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ANTIOXIDANT AND NUTRITIONAL IMPORTANCE OF SOME *PLEUROTUS* SPECIES

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ABSTRACT

The nutrient compositions (dry matter, moisture content, ash content, fat content, crude fibre, total carbohydrate and crude protein), antioxidant and yielding potential of *Pleurotus pulmonarius* (LAU 09), *P. cornucopiae* (NE 02), *P. sapidus* (NE 07) and *P. ostreatus* (LAU 10) were evaluated. The highest percentage spawn productivity (28.33%) was obtained in NE 02, while the lowest value of 23.33% obtained in NE 07. The highest total weight of fresh mushroom (580g), highest biological efficiency (59.10±12.41%) and production rate (1.85±0.65%) were obtained in LAU 09. The phytochemical screening of the mushroom extract revealed the presence of alkaloids, saponins, steroids, phlobatannins, flavonoids and anthraquinones. The antioxidant activity of all evaluated mushroom extracts gave a positive result with free radical scavenging potentials found to be in the order of NE 02 > NE 07 > LAU 09 > LAU 10 considering all used *in vitro* methods. The highest percentage protein of 34 ±3.06% was produced in NE 02, while other strains gave satisfactory yields in terms of nutritional and mineral compositions. The results obtained from this study showed the nutritional and antioxidant potential of mushroom species.

Keywords: Mushroom, yield, antioxidant, proximate, phytochemical

INTRODUCTION

Pleurotus species are refers to as one of the commercially important edible mushrooms throughout the world (**Kibar and Perksen, 2008**). Mushrooms are group of fleshy macroscopic fungi, which until recently, as other fungi, were included in the plant Kingdom because of cell wall and spores (**Lindequist *et al.*, 2005**). Mushrooms have been valued throughout the world as both food and medicine for thousands of years (**Wright, 2004**). Mushrooms have long been valued as tasty, nutritional foods, by different societies worldwide. The relationship between mushrooms and man can be traced far back into antiquity.

Mushrooms are considered as good source of digestible proteins, with protein content above vegetable and less than milk and meats (**Breece, 1990**). Protein content can vary from 10-40% on a dry weight basis which contain all the essential amino acids but can be reduced in sulphur-containing amino acids, crystine and methionine (**Breece, 1990**). Edible mushrooms are well known as the ideal materials for the dietetic prevention of atherosclerosis due to their high content of fiber, proteins, microelements and their low fat content (**Crisan and Sands, 1978**). Fresh mushrooms contain 3-21% carbohydrates and 3-35% fiber, with low calorific value. Mushrooms are excellent source of minerals and vitamin. The main classes of lipid compounds include free fatty acids, which are generally low level, around 2-8% of mushrooms dry weight (**Breece, 1990**). There are many varieties of mushrooms species; of which *Pleurotus* are characterized by a white spore print, attached to the gills, often with an essentric stip, or no stip at all. They are commonly known as Oyster mushrooms (**Khanna and Garcha, 1984**). Mushrooms accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids. Also, a mushroom phenolic compound has been found to be an excellent antioxidant and synergist that is not mutagenic (**Ishikawa *et al.*, 2001**). Antioxidant compounds prevent oxidative damage related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis. Mushrooms that contain antioxidants or increase antioxidant enzyme activity may be used to reduce oxidative damage in human (**Blois, 1958**).

Free radicals are types of Reactive Oxygen Species (ROS), which include all highly reactive, oxygen-containing molecules, the hydroxyl radical, the super oxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All these are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules, resulting in cellular damage. In living organisms, various ROSs can be formed in different ways, such as normal aerobic respiration, stimulated polymorphonuclear leukocytes and macrophages and peroxisomes. These appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents and pesticides.

