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Ethnomycology, myco-chemical analyzes and antioxidant activity of eleven species of the genus *Amanita* (*Basidiomycota*, fungi) from Benin (West Africa)

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Abstract
Fungi of the genus *Amanita* (*Basidiomycota*) contain secondary metabolites very useful for human welfare. They are much exploited by tropical African people for various purposes. The objective of this study is to identify the myco-chemical groups and evaluate the antioxidant activity of eleven macromycetes of the genus *Amanita* harvested in woodlands of Benin. The species were selected based on ethnomycological surveys conducted on a sample of 68 randomly selected persons from three ethnic groups in the Angaradebou village (Borgou Province, central Benin). All target species were subjected to chemical screening according to standard methods. The antioxidant activity was determined using the 1,1-diphenyl-2-picrylhydrazyl. Ethnomycological investigates reported three (03) edible and eight (08) inedible species with chemical compounds and antioxidant activity. The eleven species of *Amanita* can be useful in primary health care of local people.

Keywords: Wild mushrooms, chemical composition, edibility, health care, local people, Benin

Introduction
Previous studies have documented the various biological activities of fungi, including antibacterial, antiviral, antitumor and hepato-protective activities [1]. Like plants, fungi possess antioxidant capacity in *in vitro* systems. They contain antioxidant compounds such as: phenolics [2], alkaloids [2], organic acids [3] and can therefore be used both as a food supplement and in the pharmaceutical industry. These compounds do not act in the human organism when there is oxidative stress. Oxidative stress is generally related to the imbalance between the production of free radicals and the body's ability to neutralize and repair various oxidative damage [4]. The oxidative stress usually affects many cells such as the oxidation of sugars and proteins, lipid peroxidation and various genetic mutations [5]. It represents a very crucial factor promoting the development of many sources of diseases in humans; like diabetes, neuro-degenerative diseases, cardiovascular problem, and aging. Nevertheless, oxidative stress can be neutralized by antioxidants, which are considered to be any substance that can retard or prevent the oxidation of biological substrates [6]. These antioxidants actually have the ability to neutralize free radicals derived from either normal essential metabolic processes in the human body or from external sources. These are mainly micronutrients such as vitamin C (ascorbic acid), vitamin E (α-tocopherol), β-carotene and vitamin A; naturally available in fungi [7], phenolic compounds (flavonoids, and phenolic acids) and nitrogen compounds (alkaloids, amino acids and amines) [8]. Certain antioxidants such as flavonoids are used in the cosmetic, pharmaceutical and food sectors [9].
Fungi are ubiquitous organisms that produce a variety of biomolecules with both nutritional and pharmaceutical properties [10]. Secondary metabolites of *Basidiomycota* are exploited fortheir pharmacological activities to face various pathologies such as chronic inflammation, pathologies associated with oxidative processes, diabetes, infections (HIV, fungi, bacteria), immunological disorders and cancer [11]. Apart from their taste and their attractive aroma, fungi are known for their content in proteins, fats carbohydrates, amino acids and vitamins [12] such as vitamins B, C and D and mineral elements [13]. Fungi are also known for their richness in different bioactive substances with antibacterial, antifungal, antiviral, antioxidant and anti-parasitic properties [1]. Antioxidants found in fungi [14] provide the same medical services as plants because of their chemical composition [15]. However, the different therapeutically valuable phytochemicals may act as antioxidants or prevent the oxidative stress underlying pathological conditions such as cancer and diabetes [16], heart disease [17] and microbial

pathogens [18]. Some species of the genus *Amanita* (*Basidiomycota*) have very important myco-chemical elements for the organism [19].

In tropical Africa, and in Benin in particular, several species of *Amanita* are consumed [20] by local people, although the genus presents a wide range of edible and toxic species [21]. Not only some species can be eaten as food, but such edible taxa can be incorporated into diets as food supplements whilst inedible species can also be exploited as a source of bioactive metabolites. Edible and non-edible *Amanita* species can therefore be powerful sources of bioactive compounds, thus conferring their important chemical characterization on the human organism. In the present study, we are attempting to document the chemical composition of the genus *Amanita*, one of the most controversial fungal taxa when it comes to edibility, and to discussing their potential importance to local inhabitants.

