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Mycochemical composition and antioxidant potential of *Macrolepiota africana* (Heim) Heinem. (Agaricaceae), an edible mushroom from Gabon

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Abstract

Macrolepiota africana is a fungus used as food in Gabon for its culinary properties. However, its nutritional value, mycochemical contents, antioxidant properties and health potential still unknown. The present study investigated the phytochemicals and antioxidant properties of this mushroom species. This chemical screening was followed by a study of the antioxidant activity and a prediction of additional pharmacological activities of *M. africana*. Using standard methodology, the mycochemical analyses were carried out on aqueous, hydro-ethanolic and ethanolic fungi extracts. The antioxidant activity of the mushroom extracts was determined using DPPH radical scavenging assay. Apart from Digitoxigenine, flavonol and gitoxigenine that were not found in any of the extracts, all other tested mycochemical were found in atleast one of the extracts. Alkaloids, flavonoids, polyphenols, oses and holosides, proanthocyanidins and coumarins were found in all extracts at different intensity. Saponosids, sterols and triterpenes, tannins gallics, reducing sugar, anthracenosides and digitoxine were found in two extracts while tannins catechics, flavone and gitoxine were detected only in one extract. The dosage of phenolic compounds confirmed the richness of this fungus in total polyphenols, its moderate richness in proanthocyanidins, the lack of flavonoids in the aqueous extract and the moderate richness of the hydro-ethanolic and ethanolic extracts in flavonoids. Regarding the antioxidant activities, the results obtained for the DPPH trapping test showed that the different extracts had low to moderate antioxidant activity with antioxidant activity index (IAA) ranged 0.29 to 0.97 respectively in aqueous and ethanolic extracts. The presence of these mycochemical compounds along with the identified antioxidant activities shows that this *M. africana* have some pharmacological potential.

Keywords: Chemical groups; Pharmacological potential; Antioxidant activity; *Macrolepiota Africana*; Gabon

1. Introduction

Mushrooms are used all over the world for their cultural, food and medicinal potential; they are especially used in traditional medicine in African and Asian. Many medicinal properties are attributed to these organisms including antiplasmodial, antibacterial, antiseptic, anti-inflammatory, antihypertensive, antioxidant, antiviral, antibacterial, antiparasitic, anticancer, hepatoprotective and antidiabetic properties [1- 5]. In Gabon, fungi are only better known for their food use. However, recent studies [6, 7] reveled the uses of local mushrooms species by native traditional healers with very few studies carried out on their medicinal properties. In Gabon and Tanzania [6, 8], the species of the genus *Macrolepiota* especially *M. africana* and *M. procera* are commonly used in traditional medicine by local populations to treat infected wounds. According to the fact that antioxidants such as polyphenols, polysaccharide and carotenoids play important roles on wound healing [9], it can be assumed that this local uses is due to the antioxidant properties of the species.

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The present study aims to highlight the main chemical groups of *Macrolepiota africana*, to assess its antioxidant activity and to determine other possible therapeutic properties of this fungus, depending on the chemical groups present.

2. Material and methods

2.1. Collection, identification and preparation of *Macrolepiota africana* studied samples

The studied samples (figure 1) was collected in the Mondah classified forest, north of Libreville, Gabon and identified following [10] according to its macroscopic and microscopic features. After collection, the mushrooms were washed with tap water to remove superficial soil particles and potential pollutants. Then they were split, dried at 60° for 24 hours using a travel dryer until they reached a constant weight, and then crushed.

Figure 1 Fruit bodies of *Macrolepiota africana*. Photograph by Hugues Calixte Eyi Ndong

2.2. Chemical analysis

2.2.1 Preparation of fungal extract

A water-ethanol extract (50/50 v / v), an ethanol extract and a water extract were prepared from the dry powder of *M. africana*. These solvents were choiced according to the fact that various organic compounds, including phenolics and flavonoids, have good solubility in ethanol and water. Here, 500 mL of each solvent or mixture of solvents were introduced into a erlenmeyer containing 50 g of dried mushroom powder and left stirring at room temperature (25°C) for 24 h. Each extract was filtered using Whatman No. 1 filter paper and the solvents were completely removed at low pressure with a rotary evaporator (Büchi, Labortechnik, Switzerland). The extracts were then concentrated, lyophilized and stored at 4°C until analysis.

2.2.2 Qualitative analysis

Chemical screening was carried out on each extract in order to highlight the different main chemical groups [11].

2.2.2.1 Total phenolic content

The total phenolic content of the extracts was determined according to Folin-Ciocalteu method [12] using gallic acid as standard [13]. Absorbance was measured at 735 nm using a multi-well plate reader (μQuant Bio-Tek Instrument, Inc, USA). All analyzes were carried out in triplicate and the results were expressed in gallic acid equivalent per gram of lyophilized sample.

