International Journal of Biomedical and Pharmaceutical Sciences ©2008 Global Science Books

Phytochemical Constituents and Antioxidant Activity of the Fruit-bodies of *Pleurotus ostreatus* var *Florida* Eger. Grown on Different Substrates and Substrate Supplementations

Ikechukwuka Cyriacus Okwulehie* • **Ikechukwu Adiele Okwujiako**

Department of Biological Sciences, Michael Okpara University of Agriculture Umudike, P.M.B. 7267, Umuahia, Abia State, Nigeria

Corresponding author: * **phylyke@yahoo.com**

ABSTRACT

The phytochemical constituents of the fruit-bodies of *Pleurotus ostreatus* var. *florida* were investigated to determine whether they are influenced by the substrates and the level of supplementation of organic manure in the substrates. The mushroom's phytochemical constituents were significantly influenced by the substrate and additive levels. Straw with 10% cow dung produced mushrooms with 0.05% alkaloids while those from the control straw contained only 0.02%. Fruit-bodies from straw with 5 or 10% poultry droppings and 5 or 10% turkey droppings yielded significantly more flavonoids than control mushrooms. The phenolic content of the fruit-bodies produced on unsupplemented *Andropogon* and *Pennisetum* straws were significantly less than those produced on straws supplemented with 5 and 10% levels of any manure ($P < 0.05$). The saponin and tannin contents of the fruit-bodies showed a trend: high level of supplementation significantly ($P < 0.05$) suppressed the quantity of both bioactive compounds. On the contrary, the 5 and 10% levels of supplementation with cow and poultry manures did not significantly influence the tannin contents, but the addition of manures in general increased the tannin contents of the fruit-bodies. Similarly, the antioxidant activity of the mushrooms produced on different substrates was investigated. The fruit-bodies from the three substrates exhibited varying degrees of activity. Those from *Andropogon* straw showed the highest activity and rice and *Pennisetum* straws had similar activity. The results of the investigations are discussed in relation to the establishment of the medicinal potentials of and domestication of *P. ostreatus* var. *florida* in commercial quantities for local consumption.

Keywords: alkaloid, flavonoid, mushroom, radical scavenging activity, saponin, tannin
Abbreviations: AS, *Andropogon* straw, **CD**, cow dung, **DPPH**, 2, 2-diphenyl-1-picrylhydazyl; **MEA**, malt-extract agar; **PD**, poultry droppings; **PDA**, potato dextrose agar, **TD**, turkey dropping