Free radicals may be defined as chemical species associated with an odd or unpaired electron. They are neutral, short lived, unstable and highly reactive to pair up the odd electron and finally achieve stable configuration. They are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, liver diseases, diabetes mellitus, inflammation, renal failure, brain dysfunction and stress among others. To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly sophisticated and complex antioxidant protection system, that functions interactively and synergistically to neutralize free radicals. Thus, antioxidants are capable of stabilizing or deactivating, free radicals before they attack cells (**Beris, 1990**). Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being. Naturally, there is a dynamic balance between the amount of free radicals produced in the body and antioxidants to scavenge or quench them to protect the body against deleterious effects. The amount of antioxidant principles present under normal physiological conditions may be insufficient to neutralize free radicals generated. Therefore, it is necessary to enrich our diet with antioxidants to prevent harmful diseases. It is interesting to note that there has been a serious interest in the food industry and in preventive medicine in the development of "Natural antioxidants" from plant materials. There has been a considerable interest by the industry and a growing trend in consumer preferences for natural antioxidants over synthetic compounds. Additionally, elimination of synthetic antioxidants in food applications has given more impetus to explore natural source of antioxidants. Thus, antioxidants are of interest to both food scientists and health professionals. It should be further stressed that there has been a convergence of interest among researchers in these fields as the role of antioxidants in the diet and their impact on human health cannot be overemphasized.

In this present study, the antioxidant activity and nutritional importance of the extracts of *Pleurotus pulmonarius, P. cornucopiae, P. sapidus,* and *P. ostreatus* fruit bodies were assayed through various in vitro models.

MATERIALS AND METHODS

Spawn Production

Four different strains of *Pleurotus* species were isolated from their wild fruit body, characterized to the species level and registered at the GenBank database. The obtained germplasts of the four strains were grown into spawn in paddy grain. Paddy grains (100g/bottle) were washed four times, boiled for 45minutes and dried. The dried grains were mixed with 1%w/w of Calcium carbonate ($CaCO_3$), dispensed in bottles and sterilized at 121^{O}C for 30 minutes. The sterile grains were inoculated with 6 plugs (6 mm) of actively growing culture, incubated at 23 ± 2^{O}C and mycelia running were recorded three days interval. The following parameters were determined; rate of mycelia ramification (RT), ramification days (RD), weight of ramified mycelia (WMR) and spawn productivity (PDT) (Adebayo *et al*., 2013).

Sporophore/fruit body production (sawdust as substrate).

The materials are Sawdust (50kg), NPK fertilizer (2.5kg), Calcium Carbonate (0.5kg) and Rice bran (1kg). The above mentioned materials except rice bran were mixed and adjusted to 60- 65% moisture content. The properly mixed substrates were piled up in co-shape to about 1meter high, for heat generation. The heap was turned everyday for 30days to allow evenly distribution of the generated heat for lignin decomposition. After 30 days, the rice bran was mixed with the fermented substrate and packed inside heat resistant polytene bags (1000g/bag) and sterilized at 121^{O}C for 30 minutes. The sterile bags were inoculated with the spawn (handful grains spawn) and incubated in the dark at 25± 3^{O}C, until fully colonized. The colonized bags were transferred into mushroom house for fruit body production. The following data were taken; Mycelia running, primodia formation day, weight of harvested fruit bodies per flush, biological efficiency and production rate. Biological efficiency (BE) was determined by Fresh weight of mushroom harvested x100/Dry weight of substrate used and Production rate (PR) was determined by %biological efficiency / Days elapsed between inoculation and final harvest (Adebayo *et al*., 2013).

Antioxidant Determination using DPPH

This is the most widely reported method for the screening of antioxidant activity of many plant drugs. DPPH assay method is based on the reduction of methanolic solution of coloured free radical DPPH by free radical scavenger (**Vani *et al.,* 1997**). The procedure involves measurement of decrease in absorbance of DPPH at its absorption maximum of 517 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. The hydrogen atom or electron donation abilities of the corresponding extracts were measured from the bleaching of the purple-coloured methanol solution of 1, 1-diphenly-2-picrylhydrazyl (DPPH). One thousand microlitres of 5mg/l and 10mg/l of the methanol extracts were added to 4 ml of 0.004% methanol solution of DPPH. After 30 minutes incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical by DPPH in percent (I%) was calculated in the following way: $\mathrm{I}(\%) = [\![(A\ blank - A\ sample¦\ \mathrm{A\ blank}) \times 100]\!]$, where A blank is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC50) was calculated from the graph plotted inhibition percentage against extract concentration (**Gezer *et al*., 2006**).

