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Selenium Content and Antioxidant Potential of Some Edible Wild Mushrooms from Bandundu Area, DR Congo

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Abstract

Nutrition is an important aspect of public health because it is linked to many significant diseases and health problems. This work is part of the promotion of traditional foods from the Democratic Republic of Congo in general and in the province of Bandundu, in particular by assessing the selenium content and the antioxidant capacity of wild edible mushrooms. Methanolic extracts from selected mushrooms were characterized for their chemical fingerprint by TLC and their *in vitro* antioxidant activities using ABTS, DPPH assays. Phytochemical screening revealed the presence of alkaloids, free amines, steroids and terpenes in all species. However, *Auricularia delicata* and *Pleurotus tuberregium* contain flavonoids. All extracts displayed a good radical-scavenging activity at the concentration range 1 - 300 µg/mL in the following order: *Auricularia delicata* > *Lentinus cf cladopus* > *Pleurotus tuberregium* > *Marasmius buzungolo* > *Schizophillum commune*. Studied mushrooms showed the interest quantity of selenium and *L. cf cladodus* had the highest concentration. Moderate selenium content of these wild mushrooms associated with their important antioxidant activities could provide health benefits to Bandundu's populations in protecting against oxidative damage under different conditions including konzo.

[*]These authors contributed equally to this article.

Keywords

Antioxidant Activity, *Auricularia delicata*, *Lentinus cf cladopus*, *Marasmius buzungolo*, *Pleurotus tuberregium*, *Schizophyllum commune*, Selenium, Traditional Food

1. Introduction

Nutrition is an important aspect of public health because it is linked to many significant diseases and health problems. The consumption of vegetable foods has been a public health issue; based on existing research, polyphenols may be applicable to public health in primary and secondary prevention, particularly concerning diseases associated with oxidative damage such as obesity, diabetes, cardiovascular disease, and cancers [1]. Finding alternative and complementary ways to reduce the oxidative processes might have a beneficial interest in the context of developed countries.

The Democratic Republic of Congo flora in general and this of Kwilu-Kwango in the Bandundu area, in particular, is rich in unexploited traditional foods. The Kwango-Kwilu area is among the top producers of cassava, and cassava flour, the main source of carbohydrates. This region paradoxically has a specific significance due to recurring outbreaks of a neglected disease called konzo, which is a distinct neurological entity with selective upper motor neuron damage. Recent studies suggest that disease development may be mediated by oxidative damage, induced by cyanide poisoning through the ingestion of poorly processed bitter cassava [2]. Cassava and its derivatives are consumed with vegetables, squash, caterpillars, mushrooms and fish in small quantities and meat. Meat, a source of sulfur amino acids, is reserved for special occasions such as festivals and other large events. In times of famine or welding, mushrooms are indeed considered as substitute foods with meat and fish [3]. It is amazing that food, too widespread and popular in tropical Africa as wild mushrooms from the Democratic Republic of Congo, suffer from such a lack of taxonomic data, phytochemical and biological activities. For this, Mbemba *et al*. (2013) assessed the nutritional value of traditional foods, in order to equilibrate the diet of the population of this area severely affected by the malnutrition [4]. Among traditional foods studied by Mbemba *et al*. (2013), we focus our research on five species of edible wild mushrooms largely consumed by the population of the Kenge town in Bandundu area: *Auricularia delicata* (Mont.) Henn., *Lentinus cf cladopus* (Lév.), *Marasmius buzungolo* Singer, *Pleurotus tuberregium* (Fr.) Singer, *Schizophyllum commune* Fr. Previous studies on nutritive value and chemical composition of selected mushrooms reported that they are potentially a good source of proteins, macro and micro elements, and fibres [4] [5] [6]. Mushrooms are a source of healthy nutrients, mainly secondary metabolites, such as terpenes, steroids, phenolic compounds, but also of some primary metabolites like organic acids, peptides, and proteins.