INTRODUCTION

Mushrooms spontaneously appear in forests and farm lands in great quantities after rain. Their gay colouration, short life span and their edible or poisonous attributes were and still are distinguishing features indicating the age-long uses of mushrooms as food, medicine and in culture (Mirko 1976; Lucas *et al.* 1957; Suzuki and Oshima 1976; Rambelli and Menini 1983; Stamets 1993; Aletor 1995; Fasidi 1996). According to Chang (1980), mushrooms are widely utilized as human food. They are eaten raw, in soups, fried and salted. They have been part of the human diet for a long time, being regarded as epicurean delicacy (Rambelli and Menin 1983), as used in soups, as meat supplement, as seasoning as well as flavouring. An array of edible mushrooms exists. Out of about 100,000 fleshy fungi known, about 150 species are edible and serve as food. Many edible mushrooms grow in Nigerian forests and farmlands. Zoberi (1979) identified about 25 good quality edible mushroom species in Nigeria. There are also mushrooms considered to have medicinal properties. Such mushrooms have been used by primitive people for healing purposes, often accompanied by tribal and magical rites. Some of the mushrooms have antibacterial and antiviral effects while some can be used to cure cancer and cardinal problems (Rambelli and Menini 1983). Studies by Gunde-Cimerman and Cimerman (1995) show that *Pleurotus ostreatus* and other closely related species naturally produce Lovastatin® (3-hydroxy-3-methylglutaryl-coenzyme A reductase), which is a drug used for treating excessive blood cholesterol. *Polyporus umbellatus* has been heralded to possess potent anti-cancer and immunopotentiating properties (Stamets 1993). Some mushrooms have anti-tumoral characteristics (Cochran 1978). The history of the medicinal implications of mushrooms dates back thousands of years, when primitive people used mushrooms for healing purposes, often accompanied by incantations and other tribal and magical rites (Rambelli and Menin 1983). According to these authors mushrooms still appear in the traditional pharmacopoeia of tropical Africa and many other countries. Although their medicinal uses have long been known, their consideration in orthodox medicine in the treatment of infections diseases is recent. Some mushrooms have anti-bacterial properties, e.g. *Trichoderma lianorum* is used against the chestnut ink disease and also in biological control programmes. *Flammulina velutipes*, *A. bisporus*, *Lentinus edodes* and *Coprinus comatus* have been reported by Rambelli and Menini (1983) to have antibiotic properties. Mushrooms have also been studied as potential metabolite producers. The metabolites are reported to be able to inhibit and treat cancer. Calvacin extracted from the sclerotium of *Calvatia gigantea* has been implicated in the treatment of cancer (Rambelli and Menini 1983). French cultivators of *A. bisporus* have cancer immunity because of their regular consumption of mushrooms. Recent studies of *Hericium* sp. have shown that polysaccharides in mushrooms inhibit a variety of cancers by enhancing the host's immune functions (Kang *et al*. 2002). It has also been suggested that the phenol-analogous compounds hericenone-C, -D and -E, which induced the synthesis of nerve growth factor, might be effective in treating patients suffering from Alzheimer's disease (Kawagishi *et al.* 1990). Antitumoural effects of mushrooms have also been reported

Received: 13 June, 2008. Accepted: 14 December, 2008.

Research Note

(Bushwell and Chang 1993). Mushroom species reputed to be antitumoural included *F. velutipes*, *L. edodes*, *P. ostreatus*, and *A. auricular* Judeae. (Rambelli and Menini 1983). Conchran (1978) investigated some substances such as Lentinan, a polysaccharide extracted from *L. edodes*, flammulin extracted from *F. velutipes* and poricin, a protein extract of *Poria corticola* including a cardiotoxic protein volvatoxin and flammutoxin, from *V. volvacea* and *F. velutipes* respectively, and found them to inhibit respiration of certain tumour cells. Viral-disease preventive properties have been discovered in *Grifola frondosa* (Jong and Birmingham 1990), *Boletus frostii*, *Calvatia gigantean* and *A. bisporus*. *Boletus edulis*, *C. gigantean*, *Suillus leteus* and *L. edodes* seem to hinder viruses in human body (Buswell and Chang 1993; Rambelli and Menini 1983). Mushrooms also have hypocholesterolemic effects; for instance, dried and ground fruiting bodies of *L. edodes* has been found to reduce the cholesterol level by 24% in mice fed on the diet for 10 weeks (Bushwell and Chang 1993). Mushrooms are considered as aphrodisiacs and hence could fend off old age (Bushwell and Chang 1993). The medicinal attributes of mushrooms could be as a result of the myco-chemical constituents of their fruit-bodies. Okwulehie and Odunze (2004b) reported the presence of such compounds as alkaloids, flavonoids saponnins, and tannins in *Pleurotus* mushrooms. The present work investigates the influence of different cereal straws and straw supplementations with levels of organic manures on the presence and quantity of some of the phytochemicals compounds in the fruit-bodies of *P. ostreatus* var. *florida.* Free radicals damage tissues and contribute to ageing and degenerate disease (Mitchell 2003). Antioxidants inactivate free radicals but also inactivate themselves in the process. For this reason a regular supply of antioxidants is needed. Jaworska *et al.* (2007) studied the antioxidant activity of frozen and canned *Boletus edulis*, claiming stronger antioxidant activity in the former. Lakshmi *et al.* (2004) evaluated the antioxidant activity of four Indian mushrooms, including *Pleurotus florida* and *P. sajucaju*, and noted that all four mushrooms showed significant antioxidant activity. The free radical scavenging properties and phenolic content of extracts from pickled edible mushroom *Agaricus bisporus* were also evaluated by Ganguli *et al.* (2006). Both antioxidant activity and total phenolics were detected in the mushroom and these decreased when the mushrooms were fried in mustard oil. In this paper we attempt to analyze the fruit-bodies of *Pleurotus ostreatus* var. *florida* for phytochemical compounds in order to evaluate its medicinal potentials, and also to investigate its free radical scavenging properties.