Antioxidant assay using β-carotene-linoleate model system

This is one of the rapid methods for screening antioxidants. It is mainly based on the principle that Linoleic acid, which is an unsaturated fatty acid, gets oxidized by "Reactive Oxygen Species" (ROS) produced by oxygenated water. The products formed will initiate the β-carotene oxidation, which will lead to discoloration. The antioxidant capacity of the methanol extracts was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation. A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade) and 25 µl linoleic acid and 200 mg Tween 20 were added. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml distilled water saturated with oxygen (100 ml/ 30 minutes) was added with vigorous shaking. Four thousand microlitres of this reaction mixture were dispensed into test tubes and 200 µl portions of the extracts, prepared at 2 mg/l and 5mg/l concentrations, were added and the emulsion system was incubated for 2 h at 50^{O}C temperature. The same procedure was repeated with synthetic antioxidant BHA, α-tocopherol, as positive control, and a blank. After this incubation period, absorbances of the mixtures were measured at 490 nm. Antioxidative capacities of the extracts were compared with those of BHA, α-tocopherol and blank (**Gezer *et al.,* 2006**).

Determination of total phenolics

The concentration of phenolic compounds in the extracts was determined as described by **Jayaprakasha *et al*. (2001)** and results were expressed as tannic acids equivalents. The extracts were dissolved in a mixture of methanol and water (6:4 v/v). Samples (0.2 ml) were mixed with 1.0 ml of tenfold diluted Folin–Ciocalteu reagents and 0.8 mL of 7.5% sodium carbonate solution. After standing for 30 minutes at room temperature, the absorbance was measured at 765 nm. The estimation of phenolic compounds in the fractions was carried out in triplicate and the results were averaged.

Determination of total flavonoid concentration

Flavonoid concentration was determined as follows: mushroom methanolic extracts solution (1 ml) was diluted with 4.3 ml of 80% aqueous methanol and 0.1 ml of 10% aluminum nitrate and 0.1 ml of 1 M aqueous potassium acetate were added. After 40 minutes at room temperature, the absorbance was determined spectrophotometrically at 415 nm. Total flavonoid concentration was calculated using quercetin as standard (**Park *et al.,* 1997**).

Evaluation of antioxidant capacity by phosphomolybdenum method

The total antioxidant capacity of methanol extracts of the mushrooms was evaluated by the method of **Prieto *et al.* (1999)**. An aliquot of 0.1 ml of sample solution (1mg/ml and 5mg/ml) was combined with 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95^{O}C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions as rest of the sample. For samples of unknown composition, water soluble antioxidant capacity was expressed as equivalents of ascorbic acid (lmole/g of extract).

Phytochemical Screening

The phytochemical analyses of the mushroom extracts were carried out following the methods of **Adebayo and Ishola (2009)**, and the following phytochemicals were evaluated:

Alkaloids

About 0.2 g of extract was warmed with 1% of aqueous hydrochloric acid for two minutes. The mixtures were filtered and few drops of Dragendorff's reagent were added. A reddish-brown colour and turbidity with the reagent indicated the presence of alkaloids.

Flavonoids

Small quantities (2 g) of the extracts were dissolved in10% of sodium hydroxide (NaOH) and Hydrochloric acid (HCl). A yellow solution that turned colourless on addition of HCl indicated the presence of flavonoids.

Anthraquinones

5 g of the extracts was shaken with 10 ml of benzene. The solution was filtered and 5 ml of 10% NH_4OH solution was added to the filtrate. A pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of anthraquinones.

Glycosides

The test method is referred to as Lieberman's test. A small quantity of the extracts was dissolved in 2 ml of acetic anhydride and cooled in ice. Sulphuric acid (conc.) was then carefully added. The colour change from violet to blue to green indicated the presence of a steroidal nucleus (that is algycone portion of the cardiac glycoside).

Tannins

5 mg of the powdered extracts was stirred with 10 ml of hot distilled water, filtered and ferric chloride was added to the filtrate and observed for blue-black, blue-green or green precipitate.

Steroids

The test for steroids was done by the Lieberman acid test. A portion of the extract was treated with drops of acetic anhydride. Concentrated H_2SO_4 was carefully

added to the side of the test tube. The presence of a brown ring at the boundary of the mixture was taken as positive result.

Saponins

0.1 g of the powdered extract was boiled in 10 ml of distilled water for 5 minutes and decanted while still hot. The filtrate was used for the following tests:

(a) Frothing test: 1 ml of filtrate was diluted with 4 ml of distilled water and mixture was shaken vigorously and observed for persistent foam which lasted for at least 15 minutes.

(b) Emulsion test: This was performed by adding 2 drops of olive oil to the frothing solution and shaken vigorously. Formation of an emulsion indicated a positive test.