To our knowledge, few investigations have been performed on the biological

properties such as antioxidant capacities of mushrooms from Bandundu. The present work aimed to investigate the antioxidant activities of methanolic extracts of edible wild mushrooms using ABTS and DPPH assays. Thin Layer Chromatography (TLC) was used to achieve phytochemical analysis.

2. Materials and Methods

2.1. Study Area

Plants have been harvested in Kenge (S04˚S1'E16˚S8'), a territory in Pelende-Nord sector, Kwango district, Bandundu Province in the Democratic Republic of the Congo. According to Koppen classification, its climate is of AW4 type. The annual average pluviometry is around 1500 mm. The monthly average temperatures vary between 22˚C and 24˚C; during the rainy season, the average maxima rise around 28˚C and in the dry season around 31˚C whereas the average minima get down between 17˚C and 13˚C. Geologically, Kenge-Kwango formations belong to two systems, which are: Kharroo and Kalahari.

This latter covers another one, and the Kalahari system is made of layer superposing of Bateke series and of soft polymorphic and stones upon hard rock's (silicious sand stones). The Kharroo is mainly made by Kusango series (upper cretaceous). These are soft clay sandstones, red brick withargillite (clay stone) and conglomerate. The soft sandstones are made of average dimension quartz grains, well rolled, spread in a mass of slender grains [7].

2.2. Materials

Mushrooms: *Auricularia delicata*, *Lentinus cf cladopus*, *Marasmius buzungolo*, *Pleurotus tuber-regium* and *Schizophyllum commune* were harvested in the state specifically in the territory of Kenge (Bandundu) on November 2014. The collected mushrooms were identified by Professor Dibaluka S, expert of the Department of Biology, Faculty of Science, University of Kinshasa, DR Congo). The mushrooms were first dried under sunlight to avoid deterioration due to moisture, before passing through the oven. Once in the laboratory, the mushrooms were dried at 65˚C in an oven while calculating the rate of moisture and grinding using an electric grinder. The powdered mushrooms were stored in the dark at room temperature and used for solvent extraction.

2.3. Chemicals and Reagents

All solvents were of analytical grade and purchased from Merck VWR (Leuven, Belgium). Gallic acid, 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2-aminoethyldiphenylborat, Folin-Ciocalteu reagent and potassium persulfate were purchased from Sigma (Bornem, Belgium) purchased from Sigma-Aldrich. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Eastman Kodak (Rochester, NY, USA).

2.4. Preparation of Extracts

Methanolic extracts were prepared by maceration of 30 g of mushroom powder

with 200 ml of methanol 80%. Evaporation of the solvent was performed under a reduced pressure (40˚C) followed by a stay of 48 - 72 h in a vacuum chamber to provide the dry extracts which were weighed and kept in hermetic and dark flasks at 4˚C.

2.5. Phytochemical Analysis

2.5.1. Selenium Content

The total selenium content was performed by a colorimetric method using 3, 3'-diaminobenzidine as a complexing reagent. All selenium compounds are reduced in selenites and selenium thus converted in selenium IV preceded by mineralization. The method was based on the measurement of the yellow color formed when 3, 3'-diaminobenzidine (DAB) reacts with Se^{4+} [8]. Selenium VI standard was prepared daily by appropriate dilution of $Na_2SeO_4 \cdot 10H_2O$ to obtain 1 µg/mL of analytic solution.

2.5.2. Thin Layer Chromatography (TLC) Analysis

The phytochemical screening was performed following the standard techniques [9]. Analytical TLC of 10 µl of solution for 10 mg/ml of aqueous extracts was carried out on normal phase Silica gel 60 F_{254} plates (Merck), using as eluent either:

a) Ethyl acetate, formic acid, glacial acetic acid and water (100:11:11:26; v/v/v/v).

b) Toluene/ethyl acetate (9:1; v/v) [10].