MATERIALS AND METHODS

Materials

The original culture of *Pleurotus ostreatus* var. *florida* used for the investigation was supplied by Dr. I. A. Okwujiako, a mushroom scientist in the Department of Biological Sciences, Michael Okpara University of Agriculture, Umudike. The mushroom culture was sub-cultured and maintained on Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA) in sterile Petri dishes. The dishes were wrapped in aluminum foil and stored in the refrigerator and used for the investigation when required. The pure culture was bulked up in sorghum grains, and the resulting spawn was used to inoculate the different substrates and the levels of supplementation. The fruit-bodies of the mushroom produced from the different substrates were screened or the presence of the phytochemical constituents; alkaloids, flavonoids, phenols, saponins and tannin.

Test for alkaloids (Harbone 1973)

About 2 g of the dry powdered sample of the mushroom was placed into a 100 ml conical flask, containing 2 ml of 5% H_2SO_4 in ethanol. The mixture was heated to boiling in a water bath, left to cool and then tested for the presence of alkaloids. To test for the alkaloids, 2 drops of Mayer's reagent was added to the mixture in a test tube and observed for yellow precipitate. Similarly, 2 drops of Wagner's reagent was added to the mixture in another test tube, and a colour change to reddish-brown precipitate confirmed the presence of alkaloids.

Test for flavonoids (Harborne 1973)

To test for the presence of flavonoids in the fruit-bodies of the mushroom, 5 ml of dilute ammonia solution was added to 5 ml of aqueous filtrate of each sample. To this mixture, about 2 drops of H_2SO_4 was added and observed for yellow colouration which would disappear on storage.

Test for phenols (Harborne 1988)

To detect the presence of phenols, 2 g of the powdered sample was mixed with 20 ml of tetraoxosulphate (VI) acid (H_2SO_4) in ethanol and heated for 5 min. Filtrate of the heated mixture (1 ml) and 2 drops of neutral ferric chloride were mixed to observe green, blue or black colouration.

Test of saponins (Harborne 1973)

The presence of saponins in the mushroom fruit bodies was tested by boiling 2 g of the dry powdered sample in 20 ml of distilled water in a water bath. After cooling, the boiled mixture was filtered. Filtrate (10 ml) was mixed with 5 ml distilled water and shaken vigorously for a stable froth. Three drops of olive oil were added to the frothing solution, and the formation of an emulsion confirmed the presence of saponins.

Test for tannins (Pearson 1976)

Dry powdered sample (0.5 g) was boiled in 20 ml distilled water in a water bath. On cooling, a drop of ferric chloride was added and observed for a brownish green or a blue-black colouration.

Determination of the amount of the phytochemicals in the mushroom fruit bodies

Determination of alkaloids

About 5 g of the dry powdered sample was used to determine the alkaloids contents of the mushroom following the method of Harborne (1973). The alkaloids were expressed as percentage as:

$$\% \text{ alkaloids} = \frac{\text{weight of residue}}{\text{weight of sample}} \times 100$$

Determination of flavonoids

The determination of flavonoids in the mushroom dry samples was done following the methods of Boham and Kocipai (1974). The flavonoid content was expressed as a percentage as:

$$\% \text{ flavonoids} = \frac{\text{weight of residue}}{\text{weight of sample}} \times 100$$

Determination of phenolic content

To determine the phenolic content of the powdered sample of the mushroom, a fat-free sample was used. About 2 g of the sample was defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 h. To extract the phenols component of the sample, the fat-free sample was boiled with 50 ml of ether for 15 min. Extract (5 ml) was pipetted into a 50 ml flask with 10 ml of distilled water, 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol. The mixture was made up to the mark and left to react for 30 min for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm. The percentage phenols were calculated (Harborne 1973; Obadoni and Ochuko 2001).