2.2.2.2 Total flavonoid content

The total flavonoid content was determined by colorimetric aluminum chloride ($AlCl_3$) assay [14] adapted to a 96-well plate, using quercetin as standard [15]. Total flavonoid content was expressed in quercetin equivalents in milligrams per gram sample (analysis average in triplicate).

2.2.2.3 Tannin content

The reference method by Sima-Obiang was used to determine the tannin content [16]. Absorbance was measured at 525 nm and tannic acid was used as a standard. The tannin contents were expressed in mg of tannic acid equivalent (TAE)/100 g of extract.

2.2.2.4 Proanthocyanidin content

The method consists in the proanthocyanidins hydrolysis in a hot acid-alcohol medium into anthocyanidins. This method makes it possible to take into account all the units of flavans-3-ols constituting the polymers [17]. The assay is carried out by mixing 50 µL of the extract with 700 µL of HCl-butanol solution at 30% (v / v). The mixture was placed in a hermetically sealed 1.5 mL Eppendorf tube and vortexed for 1 min. the tube was then heated for 2 h at 100°C. After cooling, 200 µL aliquots were placed into a 96-well plate and the absorbance was read at 550 nm. Apple procyanidins (DP ≈ 7.4) treated as mentioned above were used as standard. The results were expressed in apple procyanidin equivalents (APE).

2.3. Antioxidant activity index

Scherer & Godoy method [18] was used to determine the antioxidant activity index (AAI). It is based on the DPPH radical test. For this, 10 mg of DPPH were dissolved in 100 mL of ethanol. Height graduated concentrations of extracts ranging from 0.781 to 100 µg / mL obtained by double dilutions was prepared and 100 µL of each dilution were mixed with 100 µL of the working solution of DPPH in a 96-well plate. After 15 min of incubation at room temperature in the dark, the absorbances were read at 517 nm. The references used for this were ascorbic acid and butylated hydroxyanisole (BHA). The ability to trap the DPPH radical was evaluated using the following equation:

$\%\ RSA = [(A_{control} - A_{sample}) / A_{control}] \times 100$, With A = Absorbance at 517 nm.

The extract concentration able to inhibit 50% of DPPH (IC_{50}) was determined using the regression curves in the linear range of concentrations. The AAI was calculated by the formula:

$AAI = [DPPH]\ (\mu g / mL) / IC_{50}\ (\mu g / mL)$

[DPPH] being the final concentration of DPPH. Fungi extracts have a weak antioxidant activity when the AAI <0.5; moderate antioxidant activity when the AAI is between 0.5 and 1; a strong antioxidant activity when the AAI is between 1.0 and 2.0; and very strong antioxidant activity when AAI> 2 [19].

2.4. Statistical analyzes

Data were expressed as the mean ± standard deviation (SD) of three independent experiments and analyzed using one-way analysis of variance and Student's t-test.

Values of $p < 0.05$ were considered to be statistically significant.

3. Results and discussion

3.1. Chemical screening

The chemical extracts screening results of *M. africana* are transcribed in table 1 that shows that the three extracts (aqueous, hydro-ethanolic and ethanolic) are rich in total polyphenols, alkaloids and coumarins; however, they are moderately rich in oses and holosides, as well as proanthocyanidins. The hydro-ethanolic and ethanolic extracts are moderately rich in flovonoids, as well as sterols and terpenes, while the aqueous and hydro-ethanolic extracts are moderately rich in reducing sugars. On the other hand, the aqueous extract is moderately rich in gallic and cathechic tannins while the ethanolic extract is poor in tannins. The presence of these compounds gives *M. Africana* various properties. The abundance of polyphenols indicates antioxidant, antiallergic and antiplasmodial [2, 3, 20].

This fungi could have anesthetic, analgesic, anticancer and vasodilating properties due to the alkaloids presence; anti-bacterial, anti-plasmodial and anti-inflammatory properties due to its high saponosides content [2]. Anti-carcinogenic and anti-mutagenic properties by its coumarins contained [19].

