The Journal of Phytopharmacology 2024; 13(4):280-285
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2024; 13(4): 280-285
July- August
Received: 12-04-2024
Accepted: 21-07-2024

doi: 10.31254/phyto.2024.13402

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Phytochemical screening and antioxidant activity of seven Wild mushrooms species used in Niger

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ABSTRACT

Mushrooms are widely used in the treatment of many chronic diseases such as: hepatitis, chronic bronchitis, hypertension, diabetes and cancer. However, despite the importance of mushrooms in the traditional pharmacopoeia in Niger, this field is very little explored. The aim of this study was to evaluate the phytochemicals screening and antioxidant activity of seven mushrooms species used in Niger. Phytochemical screening was carried out according to colorimetric reactions. The total polyphenols content was determined spectrophotometrically by Folin-Ciocalteau method, total flavonoid content was measured by the aluminium chloride method and condensing tannin content was measured by using vanilic acid method. The antioxidant activity was evaluated using the DPPH method by varying the concentration. Phytochemical screening revealed the presence of several secondary metabolites: alkaloids, polyphenols, flavonoids, steroids, terpenoids and quinones. The total phenol contents varied between 03.38±0.36 and 121.57±3.54 mg EAG/g. The tannins content varied from 0.34±0,03 to 2.02±0,042 mg TAE/g and total flavonoids ranged from 1.82±0.12 to 24.95±1.63mg EQ/g. The concentration IC_{50} ranged from 78.05±2.42 to 8790 ±70.15 µg/mL. The phytochemical and antioxidant activity of *Agaricus subsaharianus* and *Phellinus alardii* are reported for the first time in the literature through this study. These secondary metabolites confer therapeutic potentialities to these mushrooms species such as the antioxidant properties.

Keywords: Phytochemicals, Antioxidant, Mushrooms, Niger.

INTRODUCTION

Mushrooms have a long history of uses for their nutritional and medicinal properties. They have been consumed by people for thousands of years [1,2]. During the recent years, mushrooms extracts and their phytochemicals compounds have received considerable attention for their pharmacological properties which include antimicrobial antioxidant, anti-cancer, anti-inflammatory, anti-cancer and immunomodulatory activities [3,4,5]. Polysaccharides isolate from mushrooms are capable of simultaneously stimulating different components of the immune system, which gives them different therapeutic properties, including antibiotic, anticarcinogenic and antiviral properties [6]. Triterpenes are able to protect normal cells from radiation-induced damage by decreasing the oxidative stress response and the formation of oxygen radicals in the intracellular environment while increasing the endogenous antioxidant activity of splenic lymphocytes [7]. They also contain active substances capable of suppressing the growth of colon cancer cells [8].

In Niger, numerous ethnobotanical studies have been carried out with the aim of cataloguing medicinal plants [9,10]. However, despite the importance of mushrooms in traditional pharmacopoeia, this field has been little explored. The first studies carried out in this field included an inventory of mushrooms species [12,13,14,15]. Among the species inventoried, *Agaricus subsaharianus*, *Macrocybe lobayensis*, *Phellinus allardii*, *Phellinus merrillii*, *Phellinus sp*, *Podaxis pistillaris* and *Pisolithus albus* are widely used in traditional pharmacopoeia to treat a wide range of illnesses, including bacterial parasites, heart disorders and cancer. Nevertheless, these species have not been extensively studied in order to better explore their therapeutic potential. The aim of this study was to phytochemically screen these mushrooms species, quantify their phenolic compounds and evaluate their antioxidant activities.

MATERIAL AND METHODS

Fungal material

The mushrooms were collected during the rainy season, from July to September 2021 in the city of Niamey and in the region of Torodi, a city located about 55 km from the capital Niamey. The identification of the specimens was carried out at the Mycology Laboratory of the Ecole Normale Supérieur de Niamey on the basis of macroscopic characters but also with the help of books and articles

dealing with the taxonomy of tropical African fungi [16,17]. After indentification, the specimens were photographed and then dried under a temperature between 45-50 °C for 24 hours and packed in plastic 'Minigrip' bags for future analysis.

Figure 1: Some mushrooms collected: a. *Agaricus subsaharianus* ; L.A.Parra, Hama & De Kesel b. *Macrocybe lobayensi* (Heim) Peg. & Lodge s; c. *Podaxis pistillaris* (L.) Fr.; d. *Pisolithus albus*; e. *Phellinus alardii* (Bres.) Ryv. ; f. *Phelllinus merrilli* (Murrill) Ryvarden; g. *Phelllinus sp*.

