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Phytochemical screening and antioxidant activity of methanolic extract of selected wild edible nigerian mushrooms

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PEER REVIEW

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Comments

This is a good study in which the authors phytochemically investigate as well as demonstrate the antioxidant activity of eight wild edible Nigerian mushrooms. DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical was used to evaluate the scavenging ability of the mushroom extracts and their reducing power were also assessed.

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ABSTRACT

Objective: To elucidate the phytochemical content and antioxidant activity of selected wild edible Nigerian mushroom species.

Methods: Phytochemical screening was carried out using standard methods while 1, 1-Diphenyl picryl hydrazyl (DPPH) radical and reductive power assays were used to evaluate the invitro antioxidant properties of the selected edible Nigerian mushroom species.

Results: The result obtained revealed the presence of alkaloids, cardiac glycosides, saponins, flavonoids, terpenes, steroids, tannins and phenols in the selected mushrooms extracts. The extract of *Pleurotus ostearus* showed a significantly ($P < 0.05$) higher total phenol and flavonoid content of $248.80 \pm 7.63 \text{ mg/g}$ and $42.63 \pm 0.63 \text{ mg/g}$ respectively compared to other mushroom extracts. *Cantharellus cibarius* had the most significant ($P < 0.05$) amount of alkaloids ($135.57 \pm 0.27 \text{ mg/g}$) and Saponins ($150.41 \pm 0.50 \text{ mg/g}$) when compared to other extracts while the tannin content ($170.56 \pm 0.74 \text{ mg/g}$) was highest in the mushroom *Temitomyces robustus*. All mushroom extracts scavenged DPPH radical in a dose dependent manner however *Lactarius deliciosus* had the highest DPPH scavenging activity compared to the other mushroom extracts. *Pleurotus ostearus* and *Lactarius deliciosus* had better reductive power than other mushroom extracts concentrations used.

Conclusions: The mushroom species analysed have been shown to be good sources of antioxidants and other phytoconstituents, thus can be used in the management of oxidative stress induced diseases.

KEYWORDS

Phytochemicals, Antioxidants, Mushrooms, 1, 1-Diphenyl picryl hydrazyl, Reductive power

1. Introduction

Free radicals are constantly formed in the human body during energy production, in the mitochondrial electron transport chain, phagocytosis, arachidonic acid metabolism, ovulation, fertilization and in xenobiotic metabolism[1] and from external sources such as food, drugs, smoke and other pollutants in the environment[2]. Living organisms are endowed with endogenous and exogenous antioxidant defense systems capable of countering the adverse reactions of free radicals [3]. The generation of free radicals in the body beyond its

antioxidant capacity actually leads to oxidative stress and this has been implicated in the etiology of a number of disorders [4]. As a result, of this much attention is being focused on the use of antioxidants to inhibit and protect damage due to free radicals and reactive oxygen species. Synthetic antioxidants such as butylated hydroxyanisole (BHA), tert-butylated hydroxyquinone and butylated hydroxytoluene are radical scavengers but are usually associated with adverse side effects [5]. Neutralization of radical damage by naturally occurring antioxidants from several sources either as food supplements or drugs is becoming one of the most acceptable modes of

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modern therapy^[6].

Mushrooms have continued to generate a lot of interest particularly in their consumption as food^[7], in the cure of diseases^[8], in bioremediation and as important items of commerce all over the world due to their nutritional, antioxidant and therapeutic values^[9, 10]. They may then be utilized to be amongst the useful candidates in the search for bioactive compounds with radical scavenging activity^[11, 12]. Although there are many studies on nutrients compositions of different mushroom species, only few studies have been carried out on the antioxidant activity in wild edible species^[12, 13]. Therefore this study was carried out to elucidate the phytochemical composition and invitro antioxidant activities of methanolic extract of the selected Nigerian wild edible mushrooms.

