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Phytochemical Constituents and in-vitro Antioxidant Activity of Aqueous Extracts of Mushrooms (Pleurotus ostreatus & Agaricus sp)

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ABSTRACT

Antioxidants help to scavenge free radicals and thus, provide health benefits. This study reports the in-vitro radical scavenging capacity of aqueous extracts from the mushrooms (Agaricus sp. & Pleurotus ostreatus). The main objective of this research was to determine the phytochemical constituents, nitric oxide scavenging activity, hydroxyl radical scavenging activity, and DPPH-scavenging activity of aqueous extracts of Agaricus & Pleurotus. These parameters would give an indication of the possible antioxidant abilities of these extracts. Extracts of the two mushrooms with concentrations ranging from 0.02 to 0.10 mg/ml were prepared and their antioxidant activities evaluated using the standard antioxidants tannic acid and ascorbic acid as the control. For percentage scavenging of nitric oxide radicals, Agaricus sp. recorded 58.74% whereas Pleurotus ostreatus had 54.62%with respect to their highest concentration (100µg/ml). For percentage scavenging of DPPH radicals, Agaricus sp. recorded 24.83% whilst Pleurotus ostreatus recorded 29.79% and for percentage scavenging of hydroxyl radicals, Agaricus sp recorded 21.74% as against 64.94% recorded for Pleurotus ostreatus with respect to their highest concentration (100µg/ml). Results demonstrated that the extracts scavenged free radicals in concentration-dependent manner, just like the standards. Aqueous extracts of the mushrooms (Agaricus sp & Pleurotus ostreatus) demonstrated antioxidant activity in-vitro and can therefore be beneficial in the prevention and treatment of diseases which occur due to reactive oxygen species and reactive nitrogen species generation.

Keywords: *Phytochemicals, antioxidants, reactive oxygen species (ROS), Pleurotus ostreatus, Agaricus sp.*

INTRODUCTION

Reactive oxygen species (ROS) are produced in living systems and the most common source of ROS is leakage of electrons to O_2 from the electron transport chain of mitochondria [1]. ROS can cause damage to biomolecules, resulting in several diseases. Living systems have specific pathways to overcome the adverse effect of various damages caused by the ROS, although sometimes these repair mechanisms fail to keep pace with such deleterious effects [2]. Moreover, antioxidant agents such as phenolic compounds can decrease the harmful effects of ROS [3]. Phyto chemicals have been detected in both edible and non-edible mushrooms [4].

These compounds were observed in different levels and types in various parts of mushrooms [5]. Phenolic compounds have attracted increasing attention for their antioxidant behaviour and beneficial health-promoting effects. It is assumed that many anti-oxidative phenolic compounds are usually present in a covalently-bound form [6]. They can act as antioxidants by donating hydrogen to highly reactive radicals, thereby preventing further radical formation [7]. Edible mushrooms (*Agaricus sp. & Pleurotus ostreatus*) are an effective alternative in the prevention and

treatment of many diseases. They are an important source of nutrients and have been known to be of medicinal and physiological benefit to mankind [8]. Edible mushrooms have been used in folk medicine throughout the world since ancient times.

Several medicinal properties of edible mushrooms have been reported, and these include inhibition of platelet aggregation, reduction of blood cholesterol levels, prevention of myocardial infarctions, reduction of blood glucose levels [9], and prevention of infections caused by bacterial, viral, fungal and parasitic pathogens [8].

This research investigated the phyto chemical constituents, nitric oxide scavenging activity, hydroxyl radical scavenging activity, and DPPH-scavenging activity of aqueous extracts of the mushrooms; *Agaricus* & *Pleurotus*.

This was done by preparing aqueous extracts of the two mushrooms with concentrations ranging from 0.02 to 0.10 mg/ml and the aforementioned parameters evaluated against the standard antioxidants tannic acid and ascorbic acid.

Materials and Methods

Sample Collection

Fresh whole mushroom (*Agaricus sp & Pleurotus ostreatus*) were collected from the University of Cape Coast Botanical Garden, Cape Coast, Ghana. The taxonomic identities of the mushroom were verified at the Department of Botany, University of Cape Coast, Ghana. The mushroom samples were washed under running tap water to remove unwanted dirt and other foreign materials. The samples were air dried under shade until there was no moisture left. The dried samples were ground into powder using an industrial blender.