Preparation of extracts

A quantity of 10g of each mushroom powder was mixed was mixed with 100 mL of methanol (99.6%) for 24 hours The mixture was then fltered using Whatman flter paper. This operation was repeated three times and filtrates were collected. The filtrates obtained were concentrated under vacuum on a rotary evaporator and stored at room temperature [18].

Phytochemical screening

Phytochemical screening was carried out according to colorimetric reactions using standard method [19,20].

Total polyphenols content

The total polyphenols content of the methanolic extracts were determined according to Folin–Ciocalteu method [21,22]. 1mL of extract solution was added with 1 mL of Folin-Ciocalteu's phenol reagent. After few minutes, 1 mL sodium carbonate solution (75 g/L) was added. The mixture was incubated for 30 minutes at room temperature. The absorbance was measured by spectrophotometer at 760nm. The calibration curve was constructed with different concentration (0. 20.40. 60. 80 and 100 mg/L) using gallic acid as reference standard. The results were expressed as milligrams of gallic acid equivalents per gram of dried sample (mg GAE/g). The total polyphenols content in the extract was calculated using the regression equation: $Y = 0.0091x + 0.1164$; $R^2 = 0.9985$

Total flavonoid content

The total flavonoid content was determined by using aluminum chloride method [22]. 100µL of methanolic extract of each mushroom (1 g/L) was added with 100 µL of $NaNO_2$ (5%), 100µL of $AlCl_3$ solution (20 mg/mL) and 200µL NaOH (4%). The mixture was incubation in the dark for 30 minutes at room temperature. The absorbance of each extract was measured at 415 nm. The total flavonoid content was calculated from a linear calibration curve, established with various concentrations (5; 10; 15; 20; 25 and 30 mg /mL) using quercetin as a reference standard. The results were expressed as mg quercetin equivalents per gram of dried sample (mg QE/g) using the regression equation:

$$Y = 0.0607x + 0.0260\ ;\ R^2 = 0.9856$$

Condensed tannin content

Condensed tannin content was determined by the acid vanillin method described by Julkunen-Titto, [23 24]. 50 µL of each extract was added to 1500 µL of the 4% vanillin solution in methanol. The resulting mixture was vigorously stirred and 750µL of concentrated hydrochloric acid added. The optical density is read at 550 nm. The resulting mixture was left to react at room temperature for 20 min. Absorbance was measured at a wavelength of 550 nm against a blank consisting of the 4% solution of vanillin in methanol. A stock solution of tannic acid was used as a reference standard for establishing the calibration curve and for quantifying condensed tannin content expressed as milligram equivalent of tannic acid per gram of dry extract (mg EAT/g). The tannin content was calculated using the regression equation: $Y = 0.1165x + 0.0003\ ;\ R^2 = 0.9825$

Antioxidant activity

The antioxidant activity of mushrooms extract was evaluated using 2.2-diphenyl-1-picrylhydrazyl (DPPH) method [25]. 50µL of methanolic extract of each mushroom at different concentrations (0.1 to 10mg/ml) was added to 2,5 ml of a methanol solution of DPPH(0.025mg/ml). The mixture was shaken and stored in a dark at room temperature for 30 min. Absorbance was measured at 517nm. Ascorbic acid was used as a standard antioxidant. All tests were carried out in triplicates. The absorbance value obtained was used to calculate the percentage of inhibition using the formula equation:

$$\text{Inhibition } \% = \frac{A_0 - A_s}{A_0} \text{ X } 100$$

$A_0 =$ Absorbance of negative control.

$A_s =$ Absorbance of sample.

IC_{50} value was calculated using a linear regression equation.

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical screening showed the presence of alkaloids, polyphenols, flavonoids, anthocyanins and coumarins in all *Phellinus* species (Table 1). However, quinones, saponosides, tannins, steroids and terpenoid were not detected. Other study on *Phelllinus gilvus*, *Phellinus fantuous* and *Phellinus sanfordii* reveals the presence of tannins, steroids and terpenoids [26]. In the *Podaxis pistillaris* and *Pisolithus albus*, tannins, total polyphenols, flavonoids, anthocyanins, coumarins, steroids and polyterpenes were found. On the other hand, Hama's work on *Podaxis pistillaris* did not reveal tannins, flavonoids or anthocyanins [15]. Finally, total polyphenols, alkaloids, steroids and polyterpenes were present in *Agaricus subsaharianus* and *Macrocybe lobayensis.* These secondary metabolites play an important role in the medicinal properties. Alkaloids are known for their various pharmacological properties that include analgesic, antiparasitic, neuroprotective, antimicrobial, antimalarial anticancer anti-hypertensive, anti-depressant, and anti-inflammatory [27,28]. Alkaloids were present in all mushrooms of this experiment. The presence of steroids and terpenoids were detected only in *Agaricus subsaharianus*, *Macrocybe lobayensis* and *Pissolothus albus*. Steroids and terpenoids have been reported to show a wide range of pharmacological benefits include antimicrobial agents, and their mode of action is the weakening of tissue and the cell wall of the microorganisms [29]. Several studies have reported the efficacy of terpenoids for therapeutic treatments of neurodegenerative, antiviral [30,31]. They also serve as antidiabetic agents and anticancer [32]. Other important components in mushrooms called phenolics compounds such as flavonoids, anthocyanin and tannins. These compounds are known for their antioxidant, antimicrobial, anticancer, anti-inflammatory, antimalarial and anticancer properties [33,34].