2. Materials and Methods

2.1. Collection of Samples

Eight indigenous wild edible Nigerian mushrooms including *Cantharelle cibarius*(CA), *Termitomyces robustus*(TR), *Termitomyces manniformis*(TM), *Pleurotus ostreatus*(PO), *Pleurotus pulmonarius*(PG), *Auricularia cularia*(AT), *Hericium erinaceus*(WM), *Lactarius deliciosus* were collected from logs of wood, palm logs and humus soil from different locations in Nigeria. They were identified by a Taxonomist, Prof. Onyekwere S. C. of Applied Biology Department, Ebonyi State University Abakaliki, Nigeria.

2.2. Sample preparation and Extraction

Mushrooms were destalked and air dried at room temperature with adequate ventilation and pulverized using a blender. The pulverized samples were extracted with methanol by reflux. Exactly 50g of the powdered samples were weighed into 400ml of methanol in a reflux flask and refluxed for 2 hours. The extracts were filtered hot using a muslin cloth and subsequently evaporated using a rotary evaporator. The semi-dry extracts were weighed, placed in sterile sample bottles and stored in a refrigerator until required for use.

2.3. Qualitative phytochemical screening

The extracts were screened for phytochemical properties

using standard methods^[14].

2.4. Quantitative determination of the phytochemical constituents in samples

Aluminum chloride colorimetric method was used for flavonoid determination^[15]while total phenol content of the extracts was determined using the method reported by Singleton et al.^[16].

The method of Oloyed^[17]was used to determine the amount of alkaloids and saponins in the mushroom extracts while tannin content was quantified with the method described by AOAC^[18]

2.5. In vitro Antioxidant Determinations

Ability of the extracts to scavenge DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical was evaluated as described by Gyamfi et al.^[19]and the reducing power of the extracts was determined by assessing the ability of the extracts to reduce FeCl₃ solution as described by Oyaizu^[20].

2.6. Statistical Analysis

All values were expressed as Mean±SEM. The SPSS program (version 16.0 SPSS Inc., Chicago, IL, USA) was used for the Analysis of Variance (ANOVA) followed by the new Duncan multiple test.

3. Results

3.1. Qualitative Phytochemical Screening

Phytochemical Screening result revealed the presence of alkaloids, cardiac glycosides, saponins, flavonoids, terpenes, steroids, tannins and phenolics in the selected mushroom extracts in varying proportions. Phlobatannins was absent in all mushrooms except *Termitomyces manniformis* and *Pleurotus ostreatus* while anthraquinone was present in all except *Hericium erinaceus*, *Auricularia cularia* and *Pleurotus ostreatus*.

3.2. Quantitative Phytochemical Analysis

The quantitative phytochemical content determination of the

Table 1

Phytochemical constituents of Selected Wild Edible Nigerian Mushroom species.

Mushrooms	Alkaloids	Anthraquinones	Cardiac glycosides	Flavonoids	Phenols	Phlobatannins	Saponins	Tannin	Terpenes	Steroids
<i>Cantharelle Cibarius</i>	+++	+	++	++	+	-	+	+	+++	+++
<i>Termitomyces robustus</i>	++	+++	+++	++	++	-	++	++	+++	++
<i>Termitomyces manniformis</i>	++	+	+++	++	++	+	+++	++	+++	+++
<i>Pleurotus pulmonarius</i>	+	++	++	++	++	-	++	+	++	++
<i>Pleurotus ostreatus</i>	++	-	+++	++	++	++	++	++	++	++
<i>Lactarius deliciosus</i>	+	++	+++	++	+	-	++	++	+++	+
<i>Auricularia auricula</i>	+	-	+++	+	+	-	++	+	+++	++
<i>Hericium erinaceus</i>	+	-	+++	+	+	-	+	+	+	+

KEY -Absent, + Faintly present, ++ Moderately present, +++ Highly present

Table 2:

Quantitative Phytochemical contents of Selected Wild Edible Nigerian Mushroom species.