Phlobatannins

Deposition of a red precipitate when an aqueous extract of the mushroom was boiled with 1% aqueous hydrochloric acid indicated the presence of phlobatannins.

Proximate Analysis

The major different nutrients analyzed are dry matter, moisture content, ash content, fat content, crude fibre, total carbohydrate, crude protein following the method of **Thimmaiah (1999)**. Mineral contents such as sulphur, phosphorus, calcium, magnesium, potassium, iron, sodium were also determined by Thimmaiah method **(Thimmaiah, 1999)** except the fat content which was determined using Fat extractor machine SOCS PLUS (SCS 6: PELICAN EQUIPMENT) .

RESULTS AND DISCUSSION

Mycelia and Sporophore Production

The isolated *Pleurotus* strains were characterized and registered with the following accession numbers; *Pleurotus pulmonarius* (LAU 09- JF736658), *P. cornucopiae* (NE 02- JF736662), *P. sapidus* (NE07- JF736664), and *P. ostreatus* (LAU10- JF736659). The highest percentage spawn productivity (28.33%) was obtained in NE 02, while the lowest value of 23.33% obtained in NE 07 (Table 1). The highest total weight of fresh mushroom (580g) (Table 2), highest biological efficiency (59.10±12.41%) and production rate (1.85±0.65%) were produced in LAU 09 (Table 3). The BE and PR values by **Vogel and Salmones (2000)** and **Philippoussis *et al.* (2001)** were lower than reported values in the present study.

Table 1 Spawn ramification rates (cm/day) of *Pleurotus* species

Strains	RT (cm/d)	RD (day)	WMR (g)	PDT (%)
LAU 09	0.412±0.19^{a}	16	4.900±0.11^{cd}	30.62±0.72^{f}
LAU 10	0.425±0.10^{a}	16	3.700±0.17^{e}	23.12±1.08^{g}
NE 02	0.359±0.03^{b}	16	5.100±0.17^{c}	31.87±1.08^{g}
NE 07	0.437±0.02^{a}	16	4.200±0.15^{cd}	26.25±0.95^{g}

The mean values on the same column followed by different superscript are significantly at 0.05 probability level according to Duncan Multiple test
RT = ramification rate, RD = ramification days,
WMR = weight of mycelia ramification,
PDT = spawn productivity

Table 2 Distribution of the total fresh mushroom production (g) over three harvests by *Pleurotus* species on saw dust

Strains	IP	PP	1st Harvst (g) (%)	2ndHarvest(g) (%)	3rdHarvest(g) (%)	Total weight (g)
LAU 09	15	31	343(59.14)	196(33.79)	41(7.07)	580
LAU 10	17	37	121(32.97)	140(38.15)	106(28.88)	367
NE 02	15	34	117(32.68)	132(36.87)	109(30.45)	358
NE 07	19	42	108(32.97)	102(30.91)	120(36.36)	330

IP= Incubation period (required days for formation of primordia), PP= Production period, starting with the formation of primordia till third harvest, Weight of mushrooms obtained in 5 replicates, Percent production for the strains during each harvest

Table 3 Biological efficiency (%) and Production rate (%) of *Pleurotus* species

Strain	BE(%)	PR(%)
LAU 09	59.10±12.41^{a}	1.85±0.65^{a}
LAU 10	36.70±11.25^{b}	0.97±0.76^{b}
NE 02	35.80±6.0^{b}	1.05±0.45^{ab}
NE 07	33.00±7.93^{b}	0.79±0.04^{b}

The mean values on the same column followed by different superscript are significantly at 0.05 probability level according to Duncan Multiple test
BE= Biological Efficiency (Productivity) of the each strain
PR= Produc tion Rate of the each strain