Phenolic compound content

The phenolic compounds contents are shown in Table 2. The total phenol was expressed as milligrams of gallic Acid Equivalent per gram of dry sample. (mg GAE/g). These contents ranged from 03.38 mg GAE/g to 121.57 mg GAE/g. The highest total phenolic content was observed in the methanolic extract of *Phellinus alardii* and the lowest total phenolic content in the extract of *Macrocybe lobayensis*. Indeed, the content of 3.38 mg EAG/g of sample in the methanolic extract of *Macrocybe lobayenis* is lower than 10.96 mg EAG/g of methanolic extract reported in India by Khatua and *al* [35]. As for Phellinus species, the levels reported in this study are higher than 36.84 mg EAG/g of sample reported in *Phellinus gilvus* species, but remain low compared with 297mgEAG/g of methanolic extract of *Phellinus linteus* [36,37]. These results show that the total phenolic content of varies from one species to another. It has been reported that the phenolic content of both plant and mushroom are affected by environmental conditions such as soil structure, climate, humidity, locality, collection time [38, 39]. Highest total polyphenols contents were observing in *Phellinus* and *Pisolithus albus* species. High concentration in total phenols content is an indication that the mushroom extract has antioxidative [40]. Total flavonoid content varied from 1.82 to 24.95mg EQ/g. The highest value in total flavonoid content was found the methanolic extract of *Pisolithus albus* and the lowest content was found in the methanolic extract of *Macrocybe lobayensis*. The presence of flavonoid content is particularly important, acting as antioxidants, protect against cardiovascular disease and certain forms of cancer [41]. Recent study has also demonstrated that flavonoids are the most important bioactive compound in medicinal mushrooms that have antioxidant activities against stress induced oxidative diseases [42]. Condensed tannin content varied between 0.34 to 2.02 mg EAT/g. Highest condensed tannin content was found in *Pisolithus albus* (2.02 mg TAE/g) and the lowest content was found in *Phellinus merrillii* extract (0.34 mg TAE/g). Tannins are bioactive compounds known for their antioxidant and wound healing properties. They are also used to treat inflamed mucous membranes and prevent cancer. [43, 44].

Antioxidant activity

The antioxidant activity was determined using DPPH method. The free radical DPPH is widely used to evaluate the antioxidant potential of mushrooms. Percentage inhibition and IC_{50} of mushrooms studied are shown in Table 3. The results show that DPPH free-radical scavenging power is concentration-dependent. In this study all mushrooms showed scavenging activity against the DPPH free radicals. The percentage inhibition of DPPH free radicals varied from 4.16% for the methanol extract of *Macrocybe lobayensis* at 125 µg/mL to 95.18% for the methanol extract of *Phellinus alardii* at 1000µg/mL The result show that *Phillinus* species showed higher antioxidant activity than the other mushroom species studied. Ascorbic acid used as a reference gave a percentage of 97.26%. The IC_{50} is the concentration required to reduce 50% of DPPH activity. This concentration is inversely proportional to a compound's antioxidant activity. The lowest IC_{50} value of the extract indicates the

highest antioxidant. The IC_{50} values reported in this study ranged from 8790 ±70.15 to 78,05±2.42µg/mL. The lowest value was obtained with the *Phellinus alardii* species, and highest value in *Macrocybe lobayensis* species (8790 ±70.15µg/mL). These values remain low compared with the ascorbic acid used as a reference 8.14±0.10µg/mL. The IC_{50} value of the methanolique extract of *Macrocybe lobayensis* reported in this study is much higher than that reported by Khatua and *al*, [35]. On the other study, the value of 156.99 µg/mL for *Phellinus merrillii* is low compared with 810 µg/mL for the ethamolic extract of *Phellinus merrillii* reported by Chang et *al* [45]. Several studies have found that *Phellinus* mushroom extract has antioxidative activity [46,47]. antioxidant activity of *Agaricus subsaharianus* and *Phellinus alardii* presented in this study is the first record for the literature. Phenolic compounds especially phenols and flavonoids are potential antioxidants [48]. It has also reported that more amount of total phenolic and flavonoid content in mushrooms have shown strong antioxidant activity [49,50]. *Phellinus* species and *Pisolithus albus* mushrooms have strong antioxidant activity against tested free radicals. Antioxidant is important in the treatment of oxidative stress induced diseases such as cardiovascular diseases, diabetes and cancer [51]. These mushrooms can be used as good source of natural antioxidants for the treatment of cardiovascular diseases, cancer, and diabetes.