Sample Preparation

Aqueous Extraction

An amount of 60g each of the powdered samples was weighed and dissolved in 600ml of sterilized distilled water and then kept in water bath for two hours under reduced pressure at a temperature of 90°C, and then filtered. The filtrates were concentrated on water bath under reduced pressure at a temperature of 90°C. After complete solvent evaporation, each of the extracts was weighed and stored in airtight bottles at 4°C for further use.

Photochemical Screening (Qualitative Analysis)

Phytochemical screening of the aqueous extracts of mushrooms *(Agaricus sp & Pleurotus ostreatus)* were carried out as per standard protocols [10, 11] to determine the presence of glycosides, terpenoids, flavonoids, carbohydrates, protein, alkaloids, phenolic compounds, tannins and saponins.

Determination of Free Radical Scavenging Activity of Extracts

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity of the extracts was determined according to the method reported by Pavithra and Vadivukkaras, [12] with slight modifications. A total of five test tubes containing different concentrations of each extract (0.02 - 0.10mg/ml) were set up. The reaction mixture in each test tube contained 0.5 mL of extracts or control (ascorbic acid and tannic acid), 1.0 mL iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 mL of 0.018% EDTA and 1.0 mL DMSO (0.85% in 0.1 mol/L phosphate buffer pH 7.4). The reaction was started by adding 0.5 ml of ascorbic acid (0.22 %) and incubated at 80°C – 90°C for 5 min. The reaction was terminated by adding 0.1 mL of ice cold TCA (17.5%). A volume of 3.0 mL Nash reagent (75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid and 2.0 mL of acetyl acetone in 1L distilled water) was added and incubated at room temperature for 15 min for colour development. The intensity of the colour formed was measured at 412 nm against a reagent blank and the percentage ability of each extract to scavenge hydroxyl radicals measured according the equation below. The procedure was repeated for the standards by replacing extract with the controls.

$$OH^{\cdot-} scavenged\ (\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

Where A_0 is the blank absorbance

A_1 is the extract absorbance or the control absorbance.

1, 1 Diphenyl-2, Picrylhydrazine (DPPH) - Free Radical Scavenging Activity

This was determined according to the method of Shimada *et al.*,[13]. A total volume of 1.0 mL of various concentrations of each extract and standard (0.02–0.10 mg/mL) was mixed with

1.0 mL of 0.135 mmol/L DPPH solution. The mixture was shaken vigorously and left in the dark for 30 min. The absorbance was measured at 517 nm against a reagent blank. The percentage ability for scavenging DPPH radical by the extracts and standards was calculated according to the equation:

$$DPPH\ scavenging\ activity(\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

Where A_0 is the blank absorbance

A_1 is the extract absorbance or the control absorbance

Nitric Oxide Scavenging Activity

Nitric oxide radical scavenging activity was measured according to the method described by Jagetia *et al*.,[14]. A volume of 2ml of sodium nitroprusside in phosphate buffer (0.02M, pH 7.4) was mixed with different concentrations (0.02-0.10 mg/ml) of extracts and controls (ascorbic acid and tannic acid). Each reaction mixture was kept at 25°C for 2 hr. Thereafter, 1.5ml of Griess reagent [1% sulphanilamide, 2% O-phosphoric acid and 0.1% of N-(1- naphthyl) ethylenediamine dihydrochloride] was added. The absorbance of each mixture was measured at 540nm after 30 min against a phosphate buffer as blank. The percentage scavenging of nitric oxide radicals by extracts and standards was calculated using the formula:

$$scavenging\ activity(\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

Where A_0 is the blank absorbance

A_1 is the extract absorbance or the control absorbance

RESULTS

Phytochemical Analysis

The preliminary phytochemical analysis demonstrated the presence of reducing sugars, proteins, phenolic compounds, flavonoids, tannins, saponins, alkaloids, terpenoids and glycosides (Table 1).

Table1. *Phytochemical constituents of the aqueous extracts of the mushrooms (Pleurotus ostreatus&Agaricus sp)*

Test	***Pleurotusostreatus* extract**	***Agaricus sp.* extract**
Alkaloids	+	+
Tannins	+	+
Saponnins	+	+
Glycosides	+	+
Reducing sugars	+	+
Flavonoids	+	+
Terpenoids	+	+
Phenols	+	+
Proteins	+	+

+ (present)