Flavonoids and phenolic acids were revealed using Natural Products-PEG reagent and observed at UV-365 nm light. Chlorogenic acid, rutin, hyperoside, and isoquercitrin were used as standards. Flavonoids were detected as yellow-orange fluorescent spots and phenolic acid as blue fluorescent spots; quinones owing to Borntrager reagent (NaOH 10% or NH_4OH 10%); alkaloids due to Dragendorff reagent; terpenoids and steroids were revealed by using sulphuric anisaldehyde and antimony 20%.

2.5.3. Determination of Total Phenolic Content

Total phenolic content of methanolic extracts (Methanol 80%) extracts was determined according to the Folin-Ciocalteu method as described previously [11]. A calibration curve of gallic acid (0.025 - 0.4 mg/ mL) was prepared, and phenolic contents were determined in triplicate from the linear regression equation of this curve. The results were expressed as milligrams of gallic acid (GA) equivalent per gram of dried matter.

2.5.4. Flavonoid Content

The flavonoid content of mushrooms extracts was determined by UV-Vis spectrophotometry method described previously [12]. Results are expressed in mg equivalent of quercetin per g (mg QE/g) of dry vegetal material using the following equation $y = 0.0232x + 0.1535$ ($R^2 = 0.945$).

2.5.5. Tannins Content

The extraction of tannins was carried out according to the adapted method used

by Zhang *et al*., 2008 [13]. Tannin extract contents were quantified by vanillin method using the procedure reported by Sun *et al*., 1998 [14]. This method is based on the ability of vanillin to react with tannins units in the presence of acid to produce a colored complex measured at 500 nm.

2.6. Evaluation of Radical Scavenging Activity

2.6.1. ABTS Radical Scavenging Capacity

ABTS assay was based on the method described by Tshisekedi *et al*. (2017) [15]. Briefly, $ABTS^{\bullet+}$ radicals were generated by mixing potassium persulfate (2.45 mM) with ABTS (7 mM) and kept overnight in the dark. Working solution of $ABTS^{\bullet+}$ was obtained by adding 100% methanol to have an absorbance of 0.80 (±0.02) at 734 nm. The $ABTS^{\bullet+}$ scavenging capacity of each extract was monitored at 734 nm after 30 minutes, and compared to control DMSO and positive control.

2.6.2. DPPH Radical Scavenging Capacity

DPPH assay was performed according to the method described previously by Tshisekedi *et al*. (2017) [15]. Gallic acid was used as positive control and $ABTS^{\bullet+}$, DPPH scavenging activities of extracts were expressed as IC_{50} values. Each sample was measured in triplicate.

3. Results and Discussion

3.1. Chemical Analysis

3.1.1. Selenium Content

Selenium, vital to human health, is a mineral essential for the functioning of the thyroid gland and also plays a role as an enzyme cofactor in antioxidant defense. The mineral analysis showed that selenium content of studied mushrooms ranging from 2.1 µg/100g to 5.6 µg/100g of dry weight (**Table 1**). *L. cf cladopus* had the highest content followed in decreasing order by *M. buzungolo*, *A. delicata*, *P. tuber-regiun* and, *S. commune*.

Studies of Ifeoma *et al*. (2009), on analysis of the mineral composition of fungi reported for *Pleurotus tuber-regium*, low selenium content (2.5 µg/100g) [16]. Studies of Mallikarjuna *et al*. (2013) on the mineral composition of mushrooms *Lentinus* and *Pleurotus* genus and showed high concentrations of selenium ranging from to 48 µg/100g to 190 µg/100g, largely superior to our samples [6]. Falandysz (2008) reported that most of the edible mushroom species are selenium-poor with quantities of selenium < 100 µg/100g dry weight.

Table 1. Ashes, selenium content and water content of studied mushrooms.