Antioxidant Analyses of dried fruiting body

The radical scavenging effect of the methanolic extracts of the strains by DPPH method is shown in Figure 1. All the strains at different concentrations of the extracts studied showed free radical scavenging activity. In the present study, the extracts of mushrooms powder were able to decolourize DPPH and the free radical scavenging potentials of the extracts were found to be in the order of LAU 09 > NE 07 > LAU 10>NE 02. The free radical scavenging activity of the *Pleurotus* species evaluated using β-carotene-linoleatemodel system (% inhibition of bleaching of β- carotene) was shown in Figure 2. Linoleic acid oxidation was compared with those of *Pleurotus species* extract, α -tocopherol and BHA. It was found that inhibition values of *Pleurotus* species extract and the standards increased with concentrations except NE 07. For example, in 2mg/ml concentration, *Pleurotus* species extract showed (63%-LAU 09, 65%- LAU 10 and 67%- NE 02), BHA (75%) and α-tocopherol (77%) of inhibition, whereas in 5mg/ml concentrations the values were (80%-LAU 09, 84%- LAU 10 and 93%- NE 02), BHA (89%) and α-tocopherol (90%) of inhibition. The total phenolic compound amount was calculated as quite high for *Pleurotus* species extracts (75% as tannic acid equivalent) (Figure 3). The highest total flavonoid compound concentration was measured as 12.20 µg mg-1 quercetin equivalent while the lowest value was 10.30 µg mg-1 quercetin equivalents (Figure 4). The antioxidant capacity of the extracts was measured spectrophotometrically through phosphomolybdenum method, which is based on the oxidation of Mo (IV) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 695 nm. The antioxidant capacity of the extracts of strains was found to be in this order, LAU 09 > LAU 10>NE 02>NE 07 (Figure 5). The essence of DPPH method is that the anti-oxidants react with the stable free radical i.e., α,α-diphenyl-β-picrylhydrazyl (deep violet colour) and convert it to α,α-diphenyl-β-picrylhydrazine with decolouration. The degree of decolouration indicates the scavenging potentials of the sample antioxidant. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, polyhydroxy aromatic compounds (hydroquinone, pyrogallol, etc.), and aromatic amines (p-phenylene diamine, p-aminophenol etc.), reduce and decolourize α,α -diphenyl-β-picrylhydrazyl by their hydrogen donating ability. It appears that the extracts from the *Pleurotus* species evaluated in this study possess hydrogen donating capabilities to act as antioxidant. According to this study, it is possible that the high inhibition value of *Pleurotus* species is due to the high concentration of phenolic compounds (**Gezer *et al.*, 2006**). The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports (**Moller *et al.*, 1999**). Polyphenolic compounds have an important role in stabilizing lipid oxidation and are associated with antioxidant activity (**Gulcin-Buykokuroglu *et al*., 2003**). The phenolic compounds may contribute directly to antioxidative action. It is observed that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily ingested from a diet rich in fruits and vegetables.

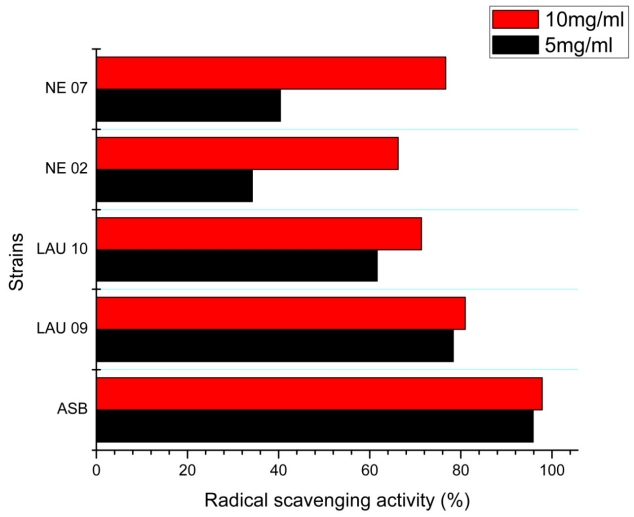

Figure 1 Radical scavenging activity of the *Pleurotus* species and Ascobic acid by DPPH method - ASB: Ascorbic acid

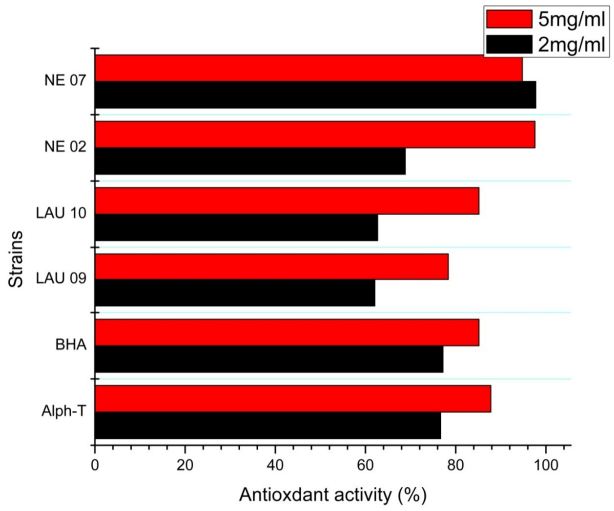

Figure 2 Antioxidant Activity of *Pleurotus* species, BHA and α- tocopherol by β-carotene-linoleate model system (%inhibition of bleaching of β- carotene)