Determination of saponins

To determine the saponins content of fruit bodies, 5 g of the sample was dispersed in 30 ml of 75% ethanol. The extract was re-extracted with 100 ml of ethyl acetate. The organic solvent was then evaporated to dryness in a crucible over a hot water bath, while the residue in the solvent was dried in an electric oven to a constant weight (Peng *et al*. 1995).

The percentage saponins was calculated as:

$$\% \text{ saponins} = \frac{\text{weight of residue}}{\text{weight of sample}} \times 100$$

Determination of tannins

To determine the tannins component of the mushroom fruit bodies, 500 mg of the powdered sample was placed into a 150 ml plastic bottle, and 50 ml of distilled water was added. This was shaken in a mechanical shaker for 1 h. Then 5 ml of the filtrate was pipitted out into a tube and mixed with 3 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M of potassium ferrocyanide. The absorbance was measured using a spectrophotometer at 120 nm. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannic acid to get 100 ppm and measured (Van Buren and Robinson 1981).

Determination of anti-oxidant activity

The antioxidant activity of the fruit bodies was evaluated with 2, 2-diphenyl-1-picrylhydazyl (DPPH) essay. DPPH radical scavenging activity was measured using a free radical which shows a characteristic absorption at 517 nm (purple) (Velaskez *et al*. 2003). A test sample solution in methanol (0.75 ml) was added to 1.5 ml of 20 mg/ml DPPH methanol solution. After shaking, the mixture was incubated for 15 min in darkness at room temperature and the absorbance was measured at 517 nm. The difference in absorbance between test sample and control methanol was taken as activity. Quercetin served as a positive control.

Statistics

The readings obtained from the investigations were subjected to analysis of variance (ANOVA). Mean separation was carried out using the Least Significance Difference (LSD) at 5% level of significance.

RESULTS AND DISCUSSION

The myco-chemical composition of mature fruit-bodies of *P. ostreatus* var. *florida* produced on different substrates and additives are summarized in **Table 1** and **Fig. 1**. The mushrooms contain alkaloids, flavonoids, phenols, saponnins and tannins in varying quantities. The presence of these metabolites indicates their medicinal value since the presence of alkaloids, phenols and phenolic compounds in certain plants has been associated with their anti-microbial properties (Okwu 2001). Rodriguez and Hesses (2000) reported that phenolic compounds in *Spondias mombin* may be the rea-

Table 1 Presence of phytochemicals in *P. ostreatus* var. *florida* grown on different substrates.

Substrate	Alkaloids	Flavonoids	Phenols	Saponins	Tannins
Andropogon + 5% CD	+	+	+	+	+
Andropogon +105% CD	+	+	+	+	+
Andropogon + 5% PD	+	+	+	+	+
Andropogon +10% PD	+	+	+	+	+
Andropogon + 5%TD	+	+	+	+	+
Andropogon + 10% TD	+	+	+	+	+
Andropogon (alone) Control	+	+	+	+	+
Pennisetum alone	+	+	+	+	+

