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Full Length Research Paper

Antioxidant activities and phenolic contents of three mushroom species, *Lentinus squarrosulus* Mont., *Volvariella esculenta* (Massee) Singer and *Pleurocybella porrigens* (Pers.) Singer

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The antioxidant properties of three mushroom samples, namely, *Lentinus squarrosulus, Volvariella esculenta* and *Pleurocybella porrigens* were investigated. The antioxidant activities, 2, 2-diphenylpicrylhydrazyl (DPPH) radical scavenging ability, reducing powers, amount of total phenolic compounds and flavonoid concentration of the extracts were determined. The mushroom samples showed differences in values for all the five parameters. *L. squarrosulus* had the highest antioxidant activity of 40.54±1.50, flavonoid concentration (61.93±2.93), total phenolic content (392.68±33.77) and reducing power activity (281.15±8.13), while *P. porrigens* had the least values for these parameters. There were statistically significant correlations between reducing power and amount of total phenolic compounds in all three mushroom extracts. The highest DPPH scavenging ability was shown by *P. porringes* (63.37±2.89) and the least value of this parameter was shown by *V. esculenta (*48.88±1.35). The mushroom samples showed significant difference ($p<0.05$) in all the parameters, except for total phenolic compounds concentration ($p>0.05$). All the three mushroom samples exhibited effective antioxidant properties which contribute to their medicinal and health values.

Key words: *Lentinus squarrosulus, Volvariella esculenta, Pleurocybella porrigens,* mushroom, antioxidant, phenolic content.

INTRODUCTION

Mushrooms represent one of the world's greatest untapped resources of nutritious food. Cultivation of saprophytic edible mushrooms may be the only currently economical biotechnology for lignocellulose organic waste recycling that combines the production of protein rich food with the reduction of environmental pollution (Obodai et al., 2003). Mushrooms are rich in protein, minerals and vitamins, and they contain an abundance of essential amino acids (Sadler, 2003). Therefore, mushrooms can be a good supplement to cereals (Chang and Buswell, 1996). Mushrooms can be saprophytic or parasitic. They include members of the basidiomycota and some members of the ascomycota. They consist of two main parts, the mycelium and the fruity body (sporocarp).

The arising awareness of the relationship between diet and diseases has evolved the concept of “functional foods” and the development of a new scientific discipline, Functional Food Science (Sadler and Saltmarsh, 1998). A food may be considered to be functional if it contains a food component (whether a nutrient or not) which affects one or more identified functions in the body in a positive manner, which are in different name forms, e.g. dietary supplements, nutraceuticals, medicinal foods, vita foods, pharma foods, phytochemicals, mycochemicals and foods for specific health uses (Hasler, 1996). The scientific community, in searching for new therapeutic alternatives, has studied many kinds of mushrooms and has found variable therapeutic activity, such as, anticarcinogenic, anti-inflammatory, immunosuppressor and antibiotic, among others (Asfors and Ley, 1993; Longvah and Deosthale, 1998). It has been known for

many years that selected mushrooms of higher basidiomycetes origin are effective against certain cancer types, and this have stirred a growing interest in such mushrooms from industry, the media and the scientific community (Wasser, 2002). Medicinal mushrooms have an established history of use in traditional oriental therapies. Mushrooms have been used for many years in oriental culture as tea and nutritional food, and because of their special fragrance and texture (Manzi et al., 1999).

The human body suffers several negative effects, such as, cancer, stroke, ageing, etc., that could be attributed to either internal or external factors that induce the production of free radicals (Oboh and Akindahunsi, 2004; Oboh, 2005).

Oxidative stress occurs when the production of harmful molecules called free radicals is beyond the protective capability of the antioxidant defences (Alia et al., 2003). Free radicals are chemically active atoms or molecular fragments that have a charge due to an excess or deficient number of electrons. Examples of free radicals are the super oxide anion, hydroxyl radical, and transition metals, such as iron and copper, nitric acid and ozone (Alia et al., 2003). Free radicals containing oxygen known as reactive oxygen species (ROS) are the most biologically significant free radicals. ROS include the radicals super oxide and hydroxyl radical, plus derivatives of oxygen that do not contain unpaired electrons, such as, hydrogen peroxide, singlet oxygen and hypochlorous acid. Free radicals are highly unstable, because they have one or more unpaired electrons (Alia et al., 2003). They scavenge in the body to grab or donate electrons; thereby, damaging cells, proteins and DNA (genetic materials). The same oxidative process also causes oil to become rancid, peeled apples to turn brown and iron to rust (Alia et al., 2003). Normally, bonds split in ways that leave a molecule with an odd, unpaired electron, but when weak bonds split, free radicals are formed (Alia et al., 2003). Antioxidants are substances that are capable of counteracting the damaging, but the normal effects of the physiological process of oxidation in animal tissue. Antioxidants are nutrients (vitamins and minerals) as well as enzymes (protein in the body that assists in chemical reactions) (Sun et al., 2002). They are believed to play the role of preventing the development of such chronic diseases as cancer, heart disease, stroke, rheumatoid arthritis and cataracts (Chu et al., 2002).