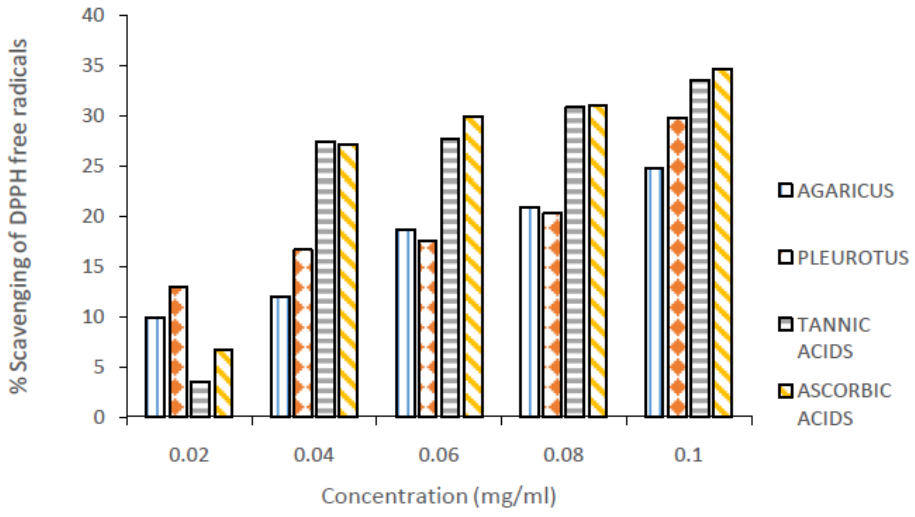

Fig1. *DPPH radical scavenging activity of aqueous extracts of mushrooms (Agaricus sp & Pleurotus ostreatus) and control (ascorbic and tannic acids)*

Antioxidant Activity

DPPH Radical Scavenging Activity

The *in-vitro* antioxidant assays performed revealed significant antioxidant potential of the mushroom extracts. The DPPH radical scavenging activities appear to increase as the concentration of the extracts and standards increase as illustrated in Fig. 1.

Hydroxyl Radical Scavenging Activity

Fig. 2 presents the hydroxyl radical scavenging activity of aqueous extracts as well as controls (tannic acid and ascorbic acid). The percentage radical scavenging activity of the extracts and controls increased with increasing concentration, with *Pleurotus* exhibiting the highest hydroxyl radical scavenging activity at all the concentrations measured.

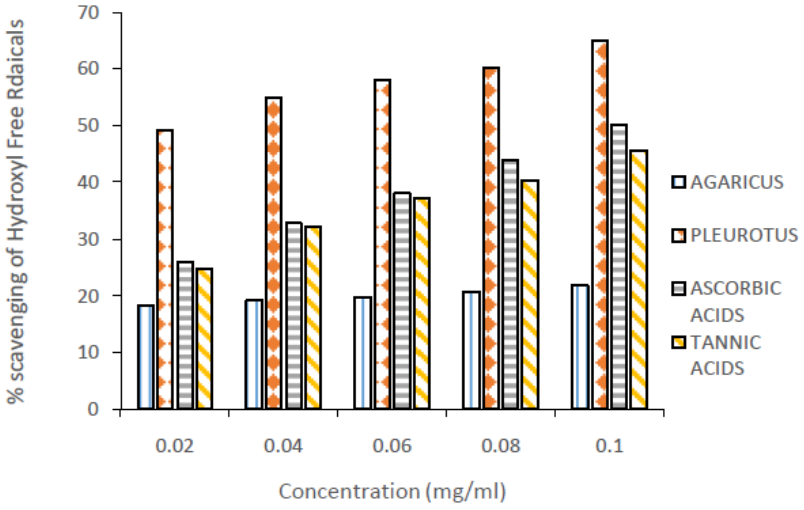

Fig2. *Hydroxyl scavenging activity of aqueous extracts of mushrooms (Agaricus sp & Pleurotus ostreatus) and standards (ascorbic and tannic acids)*

Nitric Oxide Scavenging Activity

The scavenging ability increased with increasing concentration of the extracts and standards, although, the activity of the controls (ascorbic acid & tannic acid) were relatively more pronounced than that of the extracts (Fig 3).

