *L. baudhanii* presented the best properties. This is the first time that wild edible mushrooms collected in Burkina Faso were submitted to this kind of studies. *L. baudhanii* can provide source of additive effects based on the presence of all the bioactive contents. Thus, *L. baudhanii* is a potential source of antioxidant compounds and AChE inhibitors. However, further investigations are needed to elucidate its valuable therapeutic use.

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