Almost all organisms are well protected against free radical damage by enzymes, such as, superoxide dismutase and catalase, or compounds, such as, ascorbic acid, tocopherols and glutathione (Mau et al., 2002). When the mechanism of antioxidant protection becomes unbalanced by factors, such as, aging, deterioration of physiological functions may occur resulting in diseases and accelerated aging. However, the antioxidants present in human diet are of great interest as possible protective agents to help the human bodies reduce oxidative damage. Researchers have reported the antimicrobial and antioxidative activities of several mushrooms (Lee et al., 1999; Gao et al., 2005; Turkoglu et al., 2006; Iwalokun et al., 2007), amongst other benefits of mushrooms. Therefore, this study is aimed at evaluating the antioxidant potential of three edible mushroom samples, namely, *Lentinus squarrosulus, Volvariella esculenta* and *Pleurocybella porrigens.*

MATERIALS AND METHODS

Mushrooms

Mushroom samples were collected from farm lands in Abraka, Delta State, Nigeria. They were identified by a mycologist, Mr. A. O. Oghenekaro, at the Department of Botany, University of Benin, Benin City, Edo State, Nigeria. Further analyses of the samples were done at the laboratory of Department of Biochemistry, Delta State University, Abraka. Fresh mushroom samples (150 g) were air dried in an oven at 40°C before analysis. Dried m ushroom sample (50 g) was extracted by stirring with 500 ml of ethanol at 30°C at 150 rpm for 24 h and filtering was done throug h Whatman No. 4 filter paper. The residue was extracted with two additional 500 ml of ethanol as described earlier. The combined ethanolic extract were then rotary evaporated at 40°C to dryne ss, redissolved in ethanol to a concentration of 10 mg/ml and stored at 4°C for further use.

Antioxidant activity

The antioxidant activity was determined by ammonium thiocyanate assay (Lee et al., 2002). 500 µl of extract, 200 µl of diluted linoleic acid (25 mg/ml 99% ethanol) and 400 µl of 0.5 m phosphate buffer (pH 4) was mixed and incubated at 40°C for 15 min. Al iquot (100 µl) from the reaction mixture was mixed with reaction solution containing 3 ml of 70% ethanol, 100 µl of ammonium thiocyanate (300 mg/ml distilled water) and 100 µl of ferrous chloride (2.45 mg/ml in 3.5% hydrochloric acid). The final reaction solution was mixed and incubated at room temperature for 3 min.

Absorbance was measured at 500 mM. Linoleic acid emulsions without extract served as control. Inhibition of linoleic acid oxidation was calculated by using the following formula:

Inhibition (%) = [(control OD – sample OD)/control OD)] ×100.

OD = Optical density

DPPH assay

Hydrogen atom or electron donation abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-coloured methanol solution of 2, 2-diphenylpicrylhydrazyl (DPPH). This spectrophotometric assay was done according to Blois (1958), with a slight modification. 500 µl of extract solution was mixed with 1 ml of 0.1 mM DPPH in ethanol solution and 450 µl of 50 mM Tris-HCl buffer (pH 7.4) was added. The solution was incubated at 37°C for 30 min and re duction of DPPH free radicals was measured by reading the absorbance at 517 nm. Control was maintained. Ascorbic acid solution was used for comparison. This activity was given as percentage DPPH scavenging and calculated according to the following equation:

DPPH scavenging (%) = [(control OD – sample OD) / (control OD)] × 100

Determination of total phenolic compounds

The amount of total phenolic compound content of the extracts was determined by the method described by Singleton et al. (1999). 500 µl of extract was transferred to a 100 ml Erlenmeyer flask and the final volume was adjusted to 46 ml by addition of distilled water. 1 ml of Folin-Ciocalteu reactive solution was added and incubated at room temperature for 3 min. 3 ml of 2% sodium carbonate solution was added to the mixture and was shaken on a shaker for 2 h at room temperature. The absorbance was measured at 760 nm.