Material and methods

Study site

The present study was carried out in the forest reserve of "Ouémé Supérieur" located in central part of North Benin, between 9 ° 11 'and 9 ° 47' N latitude and 1 ° 58 'and 2 ° 28' E longitude. The specimens used in the present study have been collected in Ceasalpinioid-dominated woodlands in Figure 1 below.

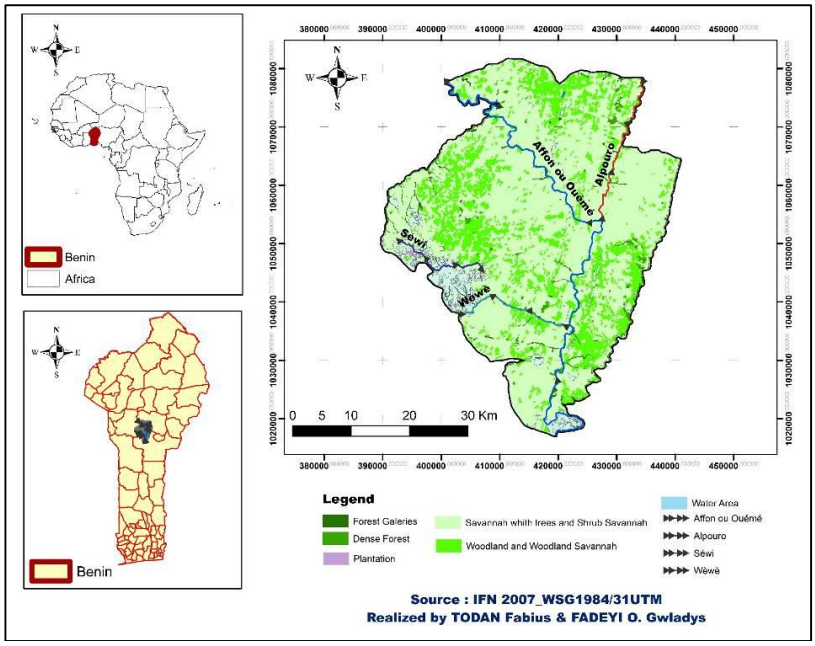

Fig 1: Vegetation map of the "Ouémé Supérieur" forest reserve showing the sampling sites in red, blue and yellow circles.

Materials

Specimens' collection, identification and preservation of specimens

Specimens sampling consisted in harvesting the whole fruit bodies by mean of the knife, taking care not to bruise any part of the fruit body necessary for a reliable identification of the species. Professional photographs (see Appendice 1) of the specimens were taking in situ by mean of the digital camera type Power Shot A2200 HD CANON (4 x Optical Zoom; 14. 1 Mega Pixels). The specimens are thereafter dried by mean of a field dryer [22] to the low heat until dehydration and stabilize their weight. After complete dehydration, the samples are kept in plastic bags type minigrip in order to avoid rehydration and deposited at mycological herbarium of the University of Parakou (UNIPAR, [23]), for further investigations. Various field books, among others [22, 24] and the serie Flore Iconographique des Champignons du Congo [25] are used for identification. Nomenclatural and spelling of the scientific names are checked in index fungorum (www.mycology.net).

Sampling of the population for ethnomycological survey

A rapid survey was done on 100 people (all ethnic groups combined) sampled randomly in the Angaradébou village. We adopted the sampling strategy of Dagnelie [26] to retain the number of people who effectively know and/or use the target fungi. By referring the methods as developed and used by [26, 27], we considered a final sampling size of 68 respondents sorted as follow into the three most represented ethnic groups in the regions: Yom (41), Peuhls (15) and Lokpa (12).

Ethnomycological surveys

The fresh specimens sampled during forest visits have been used to make ethnomycological surveys. The surveys were carried out following semi-structured interviews in which each respondent or group of respondents were subjected to a questionnaire addressing the main lines related to our study. Fresh specimens of *Amanita*, are presented to local populations. The ultimate goal of the ethnomycological surveys is to identify the use and kind of use made of *Amanita* species, but also to rank the species according to their utility and importance.

Evaluation of endogenous knowledge of local populations

To assess the endogenous knowledge of local populations with respect to the different uses
made of wild species of the genus *Amanita*, scores were used to calculate the reported Value Use of Gomez Beloz (RVU), Ethnobotanical Use Value (EUV), Total Ethnobotanical Use Value (TUV) and the K Sorensen index to evaluate homogeneity degree of knowledge among all three ethnic group. We refer to [26, 27] for the calculation of the various indices mentioned above. These scores attributed are following: the species is not used at all: 0, the species is rarely used: 0.5, the species is frequently used: 1 and the species is very frequently used: 1.5.