+ = present
- = absent

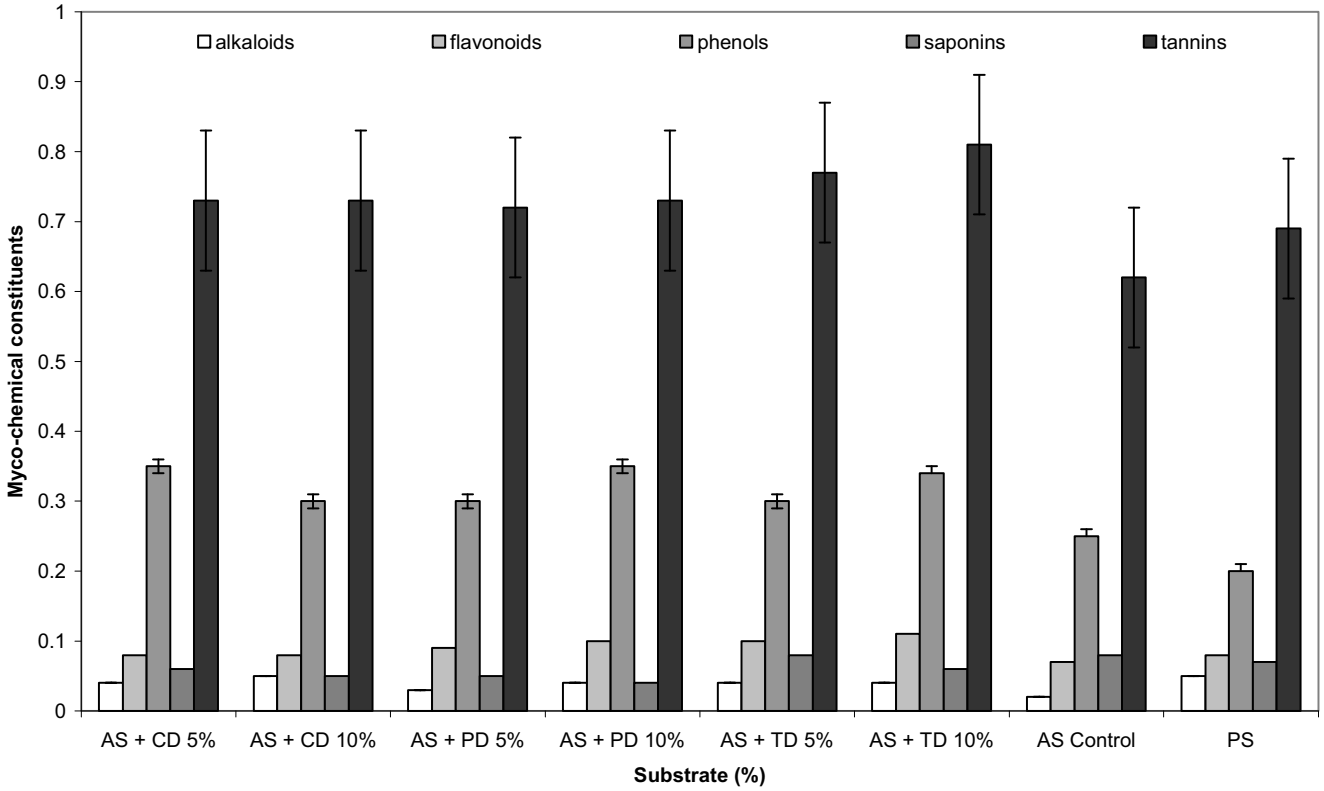

Fig. 1 Myco-chemical constituents *of P. ostreatus* var. *florida* on different substrates (relative %).

Table 2 Antioxidant activity of the fruit-bodies of *P. ostrestus* var *florida* grown on different substrates.

	Rice straw	*Pennisetum* straw	*Andropogon* straw
1.	0.649 ± 0.16	0.411 ± 0.08	0.605 ± 0.10
2.	0.352 ± 0.12	0.605 ± 0.14	0.404 ± 0.13
3.	0.381 ± 0.11	0.362 ± 0.08	0.437 ± 0.12
Mean ± SD	0.46 ± 0.163 a	0.46 ± 0.15 a	0.39 ± 0.06 a

son for its therapeutic, antiseptic anti-fungal and anti-bactericidal properties. The effects are also associated with flavonoids which, according to Godwin and Mercer (1972), are phenolic glycosides i.e. they exist *in vivo* as glycosides. Flavonoids have been associated with the flavouring properties of *P. tuber-regium* (Okwulehie and Odunze 2004b). They have been reported to show antioxidant activities and are implicated in combating carcinogenesis as well as the ageing process (Hilang and Ferraro 1992). Similarly, alkaloids were ranked as the most efficient therapeutically significant plant substance (Okwu and Okwu 2004). Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, anti-spasmodic and bactericidal effects (Stray 1998). Tannins, on the other hand, have been reported to exhibit astringent properties, hasten wound healing and healing of inflamed mucous membranes (Okwu and Okwu 2004).