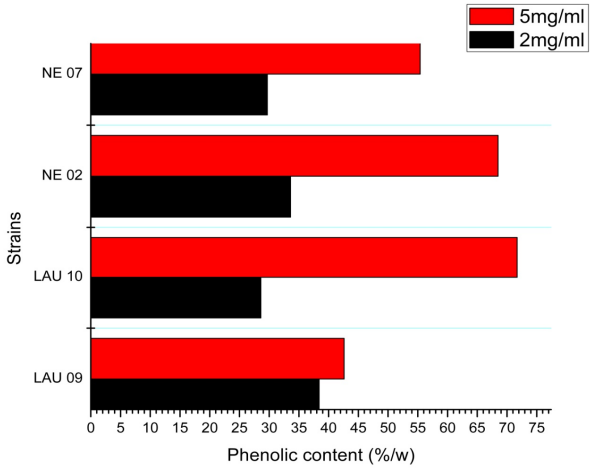

Figure 3 Phenolic content (as tannic acid equivalent) of *Pleurotus* species

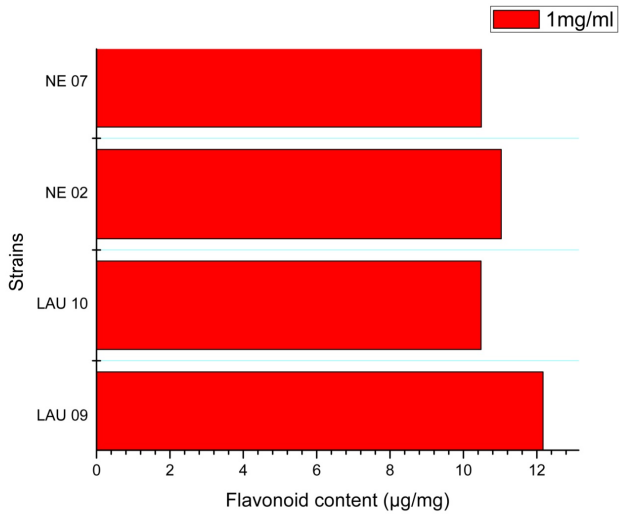

Figure 4 Total flavonoid content (as quercetin equivalent) of *Pleurotus* species

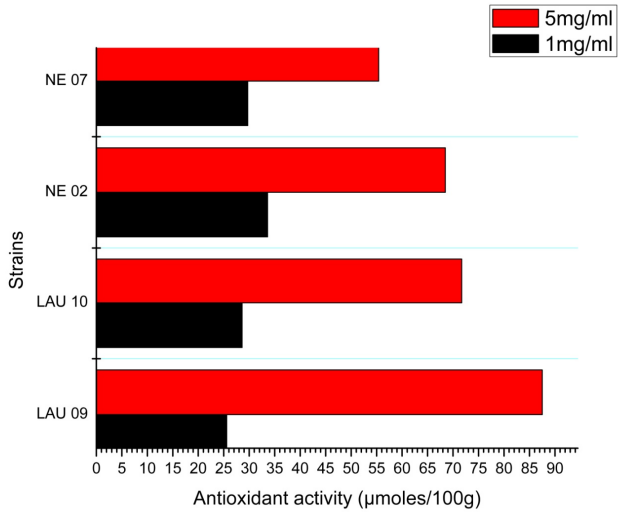

Figure 5 Antioxidant capacity (as equivalent to Ascobic acid) of the *Pleurotus* species by phosphomolybdenum method