Preparation of extracts for mycochemical screening

To obtain the extract, one hundred (100) g of powder of each mushroom species were macerated in 1 liter of distilled water and then heated for 30 min. The decocts obtained were filtered on wattman filter paper 3 mm thick. The aqueous extracts obtained were stored in the freezer for all subsequent uses.

Qualitative chemical analyzes of selected species

Qualitative mycochemical screening was performed on the powder samples after extracted with aqueous solvent, using the standard method based on staining and precipitation as described [28] and used by [29, 30].

Determination of the antioxidant activity (EC_{50}) of the extracts

For this test, the samples were prepared by dissolution in distilled water [31]. Each stock solution is diluted in a geometric series of reason 2 to have different concentrations. In dry test tubes, 1 ml of the solution of the extract to be tested, added to 2 ml of the DPPH solution (0.08 mg / ml), are introduced. Then the tubes are protected from light for 30 minutes. The absorbance is read at 517 nm on the spectrophotometer. The positive control is represented by ascorbic acid and is treated under the same conditions as the test sample.

Data processing and analysis

The antioxidant activity of the extract was expressed in EC_{50}. The IC_{50} values were obtained from a linear regression between optical densities (OD) and concentrations.

Results

Diversity, use and ethnomycological use value of wild mushrooms investigated

A total of 11 species is recorded. Ethnomycological investigation allowed record three (3) edible species in the study region. The list of species collected in the study and their uses by local people area are presented in table 1 below.

Table 1: List of species of the genus *Amanita* and their uses according to ethnic groups

Voucher number of the specimen	Scientific names	Edibility by ethnic group		
		Yom	Peuhls	Lokpa
FOG 0546	*Amanita crassiconus* Bas	-	-	-
FOG 0655	*Amanita afrospinosa* Pegler & Shah-Smith	-	-	-
FOG 0656	*Amanita* cf. *xanthogala* nom.prov.	-	-	-
FOG 0657	*Amanita* cf. *xanthogala (*witish form) nom.prov.	-	-	-
FOG 0163	*Amanita craseoderma* Bas	-	-	-
FOG 0658	*Amanita loosii* Beeli	+	+	-
FOG 0325	*Amanita masasiensis* Härk. & Saarim.	+	+	-
FOG 0659	*Amanita pulverulenta* Beeli	-	-	-
FOG 0660	*Amanita subviscosa* Beeli	+	+	+
FOG 0305	*Amanita virido-odorata* nom.prov.	-	-	-
FOG 0657	*Amanita strobilaceo- luteotacta* nom. prov.	-	-	-

Legend: (+) Edible species (-) Inedible species

Three (03) species are reported to have trade-therapeutical importance by Yom people (see table 2 below).

Table 2: List of therapeutic species and patterns of use

	Yom		
Species	Disease Treated	Potency Preparation	Dosage
Amanita masasiensis	Ulcer or heart disease	Boil the mushroom and add a little salt	Drink during the discomfort
Amanita crassiconus	Heals the pimples on body	Boil and pass to the infected parts	Applied as needed
Amanita subviscosa	Hard feet or hard hands	Boiling with water without salt + adding shea butter Next	Qualitative chemical analyzes of selected species

Qualitative myco-chemical content of the species

The results of the phytochemical analysis (Table 3) of the eleven (11) species reveal the presence of several compounds at levels variables.

Table 3: Chemical compounds present in the eleven (11) species of the genus *Amanita*

Phytochemical compound	Species										
	1	2	3	4	5	6	7	8	9	10	11
Alkaoids	+	+	+	+	+	+	+	+	+	+	+
Anthocyanins	-	-	-	-	-	-	-	-	-	-	-
Free Antracenic	-	-	-	-	-	-	-	-	-	-	-
Cardenolides	-	-	-	-	-	-	-	-	-	-	-
C-Hétérosides	-	-	-	-	-	-	-	-	-	-	-
Reducing compounds	+	+	+	+	+	+	+	+	+	+	+
Coumarines	-	-	-	-	-	-	-	-	-	-	-
Cyanogenic derivatives	-	-	-	-	-	-	-	-	-	-	-
Quinone derivatives	-	-	-	-	-	-	-	-	-	-	-
Flavonoids (Flavones)	+	+	+	+	+	+	+	+	+	+	+
Leuco-anthocuyanes	+	+	+	+	+	+	+	+	+	+	+
Mucilages	+	+	+	-	±	±	±	-	-	+	-
O-Hétérosides	-	-	-	-	-	-	-	-	-	-	-