Phytochemicals	Cantherale cibarius	Temitomyces robustus	Temitomyces manniformis	Pleurotus pulmonarius	Pleurotus ostreatus	Lactarus deliciosus	Auricularia auricula	Hericium erinaceus
Alkaloids($\mu\text{g/g}$)	135.57 \pm 0.27 ^h	85.29 \pm 0.04 ^f	112.51 \pm 0.04 ^g	46.90 \pm 0.73 ^c	81.51 \pm 0.73 ^c	52.99 \pm 0.07 ^d	16.3200 \pm 0.46 ^b	8.1250 \pm 0.40 ^a
Saponins (mg/g)	150.41 \pm 0.50 ^h	87.56 \pm 0.81 ^f	105.30 \pm 4.53 ^g	34.76 \pm 1.46 ^c	84.95 \pm 5.03 ^c	20.35 \pm 1.82 ^b	71.21 \pm 0.50 ^d	10.17 \pm 1.09 ^a
Tannins(mg/g)	76.29 \pm 0.74 ^c	170.56 \pm 0.74 ^g	169.19 \pm 0.5 ^g	88.91 \pm 0.16 ^d	146.30 \pm 0.8 ^f	85.73 \pm 0.8 ^a	66.9250 \pm 0.33 ^b	59.27 \pm 0.00 ^b
Flavonoids (mg/g)	11.50 \pm 0.50 ^b	23.88 \pm 1.13 ^{cd}	25.66 \pm 0.74 ^d	22.17 \pm 0.88 ^c	42.63 \pm 0.63 ^f	34.58 \pm 0.93 ^e	6.41 \pm 0.53 ^a	6.75 \pm 0.38 ^a
Phenols(mg/g)	97.16 \pm 0.94 ^a	177.96 \pm 1.72 ^c	211.82 \pm 2.42 ^d	223.11 \pm 0.02 ^e	248.80 \pm 7.63 ^f	115.68 \pm 2.1 ^b	115.99 \pm 0.80 ^b	105.09 \pm 3.3 ^a

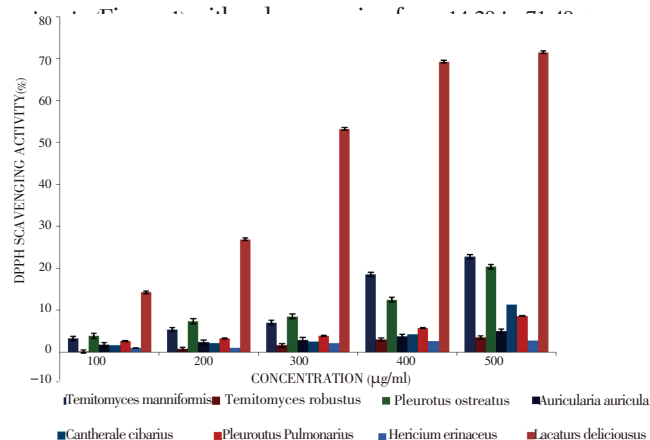
Results are presented as mean \pm SEM. Letters represent the level of significance

methanolic extract of the selected mushroom result as given in Table 2 showed that alkaloid contents of the extracts ranged between (8.125– 135.57 $\mu\text{g/g}$), tannin contents of the extract ranged between 59.27 \pm 0.00–170.56 \pm 0.74mg/g, saponin (10.17 \pm 1.09 – 150.41 \pm 0.50mg/g), total phenols (97.16 \pm 0.94 – 248.80 \pm 7.63mg/g) and total flavonoid contents 6.41 \pm 0.53 – 42.63 \pm 0.63mg/g). The extracts of Pleurotus ostreatus and Lactarus deliciosus showed a significantly high ($p < 0.05$) total flavonoid content of 42.63 \pm 0.63mg/g and 34.58 \pm 0.93mg/g respectively compared to all other mushroom extracts. Methanolic extract of Pleurotus ostreatus also showed the highest total phenol content of 248.80 \pm 7.63mg/g while Cantherale cibarius had the most significant amount of alkaloids (135.57 \pm 0.27mg/g) and saponins (150.41 \pm 0.50mg/g) when compared to other extracts.