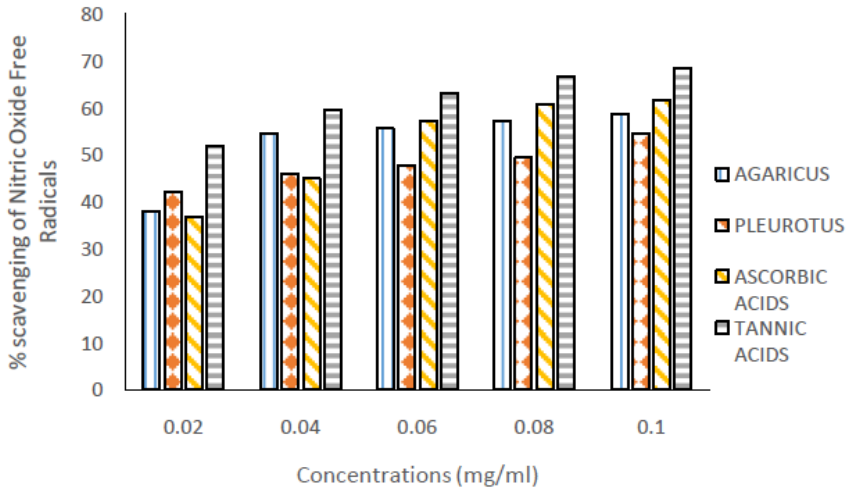

Fig3. *Nitric oxide scavenging activity of aqueous extract from mushrooms (Agaricus sp & Pleurotus ostreatus)*

DISCUSSION

This study set out to investigate the phytochemical constituents and in-vitro nitric oxide scavenging activity, hydroxyl radical scavenging activity and DPPH-scavenging activity. Phenolic compounds like flavonoids, alkaloids and tannins are considered to possess high antioxidant activities, which can prevent or treat many diseases, including cancer [15]. Therefore, the presence of appreciable to moderate amounts of these phytochemicals in

the extracts can confer some possible significant medicinal properties/potentials on the mushrooms (*Pleurotus ostreatus & Agaricus sp*). The DPPH radical scavenging activities appear to increase as the concentration of the extracts and controls increase. DPPH is a stable free radical and has been used extensively to test the ability of natural products to act as free radical scavengers or hydrogen donors [16]. Free radical scavengers either transfer an electron or a hydrogen atom to DPPH, thus neutralizing a number of DPPH molecules which equals to that of hydroxyl groups [17]. The free radical scavenging abilities of *Agaricus sp. & Pleurotus ostreatus* appears to be concentration dependent, with the highest scavenging activity observed at the highest concentration (0.1mg/ml) of extracts or controls investigated.

Hydroxyl radicals are known to react with all components of DNA molecule: the purine and pyrimidine bases as well as the deoxyribose backbone [18] and results in a series of reactions that cause the formation of malondialdehyde (MDA) which is an indicator or marker of oxidative stress. Ascorbic acid demonstrated the highest percentage of hydroxyl radical scavenging ability of 68.09 followed by crude extract of *Pleurotus ostreatus* mushroom 64.91% and tannic acid 48.97%. Crude extract of *Agaricus sp.* recorded the least percentage of radical scavenging ability, which is 21.74%. The inhibitory action of the extracts of the mushrooms *(Agaricus sp. & Pleurotus ostreatus*) on hydroxyl radicals mediated oxidation might protect against damage o several biomolecules like DNA, lipids and proteins in the body and could also prevent ROS generation or reduce the damage caused by ROS.

In addition to the presence of phytochemicals, the extract also showed nitric oxide scavenging activity. The toxicity and damage caused by NO and superoxide anion is heightened as they react to produce reactive peroxynitrite ($ONOO^-$), which leads to serious toxic reactions with biomolecules such as proteins, lipids and nucleic acids [14].

Tannic acid recorded the highest % nitric oxide scavenging ability, followed by ascorbic acid, *Agaricus sp.* and *Pleurotus ostreatus* mushroom extracts, i.e. 68.48%, 61.59%, 58.74% and 54.62% respectively for the highest concentration tested (100µg/ml). The NO radical scavenging activity of mushrooms (*Agaricus sp & Pleurotus ostreatus*) may help arrest chain reactions which are initiated by excess NO reacting with certain radical species in our biological systems, this mostly leads to the generation of highly reactive radicals like $ONOO^-$. $ONOO^-$ is detrimental to the cells, tissues and organs of most organisms as it turns to react with these biological entities leading to their malfunction or outright death. The presence of a number of phytochemicals and the ability of the crude extracts of these mushrooms to scavenge certain radicals*in-vitro* lends more credence to the belief that, edible mushrooms are good sources of healthy food.

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CONCLUSION

This study detected the presence of several phytochemicals in the aqueous extracts of mushrooms *(Agaricus sp & Pleurotus ostreatus)*. The mushroom extracts also demonstrated free radical scavenging abilities in a concentration dependent manner.

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