Saponosides	+	+	+	+	+	+	+	+	+	+	+
Steroids	-	-	-	-	-	-	-	-	-	-	-
Catechin tannins	+	+	+	+	+	+	+	+	+	+	+
Gallic tannins	-	-	-	-	-	-	-	-	-	-	+
Terpénoïdes	-	-	-	-	-	-	-	-	-	-	-

1: *Amanita crassiconus*; 2: *Amanita pulverulenta*; 3: *Amanita loosii*; 4: *Amanita strobilaceo-luteotacta*; 5: *Amanita* cf. *xanthogala*; 6: *Amanita craseoderma*; 7: *Amanita virido-odorata*; 8: *Amanita* cf. *xanthogala* (witish form); 9: *Amanita afrospinosa*; 10: *Amanita masasiensis* et 11: *Amanita subviscosa.*

Radical activity of aqueous extracts of Amanites species

From the table 4 below, species with low value of IC_{50} or EC_{50} and high ARP are the most active.

Table 4: Values of inhibitory concentration (IC_{50}); efficient concentration (EC_{50}) and antiradical power (ARP)

Solution tested	IC_{50} (µg/ml)	EC_{50} (µg/mg/DPPH)	ARP
AC. Ascorbique	1,683±0,000	0,032±0,000	31,681±0,042
A. stobilaceo luteotacta	8,919±0,00002	0,167±0,0005	5,979±0,0185
A. pulverulenta	14,65±0,0001	0,275±0,001	3,641±0,018
A. cf *xanthogala*	15,275±0,007	0,286±0,125	3,861±1,689
A. crassiconus	16,679±0,002	0,313±0,031	3,214±0,321
A. loosii	20,045±0,0001	0,376±0,001	2,661±0,010
A. craseoderma	116,053±0,022	2,176±0,404	0,468±0,087
A. masasiensis	128,303±0,004	2,406±0,079	0,416±0,014
A. cf. *xanthogala* (witish form)	133,335±0,002	2,500±0,030	0,400±0,005
A. subviscosa	133,945±0,0003	2,511±0,007	0,398±0,001
A. afrospinosa	251,762±0,001	4,721±0,025	0,212±0,001
A. virido-odorata	875,07±0,039	16,408±0,745	0,061±0,003

Discussion

Diversity and exploitation of wild mushrooms of the genus *Amanita*

Amanita masasiensis, *A. subviscosa* and *A. loosii* are exploited by local populations as food. Eight (8) species are reported as inedible. The low consumption of species of the genus *Amanita* is due to unpleasant colors and forms. The low diversity of useful species can also be attributed to the sedentarily habit of the ethnic groups, who for the most part are neither nomadic nor transhumant and therefore do not know the species consumed by other peoples or ethnic groups from other regions. The differential use of fungal taxa reported in our study were already reported in tropical Africa [26] demonstrated that sociolinguistic groups living in the same village use the fungal resources differently. This is mostly attributed to the cultural and culinary background of each ethnic group, but also to their installment history in the area. The more an ethnic group is ancient, the deeper the ethno biological knowledge [32]. Our results confirm the globally recognized reputation of this genus as having enough toxic species. Globally, only seven (07) species are reported to be edible for the whole West Africa, from a putative diversity of 70 [33]. In addition, this low number of edible species is also reported in Togo where the same species (*Amanita loosii*, *A. masasiensis* and *A. subviscosa*) are also consumed [34]. Similarly, three (03) species are consumed by the Bobo Madaré, Mooré and Gouindougouda ethnic groups in Burkina Faso [35]. Nevertheless, the rejection of a species by a people does not necessarily justify this species being inedible [20, 32]. Some species considered inedible by the local populations of our region are consumed and appreciated in other localities. For example, *Amanita craseoderma* is consumed by the Yom in the N'Dali region [22] *A. crassiconus* and *A.* cf. *xanthogala* are mentioned as edible by Nagot people in Benin [22], whilst these species are rejected by the ethnic groups of our study area [36]. These results confirm that mycophagy does indeed vary from one region to another on the same territory [35], but also between populations living in the same forest villages [32].