Gallic acid was used as the standard for a calibration curve. The phenolic compound content was expressed as gallic acid equivalent.

Determination of total flavonoid concentration

Flavonoid concentration was determined as follows: Mushroom ethanolic extracts solution (1 ml) was diluted with 4.3 ml of 80% aqueous ethanol and test tubes was added to 0.1 ml of 10% aluminum nitrate and 0.1 ml of 1 M aqueous potassium acetate. After 40 min at room temperature, the absorbance was determined spectrophotometrically at 415 nm. Total flavonoid concentration was calculated using quercetin as standard (Park et al., 1997).

Test for reducing power

Phosphomolybdenum (PMo) assay according to Prieto et al. (1998) with slight modification was used to estimate the capability of the samples to reduce transition metal ions. The reagent solution contained ammonium molybdate (4 mM), sodium phosphate (28 mM), and sulfuric acid (600 mM) mixed with the samples diluted in ethanol. The samples were incubated at 90°C for 90 mi n, cooled down to room temperature, and the absorbance of the green phosphomolybdenum complex was measured at 695 nm. The reducing capacity of the extracts was calculated using the following equation:

ABS final = ABS sample - ABS blank - ABS extract

where ABS extract = absorbance of sample where molybdate solution was replaced by water; ABS blank = absorbance of blank containing methanol (400 µl) instead of extract sample.

For reference, the appropriate solutions of ascorbic acid have been used, and the reducing capacity of the analyzed extract was expressed as the ascorbic acid equivalent (AAE) per gram of sample dry weight.

RESULTS

The reducing power, total phenolic compounds and flavonoid concentration of ethanolic extracts of *L. squarrosulus, V. esculenta* and *P. porrigens* were determined, and the results are as shown in Table 1. The reducing power PMo test shows that *L. squarrosulus* had the highest value (281.15±8.13), and the least value was dispayed by *N. porringes*. Comparatively, all the three mushrooms significantly differed ($p<0.05$), when compared against one another. The total phenolic compounds values in Table 1 shows that the highest and least value were dispayed by *L. squarrosulus* and *P. porrigens*, respectively, but there was no statistical difference($p>0.05$), when compared against one another.

On the other hand, there was significant difference ($p<0.05$), when the flavonoid concentration values were compared.

The result of the DPPH scarvenging ability and antioxidant activity (Table 2) showed that *P. porrigens* and *L. squarrosulus* had the highest and least value for the DPPH scarvenging ability while, the lowest value for antioxidant activity was shown by *P. porrigens*, and the highest value dispayed was by *P. squarrosulus*, whereas, there was significant difference ($p<0.05$), when all the three mushrooms were compared for the antioxidant activity. This was however not seen in the percentage DPPH scarvenging ability, where the comparative value at $p<0.05$ was not significant when *L. squarrosulus* was compared with *V. esculenta*, but there was statistical significant difference ($p<0.05$), when *L. squarrosulus* was compared with *P. porrigens,* and when *V. esculenta* was compared with *P. porrigens.*

DISCUSSION

Mushrooms have been shown to contain vast varieties of biologically active substances with immunostimulatory, anti-cancer and antioxidant properties (Silva, 2004). Mushrooms from various researches have demonstrated several medicinal importances, which is attributed to the presence of many bioactive components which may include polysaccharides, proteins, vitamins and aromatic compounds like polyphenols (flavonoids, tannins, saponins, alkaloids, etc). All the extracts of the three mushrooms (*L. squarrosulus*, *V. esculenta* and *P. porringes*) exhibited antioxidant properties. The results of this study, as shown in Tables 1 and 2 demonstrate a good correlation between total content of phenolic compounds and the antioxidant activity of the three mushrooms investigated, and this is in agreement with previous findings (Cai et al., 2004; Beta et al., 2005; Othman et al., 2007; Tawaha et al., 2007).

The most effective free radical scavenging activity was shown by the extract of *P. porringes* (63.37±2.89), the least activity was observed in *V. esculenta (*48.88±1.35) (Table 2). The most effective antioxidant potential was shown by the extract of *L. squarrosulus (*40.54±1.50), and the least activity was shown by *P. porrigens (*20.06±0.60).