Parameters	*Auricularia delicata*	*Lentinus cf. cladopus*	*Marasmius buzungolo*	*Pleurotus tuber-regium*	*Schizophillum commune*
Ashes (%)	8.8 ± 0.1	2.29 ± 0.01	2.74 ± 0.02	1.04 ± 0.02	1.22 ± 0.05
Selenium (µg/100g)	3.3 ± 0.01	5.6 ± 0.2	4.1 ± 0.13	2.2 ± 0.01	2.1 ± 0.13
Water content (%)	7.15	14.43	6.65	6.46	18.37

Our samples contain the quantity of selenium relatively less and inferior to vegetables and fruits such as papaya, pineapple, guava, mango and raspberry which contain quantities of selenium ranging from 60 µg/100g to 1600 µg/100g [17]. The amount of selenium in any food varies greatly depending on the quality of the soil on which the food was produced, and cultured. Less selenium content of studied mushrooms is probably related to the poor amount of the selenium available in soils of Kwilu-Kwango district characterized by Kalahari soil type with texture and composition poor [18]. A low dietary selenium intake is associated with oxidative stress-related conditions. However studied mushrooms and others foods rich in selenium are needed to be consumed and valorized by Congolese population, especially population of Kwilu-Kwango severely affected by konzo. Bumoko *et al.* (2015) reported that selenium deficiency contributes to the pathogenesis of konzo through mechanisms that are responsible for oxidative damage [19].

3.1.2. Phytochemical Screening

The results of phytochemical screening are presented in Table 2. Alkaloids, free amines, terpenes, steroids are present in all species studied. TLC analysis of methanolic extracts showed the presence of polyphenolic compounds in all extracts. Phenolic acids were the most abundant, however, *A. delicata* and *P. tuber-regium* contain flavonoids.

There exist few reports on the phytochemical screening of these Congolese wild edible mushrooms. Further chromatographic and spectroscopic studies are needed to characterize the others unknown compounds.

Results from the quantitative determination of flavonoids and total phenolic contents were obtained from the linear regression equation and are summarized in Table 3.

Flavonoids as quercitrin equivalents in milligrams per gram of dry weight (QE/g DW) and total polyphenol contents were calculated as gallic acid equivalents in milligrams per gram of dry weight (mg GAE/g DW). The total phenolic contents varied significantly ($P < 0.05$) between the studied mushrooms. Results

Table 2. Results of preliminary phytochemical screening.

Secondary metabolites	*Auricularia delicata*	*Lentinus cf. cladopus*	*Marasmius buzungolo*	*Pleurotus tuber-regium*	*Schizophillum commune*
Alkaloids	+	+	+	+	+
Anthocyanins	–	–	–	–	–
Cardiac glycosides	–	–	–	–	–
Flavonoids	+	–	–	+	–
Free amines	+	+	+	+	+
Reducing sugar	–	–	–	–	–
Tannins	–	–	–	–	–
Terpenes, steroids	+	+	+	+	+

Table 3. IC_{50} values (μg/mL) on ABTS and DPPH assays, total phenolic content (mg GAE/gDW), flavonoid content (mg QE/gDW) of selected wild mushrooms (Mean ± SD, n = 3).

	ABTS	DPPH	Total phenolic contents	Flavonoid contents
Samples	IC_{50} (μg/mL)	IC_{50} (μg/mL)	(mg GAE/gDW)	(mg QE/gDW)
Auricularia delicata	25.47 ± 0.98	76.38 ± 0.98	47.98 ± 0.43	4.38 ± 0.23
Lentinus cf cladopus	112.95 ± 0.98	291.07 ± 0.98	22.79 ± 0.21	nd
Marasmius buzungolo	187.068 ± 0.97	346.74 ± 0.94	31.68 ± 0.29	nd
Pleurotus tuber-regium	153.815 ± 1,01	281.19 ± 0.95	23.37 ± 8.16	0.89 ± 0.15
Schizophylium commune	197.242 ± 0.94	319.15 ± 0.97	15.35 ± 0.14	nd
Gallic acid	0.71 ± 0.08	1.07 ± 0.10		

nd = not determined.

showed that *A. delicata* is the sample with the highest amount of total phenolic compounds followed by *M. buzungolo*. Further, *A. delicate* gives flavonoids moderately high values compared to the others. Studies of Arbaayah (2013) on the evaluation of antiradical activity of the ethanol extract of *Pleurotus* spp. and *Schizophillum commune* showed that the variety of *Pleurotus djamor* was the richest with total polyphenol contents and in flavonoids [20]. Contrary to our results, Ifeoma *et al*. (2009) reported flavonoids in *P. tuber-regium*. In general, flavonoids represented a very small percentage of the total polyphenol contents in mushrooms and phenolic acids constitute their main phenolic compounds [21].