Qualitative chemical analyzes

The mycochemical screening reveals the presence of phytochemicals such as alkaloids, flavonoids, saponosides, catechin tannins, gallic tannins, leuco-anthocyanins, reducing compounds and mucilages that vary with species. These results are consistent with earlier works on selected fungi in Nigeria and Sudan [37] and are somewhat consistent with results by [38] on fungi from Kenya. The active compounds identified are biological substances of plant origin, which have the capacity to strengthen the immune system of the human body. Alkaloids are known for their antimalarial, anti-tumor, anti-bacterial properties [39]. Saponosides have a wide range of beneficial pharmacological properties, such as anti-inflammatory and anti-diabetic effects, expectorant and immune stimulant effects [40]. Phenolic compounds are broad-spectrum antioxidants with medicinal properties such as anti-cancer, anti-inflammatory and diabetic effects [41]. This is the case for flavonoids that have pharmacological properties including anti-cancer, antimalarial, anti-diabetic, antiviral, anti-fungal, anti-inflammatory, and anti-allergic [42]. The different secondary metabolites identified in species of the genus *Amanita* could explain the satisfaction that its last ones provide in traditional medicine.

Mcotherapy in this region is practiced by men, the edible species *Amanita masasiensis* and *A. subvicosa* are used to treat ulcers and to treat hard feet and slit hands respectively. The inedible species *Amanita crassiconus* is used to treat pimples on the body. The therapeutic properties reported by local populations is therefore due to the presence of some most important secondary metabolites: alkaloids, saponosides and tannins [43].

Antioxidant activity of the species investigated

According to [40], any extract with an inhibitory concentration

(IC_{50}) of less than 10 mg/ml has an antioxidant activity. As such, the 11 taxa investigated in this study therefore have an effective antioxidant activity. That some taxa are edible attests of the utility of these fungi for local inhabitants for antioxidant activities. Although organisms have antioxidants and natural repair systems to protect them from oxidative damage, these systems are not enough to prevent them completely [30]. Hence, the importance of antioxidants in food as protective agents to help humans in the reduction of oxidative damage. Nevertheless, the values of the effective concentrations observed in the present study are all superior to ascorbic acid, a powerful antioxidant [44]. This means that ascorbic acid (vitamin C) has a better antioxidant activity than the *Amanita* extracts because of its low IC_{50} values (1.683 µg / ml); EC50 (0.032 µg / mg / DPPH) and its high ARP value (31,681). At the same time, *Amanita vaginata* [16]; *Tricholoma giganteum* [16]; *Volvariella volvacea* and *Ramaria aurea* [45], whose antioxidant activity is determined with methanoic extracts, also give low EC_{50} respectively 1.48, 0.75, 0.265 and 0.857 mg/ml; all higher than that of 'ascorbic acid.

It has been demonstrated that a strong correlation between the antioxidant activity of plant materials and their phenolic compounds [46]. Edibles fungi consumed by local people have all of the phénolitic compounds; *Amanita loossi* and *A. masasiensis* with their catechiques tannin and flavonoid (flavones) and *A. subviscosa* which consists not only of catechiques tannin, flavonoids but also of Gallic tannins. The same observations were made for *Amanita loosii* with its phenol and flavonoids composition [46]. Hence; in growing demand of food and pharmaceutical industries, such wild edible mushrooms can be important candidates [47]. This is for the first time; *Amanita loosii*, *Amanita masasiensis* and *Amanita subviscosa* were subjected to preliminary biochemical analysis. New studies on nutritional values and on their heavy metal composition will give more certainty on their edibility.

Annex

Fresh specimens of the genus *Amanita*

Plate 1**:** *Amanita* cf. *xanthogala* (whitish form) *non.prov*, 2- *Amanita craseoderma*, 3- *Amanita crassiconus*, 4. *Amanita loosii*, 5- *Amanita masasiensis*, 6- *Amanita pulverulenta*, 7-*Amnita qfrospinosa*, 8- *Amanita strobilaceo-luteotacta non.prov*, 9- *Amanita subviscosa*, 10- *Amanita virido-odorata non.prov* 11- *Amanita* cf. *xanthogala non.prov.* Photo de terrain 2015

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