3.3.Antioxidative Properties

3.3.1.Diphenyl picryl Hydrazyl radical Scavenging Activities

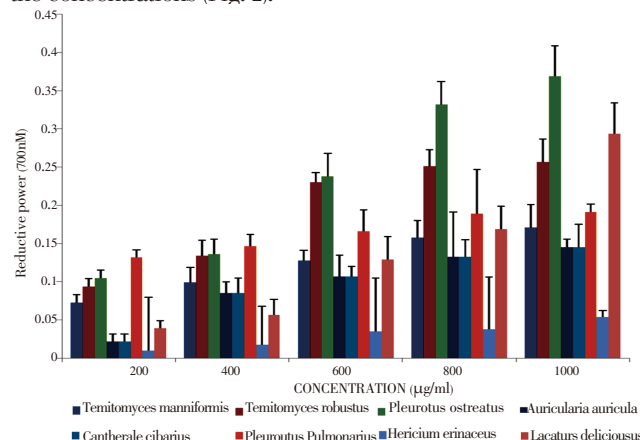
The DPPH radical scavenging activity of the selected wild edible Nigerian mushroom species is shown in Fig. 1. All the mushroom extracts scavenged the DPPH radical in a dose dependent manner. However Lactarus deliciosus had the highest DPPH scavenging activity compared to other mushroom

**Figure 1:** DPPH Scavenging Activity of Selected Wild Edible Nigerian Mushroom species.

3.3.2.Reductive Power

Result of the reductive power of the selected wild edible Nigerian Mushroom species indicate that activity of the extracts were proportional to concentration (200mg/ml– 1000mg/ml). Pleurotus ostreatus and lactarus deliciosus had better reductive power than other mushroom extracts irrespective of

the concentrations (Fig. 2).

**Figure 2:** Reductive power of Selected Wild Edible Nigerian Mushroom species.

4.Discussion

The result obtained from the qualitative phytochemical screening of methanolic extracts of the selected wild edible Nigerian Mushroom species showed the presence of tannin, saponins, cardiac glycosides, alkaloids, steroids, terpenes, phenols, and flavonoid in varying concentrations. These results agree with previous work on some selected Mushrooms in Nigeria and Sudan[12,21]and conform to some a certain degree with the study on some mushrooms from Kenya[22]. The absence of Anthraquinones in Hericium erinaceus, Auricularia auricula and Pleurotus ostreatus correlates with that of previous reports in the literature[12,22]. These phytoconstituents play a significant role in the medicinal properties of many plants.

Saponins for instance comprise a large family of structurally related compounds containing a steroid or triterpenoid aglycone. They are reported to have a wide range of pharmacological properties are beneficial, such as anti-inflammatory and anti-diabetic effects[23]. Thus these mushrooms can be used in the management of diabetes and inflammation related diseases.

Terpenoids have been reported to show a wide range of pharmacological benefits that include anti-malarial, anti-inflammatory and anti-cancer effects among others[24, 25].

The valuable pharmacological properties of many mushrooms have also been attributed to the presence of alkaloids on the autonomic nervous system, blood vessels, respiratory system, gastrointestinal tract, uterus, and have been shown to be effective against malignant diseases, infections and malaria[26]. Phenolic compounds are antioxidants, and exhibit a wide range spectrum of medicinal properties such as anti-cancer, anti-

inflammatory and diabetic effects[27,28].

Flavonoids are one of the most diverse group of natural compounds that have been shown to possess a broad spectrum of chemical and biological activities including radical scavenging properties, anti-allergenic, antiviral, anti-inflammatory, and vasodilating actions[29,30]. Thus the extracts of the studied mushrooms may be good alternatives for the treatment of diseases associated with excessive free radical generation and damage.