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). ROS and associated free radicals have been implicated in the etiology of various human diseases, including inflammation, metabolic dis-orders, cellular aging and atherosclerosis, heart disease, stroke, diabetes mellitus, cancer, malaria, rheumatoid arthritis and HIV/AIDS (Alho and Leinonen, 1999; Odukoya et al., 2005). Therefore, radical scavengers give promising indications of new therapeutic approaches

Table 1. Determination of reducing power, total phenolic compounds and flavonoid concentration of aqueous extract of *L. squarrosulus, V. esculenta* and *P. porrigens*.

Parameter	*L. squarrosulus*	*V. esculenta*	*P. porrigens*
Reducing power by PMo test (AAE mg/g) ±SEM	281.15±8.13*	211.60±8.64*	137.07±4.58*
Total phenolic compounds (µg GAE/100g)	392.68±33.77	295.71±4.46	289.71±6.53
Flavonoid concentration (mg/g)	61.93±2.93*	32.17±1.64*	20.70±1.18*

All values are expressed as mean ± standard error of mean (SEM) (n=3). *, Differ statistically at p<0.05; n = number of determinations; GAE, gallic acid equivalents; AAE, ascorbic acid equivalent.

Table 2. Determination of DPPH scarvenging ability and antioxidant activity of *L. squarrosulus, V. esculenta* and *P. porrigen.*

Parameter	*L. squarrosulus*	*V. esculenta*	*P. porrigens*
DPPH scarvenging ability (%)	53.04±2.63	48.88±1.35*	63.37±2.89*
Antioxidant activity (ammonium thiocyanate assay % of mushrooms)	40.54±1.50*	21.18±1.26*	20.06±0.60

Data are expressed as mean ± standard error of mean (SEM) for three determinations. *, Differ statistically (p<0.05).

(Cho et al., 2003).

The significance of antioxidant evaluation of these three mushrooms extracts lie in the structural requirement of the chemical constituents that could be linked to flavonoids, which have been found present in the all the three extracts evaluated (Table 1). Many flavonoids have shown strong antioxidant properties (Raj and Shalini, 1999). The capacity of plant extracts to act as reducing agents, hydrogen donors, singlet oxygen quenchers, metal chelators or free radical scavengers indicates their potential therapeutic properties for treating diseases related to free radical reactions. The results of this study showed that the reducing power activity, amount of total polyphenols and concentration were higher in the extract of *L. squarrosulus* than the other two mushrooms (Table 1). There were statistically significant correlations between reducing power and amount of total phenolic compounds in all three mushroom extracts, which is in agreement with the findings of other researchers. Yildrim et al. (2001) have suggested that there may be relationship between phenolic compounds and reducing powers. The reducing capacity of compounds could serve as indicator of potential antioxidant properties (Meir et al., 1995).

The presence of phenolic compounds might be the reason for reducing power. Phenolic compounds are also thought to be capable of regenerating endogenous α-tocopherol, in the phospholipid bilayer of lipoprotein particles, back to its active antioxidant form. They are also known to inhibit various types of oxidizing enzymes. These potential mechanisms of antioxidant action make the diverse group of phenolic compounds an interesting target in the search for healthbeneficial phytochemicals (Halliwell and Gutteridge, 1989; Hall and Cuppett, 1997). The key role of phenolic compounds as scavengers of free radicals is emphasised in several reports (Komali et al., 1999; Moller et al., 1999). Phenols are important components of plants. They are reported to eliminate radicals due to their hydroxyl groups (Hatano et al., 1989), and they contribute directly to antioxidant effect of system (Duh et al., 1999). Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity (Yen et al., 1993; Gulcin et al., 2003). The phenolic compounds may contribute directly to antioxidative action (Duh et al., 1999). It has been suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g is ingested daily from a diet rich in fruits and vegetables (Tanaka et al., 1998).

Like phenol compounds, the contribution of flavonoids to antioxidant activity is known. It has been reported that buthylated hydroxytoluene (BHT) I3, II8-biapigenin and hypericine which have the structure of biflavonoid have a very high antioxidant effect. This effect was proposed to stem from hydroxyl groups in the structure of the flavonoids (Cakir et al., 2003). Therefore, the mushrooms extracts investigated in this study could be said to compete favourably with butylated hydroxyanisole (BHA) and α-tocopherol in β-caroten-linoleic acid system used to determine the antioxidant capacity.

In conclusion, the results of this study indicate that the mushrooms investigated are enriched with antioxidant potential and would be the choice of selection for commercialization and are therefore recommended for consumption, since they have health benefits.

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