Table 1: Phytochemical screening

Secondary metabolites	**Mushrooms species**						
	Agaricus subsaharianus	*Macrocybe lobayensis*	*Podoxis pistillaris*	*Phellinus alardii*	*Phellinus merrilii*	*Phellinus sp*	*Pissolithus albus*
Total polyphenols	+	+	+	+	+	+	+
Flavonoids	-	-	+	+	+	+	+
Coumarins	-	-	-	+	+	+	-
Anthocyanins	-	-	+	+	+	+	+
Steroids-terpenoids	+	+	+	-	-	-	-
Tannins	-	-	+	-	-	-	+
Quinons	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	-
Alkaloids	+	+	+	+	+	+	+

+ Presence; - absence

Table 2: Phenolic compound content

Mushrooms species	**Total Polyphenols (mg EAG/g)**	**Total Flavonoids (mg EQ/g)**	**Condensed Tannin (mg EAT/g)**
Agaricus subsaharianus	07.75±0.36	1.82± 0.12	0.88±0.15
Macrocybe lobayensis	3.38± 0.36	2.06±0.44	0.84±0.06
Podoxis pistillaris	20.22± 0.65	5.08±0.10	1.82±0.12
Phellinus alardii	121.57 ±3.54	11.37± 0.10	0.48± 0.02
Phellinus merrillii	108.35 ± 2.67	10.53± 0.54	0.34±0.03
Phellinus sp	100.75 ±5.07	10.72 ±0.35	0.74 ±0.021
Pisolithus albus	71.12± 2.44	24.95 ±1.63	2.02±0.042

Table 3: Inhibition % and IC_{50} values of mushrooms extracts

Concentration (µg/ml)	125	250	500	750	1000	
Sample	**Inhibition (%)**					**IC_{50}(µg/mL)**
Agaricus subsahanus	8.48±0.25	10.76±0.55	16.16±0.69	20.75±1.04	25.36±1.25	6618± 21.25
Macrocybe lobayensis	4.16±0.09	6.65±0.15	10.36±0,37	14.25±0.22	16.18±0.78	8790 ±70.15
Podaxis pistillaris	14.52±0.32	19.21±0.72	25.96±1.03	32.65±0.77	38.14±1.32	2981±20.18
Phellinus alardii	75.86±0.83	80.02±0.69	88.05±0.83	92.24 ±1.07	95.18±1.58	78.05±2.42
Phellinus merrilii	72.53±0.38	76.41±0.21	82.08±0.10	85.52±0.31	89.35±0.85	156.99±2.52
Phellinus sp	47.65±1.28	53.05±1.72	66.51±1.37	69.05±1.72	83.52±0.31	293.12±12.71
Pisolithus albus	45.74±0.52	56.24±1.25	62.98±2.20	67.24±2.35	81.15±0.78	299.82±9.85
Ascorbic acid	91.87±0.20	97.26±0.22	98.12±0.13	98.90±0.35	99.32±0.36	8.14±0.10

CONCLUSION

This study involved phytochemical screening and assessment of the antioxidant activity of seven mushrooms species used in traditional pharmacopoeia in Niger. The results revealed the presence of numerous secondary metabolites. In addition, these species were found to contain appreciable levels of phenolic compounds. They also possess significant antioxidant activity. Phytochemical screening and antioxidant activity of *Agaricus subsaharianus* and *Phellinus alardii* presented in this study is the first record for the literature. The mushrooms species studied can be considered as a potential source of natural antioxidant agents, and can be associated with the treatment of numerous diseases such as cancer and cardiovascular disease.

Conflict of interest

The authors declare that they have no conflict of interest.

Financial Support

None declared.

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HOW TO CITE THIS ARTICLE
Ousmane ZC, Chaibou M, Doka DT, Ikhiri K. Phytochemical screening and antioxidant activity of seven Wild mushrooms species used in Niger. J Phytopharmacol 2024; 13(4):280-285. doi: 10.31254/phyto.2024.13402