Phytochemical assay of dried Fruiting body

The phytochemical screening of the mushroom extract revealed the presence of alkaloids, saponins, steroids, phlobatannins, flavonoids, and anthraquinones as shown in Table 4. Over 4000 structurally unique flavonoids, i.e., diphenylpropanes, have already been identified in plant sources. These low molecular weight substances are phenylbenzopyrones (phenylchromones) with an assortment of structures based on a common three-ring nucleus cconsisted of two benzene rings (A and B) linked through a heterocyclic pyran or pyrone (with a double bond) ring in the middle. Some flavonoids have been found to possess antilipoperoxidant (*2* antitumoral , antiplatelet, antiischemic, antiallergic, and anti-inflammatory activities (**Kim *et al.*, 2006**). Alkaloids have been reported as a group of basic organic substances of plant and microbial origin, containing at least one nitrogen atom in a ring structure in the molecule. The first microbial alkaloids to be recognized and studied were those of *Claviceps purpurea*, the agent causing ergot of rye. These alkaloids can be isolated from the sclerotia formed after infection of the ovaries of the plant by *Claviceps ascospores* or *conidia*. Besides the sclerotia of *Claviceps*, other fungi and several higher plants are known to contain, *ergot alkaloids*. The presence of saponins, tannins, alkaloids and steroids in the extract is an indication that the strains are of pharmacological importance (**Hostettmann and Marston, 1995**). Glucosinolates and their isothiocyanate hydrolysis products are well-known protectors against carcinogenesis. The relatively large consumption of glucosinolates by many individuals, in comparison with other plants as potential sources of chemoprotective activity, adds special significance to these compounds. Glucosinolates are β-thioglucoside *N*-hydroxysulfates. They are the precursors of isothiocyanates (mustard oils) (**Fahey *et al.*, 1997**).

Table 4 Phytochemicals of the dried powder of *Pleurotus* species

Phytochemicals	LAU 09	LAU 10	NE 02	NE 07
Alkaloid	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Steroids	+	+	+	+
Cardiac	+	-	+	+
Glycosides	-	-	+	-
Phlobatannins	+	+	+	+
Anthraquinones	+	+	-	+
Tannins	+	+	+	+

Legend: +, present; -, absent.

Nutrition Composition of dried Fruiting body

The nutrition composition analyses gave higher content of protein (34.01%) and carbohydrate (9.67%/100mg). In the study, the highest yield of fat content (3.75±0.46%), ash content (13.96±3.02%) by LAU 09 and crude fibre (8.71±1.06%) contents with appreciable yield of crude fibre content (8.71±1.06%) were obtained (Table 5). The present study indicates a much higher protein in the fruit body than that reported by **Akindahunsi and Oyetayo (2006)**. This protein content compares well with that of several leguminous seeds. This is an indication that fruit bodies of *Pleurotus* species could serve as a suitable meat substitute. Protein evaluation has shown that mushroom proteins have a higher quality than green leafy vegetables (**Chan, 1981**). The average carbohydrate yield of 9.67%/100mg is also higher than 56.2 g/100g (**Akindahunsi and Oyetayo, 2006)**. A considerable fraction of this carbohydrate is contributed by the presence of oligosaccharides. Several health benefits have been attributed to mushroom oligosaccharides including immunomodulatory effects which have been shown to be beneficial to individuals living with HIV (**Jong *et al.,* 1991**). It has been shown in the study that, the highest yield of fat content (3.75±0.46%), ash content (13.96±3.02%) and crude fibre (8.71±1.06%) obtained in LAU 09 agreed with the previous findings of Akindahunsi and Oyetayo **(2006)**. The present finding gave appreciable yield of crude fibre content (8.71±1.06%), an indication that incorporating the *Pleurotus* species in diet could aid bowel movement as well as reduce the incidence of colon cancers in its users. Epidemiological studies have found an association between high fibre diets and a lower incidence of cardiovascular diseases and large bowel cancers **(Honda *et al.,* 1999)**.

Table 5 The nutrient composition of *Pleurotus* species

Strains	DM[a] (%)	MC[b] (%)	AC[c] (%)	FC[d] (%)	CF[e] (%)	CC[f] (%)	CP[g] (%)
LAU 09	92.40±7.61	7.60±0.61	11.70±0.71	3.75±0.46	8.15±1.25	8.08±0.81	27.08±3.01
LAU 10	91.29±9.10	8.71±1.43	13.96±3.02	2.77±0.46	7.67±1.63	5.89±0.84	21.52±2.51
NE 02	91.64±8.40	8.36±1.38	11.61±1.48	2.01±078	8.71±1.06	9.67±1.41	34.01±3.06
NE 07	89.71±5.63	10.29±2.04	12.06±2.51	3.10±0.84	6.95±1.03	9.21±1.38	17.77±1.76

*Values are mean ± S.D:
[a]Dry matter, [b]Moisture content,[c]Ash content,[d]Fat content,[e] Crude fibre,[f] Crude carbonhydrate [g] Crude protein (N X 4.38).