Table 1 Qualitative mycochemical screening of *M. africana* extracts

Chemical groups /compound		Extact type		
		Aqueous	Hydroalcoholic	Ethanolic
Saponosids		+++	+++	-
Sterols and triterpenes		-	++	++
Alkaloids		+++	+++	++
Tannins gallics		++	++	-
Tannins catechics		++	-	-
Reducing sugar		+++	++	-
Polyphenols		+++	+++	+++
Total flavonoides		+	++	++
Oses and holosides		++	++	++
Proanthocyanidins		++	++	++
Anthracenosides		+++	+++	-
Cyanidine test	Flavonol	-	-	-
	Flavone	-	-	+
	Flavanone	+++	+++	-
	Coumarins	+++	+++	+++
Cardiotonic heterosides test	Digitoxine	+++	+++	-
	Digitoxigenine	-	-	-
	Gitoxine	-	-	+++
	Gitoxigenine	-	-	-

+++: very abundant; ++ abundant; +: not abundant; -: not detected.

3.2. Phenolic compounds of *M. africana* extracts

The results of phenolic compounds measurement in extracts are presented in table 2.

Table 2 Phenolic compounds content of *M. africana* extracts

Extracts	Total phenolic content (TPC) (EAG/mg)	Total flavonoids content (EQ/mg)	Total tannins content (EAT/mg)	Total proanthocyanidins content (EPP/mg)
Aqueous	2024.57±19	77.2125±0	628.78±154	351.05±4
Hydro-ethanol	1051.25±18	317.43±1	255.18±23	204.15±7
Ethanolic	3299.63±233	148.28±2	0	331.15±18

According to it, this fungus is rich in total polyphenols as its concentration were high in all the extracts. The sample shown low to moderate content of total flavonoids, total tannins and proanthocyanidins in all extracts with even the absence of tannins in ethanolic extract. The absence of tannins was also observed in alcoolic extract of *Daldinia concentrica* and *Pheolus schweinitzii* [21]. Proanthocyanidins was also found in moderate quantities in extracts of *Tricholoma nudum, Psalliota campestris, Flammulina sp*., *Trichaptum sp*., and Boletus sp. from Nigeria [22].

3.3. Antioxidant activities of the different extracts of *M. africana*

The results of antioxidant activity of the extracts of the fungi studied are mentioned in table 3. This table shows low value of the Antioxidant Activity Index (IAA) of the ethanolic extract (0.29), while aqueous and hydro-ethanolic extracts have the highest value of IAA with 0.97 and 0.76 respectively. The antioxidant activity of an extract depends on its content of phenolic compounds (total polyphenols, flavonoids, total tannins and proanthocyans), these data are consistent with the results of Table 2 that shown a richness in total polyphenols of all extracts of *M. africana*, a moderate richness in flavonoids and proanthocyans, but also the absence of tannins in the ethanolic extract. The ethanolic extract, which is rich in total polyphenols and moderately rich in flavonoids, but low in tannins, has a low value of IAA, and therefore a low antioxidant activity. This could be explained by the absence of tannins in this extract. Indeed, Touaibia and Chaouch [20] have shown that the antioxidant activity of *Myrtus communis* is dependent on its tannin and anthocyanin content; they demonstrated that the ability of tannins to reduce iron is close to that of ascorbic acid, and that anthocyanins had an iron-reducing capacity similar to that of ascorbic acid. The absence of tannins can therefore perfectly explain the low antioxidant activity observed in the ethanolic extract of our sample.

Table 3 Antioxidant activity: DPPH free radical scavenging activity *M. Africana*

Extracts	Equation	R^2	IC_{50}	IAA	Activity
Aqueux	Y= 161.61X +41.674	0.9716	51.51	0.97	Moderate
Hydroalcoholic	Y= 154.76X + 39.89	0.9905	65.32	0.76	Moderate
Ethanolic	Y= 67.159X + 38.393	0.9976	171.90	0.29	Poor

4. Conclusion

The present study based on a chemical screening and an analysis of antioxidant activity of *M. africana* made it possible not only to highlight some of the bioactive compounds produced by this mushroom species. These bioactive antioxidant compounds include Alkaloids, flavonoids, polyphenols, oses and holosides, proanthocy Saponosids, sterols and triterpenes, tannins gallics, reducing sugar, anthracenosides and digitoxine anidins, coumarins, tannins catechics, flavone and gitoxine. It also point out the antioxidant activity of this species in aqueous, hydro-ethanolic and ethanolic extracts. These results also displayed a correlation between the antioxidant activity and the phenolic compounds contents of the different studied extracts; however, the aqueous extract, yet moderately rich in total polyphenols but poor in flavonoids, has a weak antioxidant activity.

The presence of the various phytochemical compounds, in addition to the antioxidant activity justifies the uses of this fungus in local medicine for the treatment of infected wounds. Hence, it could also be assumed that, the fungus has anesthetic, analgesic, anticancer, antiallergic, antibacterial, anti-inflammatory, antimutagenic, antiplasmodial, vasodilatory and above all antioxidant properties; making it a potential remedy against common metabolite and infestuous.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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