3.2. Antioxidant Activity

Radical scavenging activity of tested food items, determined by two different methods namely ABTS and DPPH is presented in **Table 3** and is expressed as IC_{50} values. IC_{50} is the amount of antioxidant necessary to decrease the initial concentration of radical by 50%. The lower IC_{50} value indicates a higher antioxidant activity.

Our results obtained from ABTS and DPPH assays show that the extracts were effective in the reduction of stable radicals $ABTS^{\bullet+}$ and $DPPH^{\bullet}$. These extracts had significant scavenging effects with increasing concentrations in the range of 20 - 350 μg/mL and with antiradical activities connected to their ability to scavenge free radicals according to their IC_{50} and gallic acid (positive control) equivalent (GA-E) values (**Table 3**). IC_{50} and GA-E values for extracts showed that *A. delicata* is the most active followed by *L. cf cladopus*, *P. tuber-regium*, *M. buzungolo* and *S. commune*. This strong radical scavenging activity might be attributed to their direct capacity for trapping free radicals by donating a hydrogen atom. The antioxidant activity of studied mushrooms might correlate with the total phenolic content, evaluated by means of the Folin-Ciocalteu assay, nevertheless, individual phenolic compounds could show markedly different antioxidant effects. Previous studies reported the remarkable antioxidant activity of

wild and cultivated mushrooms [20] [21] [22] [23] [24]. By comparing with previous literature, all wild tropical studied mushroom samples showed higher antioxidant activity performed by ABTS and DPPH assays.

In our study, the antioxidant response of extracts appeared to be correlated with the method used. Extracts tested with a high GAE in ABTS assay also had a high GAE in the DPPH assay, but ABTS values appeared higher than those of DPPH in all the extracts. This can be explained by the different mechanisms of the analytical methods. ABT assay is applicable for both hydrophilic and lipophilic antioxidants while DPPH assay only hydrophilic antioxidants. In addition, some colored compounds can interfere at specific wavelengths of DPPH, leading to the underestimated antioxidant activity of extracts [25] [26].

Mushrooms are arich source of nutrients and nutraceuticals responsible for their pharmacological properties such as antioxidant, antimicrobial, immunomodulatory, antiatherogenic, and hypoglycemic activities [27] [28]. Wild mushrooms are becoming more important in our diet due to their nutritional value, related to the high protein and low fat/energy contents. Mbemba *et al*. (2013) reported the nutritional value of Congolese wild edible mushrooms and indicated that they were rich in proteins and certain essential amino acids, lipids, micronutrients [4]. Polyphenols have the potential to confer benefit in diverse neurodegenerative disorders associated with oxidative damage [29]. The antioxidant potential and selenium content of studied mushrooms could have a beneficial health for Congolese people, in particular, the population of Kwilu-Kwango, an area severely affected by konzo, a permanent and non-progressive paralytic disease associated with oxidative damage.

4. Conclusion

Chemical analysis of studied wild edible mushrooms showed that they contain alkaloids, free amines, terpenes, steroids and phenolic compounds. *A. delicata* and *P. tuber-regium* contain also flavonoids. Methanolic extracts of studied mushrooms showed an evident antioxidant activity in the selected *in vitro* chemical antioxidant assays. Valorization of traditional foods of Kwilu-Kwango's area with high antioxidant capacity could contribute to providing benefits protecting against oxidative damage under different conditions including konzo. The antioxidant potential and selenium content of these wild edible mushrooms could justify their use as functional foods but further studies are needed, especially on cellular models and *in vivo*, to demonstrate the benefit of these extracts on nutrition and health.

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