Mineral Composition of dried Fruiting body

The mean of obtained Zn value ranges from 8.91±1.7mg/kg (NE 07) to 12.41±2.4mg/kg (LAU 10), Phosphorus content ranged from 86.67±14.5mg/kg (LAU 09) to 105.51±5.8mg/kg (Table 6). There are wide variations in the content of Mg present in the different species of *Pleurotus* with highest value of 103.21±10.3mg/kg (LAU 10), while the lowest value of 56.91±4.9mg/kg was obtained by LAU 09 (Table 6). The Ca levels obtained range from 55.99±4.3mg/kg (NE 07) to 81.16±5.7mg/kg (mg/kg) (NE 02). Mineral composition analyses indicate that the fruit body is rich in mineral content. The mean of obtained Zn value ranges from 8.91±1.7mg/kg (NE 07) to 12.41±2.4mg/kg (LAU 10), this agrees with **Kikuchi *et al.* (1984)** previous work , which reported that Zn values of *Pleurotus* species ranges between 9.31 and 11.81mg/kg. In the current research, Zn values of *Pleurotus* species obtained were similar with the obtainable ones from the litrature. Research and Development Initiative (RDI) of Zn is 15mg for adults and 3-5mg for babies (**Sencer, 1983**). Phosphorus content of *Pleurotus* species obtained in this study ranged from 86.67±14.5mg/kg (LAU 09) to 105.51±5.8mg/kg. This is less than the values reported by **Caglarirmak (2007)**, which reported the P values of *P. ostreatus* and *P. sajor-caju* as 998.47 and 716.31 mg/ kg respectively. The fruit bodies from *Pleurotus* species can contribute to human nutrition for P intake, since recommended daily intake of P is 0.7g (**Demicri, 2006**). The Mg values obtained in this study are similar with the previous report by **Manzi *et al.* (1999)** and **Mattila, *et al* (2001)**. Mg contents of these mushrooms have nutritive value for human health. The Ca contents determined in this research were generally in accordance with previous studies **(Kikuchi *et al.*, 1984 ; Manzi *et al.*, 1999)**.
Also, the amount of K and Na obtained are in accordance with the previous work by **Kikuchi *et al.* (1984)** and **Mattila *et al.* (2001)**. There is a good balance between high content of K and low content of Na for treating high blood pressure. The results of this research work have shown nutritive potential of the *Pleurotus* species. Futhermore, It is an evidence that *Pleurotus* species could be of medicinal and nutritional benefit.

Table 6 The mineral composition (mg/kg) of *Pleurotus* species

Strains	Zn	P	K	Mg	Fe	Na	Ca
LAU 09	10.36±0.7	86.67±14.5	69.56±6.7	56.91±4.9	8.06±1.9	74.71±5.5	56.91±2.9
LAU 10	12.41±2.4	105.51±5.8	110.56±16.0	103.21±10.3	8.41±2.0	76.06±3.9	60.71±5.1
NE 02	11.31±2.2	151.31±9.7	120.00±18.1	96.37±6.4	6.04±0.9	68.36±4.5	81.16±5.7
NE 07	8.91±1.7	96.93±7.3	93.31±9.4	89.61±5.8	6.71±1.0	89.93±5.1	55.99±4.3

*Values are mean ± S.D:
Zn: Zinc, P: Phosphorus, K: Potassium, Mg: Magnesium, Fe: Iron, Na: Sodium, Ca: Calcium.

CONCLUSION

The obtained results from the present study ; the high antioxidant potential, high nutrients and minerals composition and presence of phytochemicals are evidences to show that these four edible mushrooms strains (*Pleurotus pulmonarius, P. cornucopiae, P. sapidus,* and *P. ostreatus)* hold tremendous promise in complementing the human diet. Consumption of these mushrooms products could be nutritionally and medicinally beneficial to human considering their potent antioxidant nature. Owing to the nutritional and medicinal importance of the *Pleurotus* species as reported in this study, the cultivation of these mushrooms must be encouraged among the populace.

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