The high flavonoid content in *Pleurotus ostreatus* and *Lactarius deliciosus* was found to be higher than that found in an edible mushroom ($2.84 \pm 0.12 \text{ mg/g}$) in a recent study[31]. The phenols content also in *P. ostreatus* and *P. Pulmonarius* were found to be higher than that of some wild edible mushrooms investigated in recent study[32].

These mushrooms can therefore be harnessed in the management of oxidative stress induced diseases since phenols and flavonoid have been shown to possess various antioxidant functions.

DPPH free radical Scavenging activity assay is one of the most common methods for the determination of antioxidant capacity. It relies on the reduction of methanolic DPPH solution in the presence of hydrogen donation compound (antioxidant). The resulting decolorisation upon abstraction of hydrogen from the antioxidant is stoichiometric with respect to the degree of reduction and absorbance measurement after a certain time corresponds inversely to the radical scavenging activity of the antioxidant[33].

Although the mushroom, *Pleurotus ostreatus* specie had the highest amount of phenol ($248.80 \pm 7.63 \text{ mg/g}$) and flavonoids ($42.63 \pm 0.63 \text{ mg/g}$) compared to other mushrooms extracts, it did not have the highest scavenging activity, rather the mushroom *Lactarius deliciosus* had the most appreciable DPPH scavenging activity amongst other mushrooms in a dose dependent manner.

Thus other non-phenolic and flavonoids compound may be responsible for the antioxidant properties of *Lactarius deliciosus* while *Pleurotus ostreatus* may have exhibited its antioxidant activity through a different mechanism.

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of antioxidant action of Phenolics[34]. *Pleurotus ostreatus* showed a higher reductive power than other mushroom extracts in a concentration dependent manner and this commensurate with the high phenol and flavonoid content in this mushroom. This supports earlier reports, correlating the presence of flavonoids and phenolic compounds to antioxidative actions[35,36]. Therefore the invitro antioxidant properties exhibited by this mushroom extract may be due the presence of these antioxidant phytochemicals inherent in it and these mushrooms

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Comments

Background

Globally mushrooms are major of food. Indigenous wild edible mushrooms are important to Nigerians. Therefore, it is necessary to the study its phytochemical composition and antioxidant activities.

Research frontiers

The present research work demonstrates in vitro antioxidant activity of methanolic extract of eight indigenous wild edible Nigerian mushrooms by screening them against 1, 1-Diphenyl picryl hydrazyl (DPPH) radical and reductive power assays.

Related reports

Experimental evidence suggests that free radicals and reactive oxygen species are involved in human diseases. Plants including indigenous wild edible mushrooms produce a lot of antioxidants to control the oxidative stress induced diseases. and such they can represent a source of new compounds with antioxidant activity. 1, 1-Diphenyl picryl hydrazyl (DPPH) radical and reductive power assays are used for its evaluation

Innovations and breakthroughs

Mushrooms are widely known their culinary and medicinal properties and very often taken as food globally. In the present study, authors have demonstrated the antioxidant activity of eight indigenous wild edible Nigerian mushrooms against 1, 1-Diphenyl picryl hydrazyl (DPPH) radical and reductive power assays.

Applications

From the literature review it has been found that some indigenous wild edible Nigerian mushrooms are safe to humans. This scientific study supports their ethnobotanical uses particularly as antioxidant agent for the management of oxidative stress induced diseases.

Peer review

This is a good study in which the authors phytochemically investigate as well as demonstrate the antioxidant activity of eight wild edible Nigerian mushrooms. DPPH (1, 1-diphenyl-2 picrylhydrazyl) free radical was used to evaluate the scavenging ability of the mushroom extracts and their reducing power were also assessed

Conflict of interest statement

We declare that we have no conflict of interest.

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