The value of the metabolites in the fruit-bodies of *P. ostreatus* var. *florida* were significantly influenced by the substrate type and additive levels. The value of alkaloids varied from 0.02% in the fruit-bodies of the mushroom from the control experiment to 0.05% in those from the straw with 10% cow dung and un-supplemented *Pennisetum* straw; while *Andropogon* straw supplemented with 5 and 10% poultry droppings and 5 and 10% turkey droppings yielded more flavonoids than fruit-bodies from the control or other supplemented media. The phenolic contents of the fruit-bodies produced in the un-supplemented *Andropogon* and *Pennisetum* straws (0.25 and 0.20%, respectively) were significantly less than those produced on *Andropogon* straw supplemented with 5 and 10% of the organic manures (0.03-0.50%). This implies that the phenolic content of the fruit-bodies showed much variation depending on the type and level of organic manure. The levels of supplementation of the straws with organic manure appeared to influence the percentage of saponins in the fruit-bodies. Tannins content tended to follow the same trend as saponin content (**Fig. 1**).

The result of the anti oxidant activity of the mushroom fruit-bodies grown on the different substrates is presented in **Table 2**. The antioxidant activity was evaluated with the DPPH assay (Valeskez *et al.* 2003). The three samples showed varying degrees of antioxidant activity, with those from *Andropogon* straw showing the lowest activity (0.39 ± 0.06), and those from rice and *Pennisetum* straw having similar activity, but all significantly equal. Although the samples have antioxidant activity, none could be said to have a very strong activity when compared to quercetin standard (0.56). The moderate anti-oxidant activity could be attributed to natural antioxidant that could be present.

REFERENCES

Aletor VA (1995) Compositional studies on edible tropical species of mushroom. *Food Chemistry* **54**, 265-268

Bohm BA, Kocipal-Abyazan R (1994) Flavonoids and tannins from leaves of Hawaian *Vaccinium raticulatum* and *V. calycinium*. *Pacific Sciences* **48**, 458-463

Buswell JA, Chang ST (1993) Edible mushrooms: Attributes and applications. In: Chang ST, Buswell JA, Miles PG (Eds) *Genetic and Bearing of Edible Mushrooms*, Gordon and Breach, Philadelphia, pp 297-324

Chang ST (1980) Mushroom production in South East Asia. *Mushroom Newsletter Tropics* **1 (4)**, 5-10

Fasidi IO (1996) Studies on *Volvariella esculenta* (Muss) Singer. Cultivation on agricultural wastes, proximate composition of stored mushrooms. *Food Chemistry* **55 (2)**, 161-163

Godwin TW, Mercer EI (1972) *Introduction to Plant Biochemistry*, Pergamon Press, Oxford, 259 pp

Gunde-Cimera NG, Cimerman A (1995) *Pleurotus* fruiting bodies contain the inhibitor of 3-hydroxy-3 methyl-Coenzyme A. Reductase-Lovastatin. *Experimental Mycology* **19**, 1-6

Harborne JB (1973) *Phytochemical Methods*, Chapman and Hall, London, pp 89-131

Harborne JB (1988) *Phytochemical Methods*, Chapman and Hall, London, pp 11-113

Hilang MT, Ferraro E (1992) Phenolic compounds in food and their effects on health. In: Hilang M, Ho T, Lee CT (Eds) *Antioxidants and Cancer Prevention*, American Chemical Society (ACS) Symposium series 507, American Chemical Society, Washington D.C., pp 8-12

Jaworska G, Bernas E Biernacka A (2007) A comparism of antioxidant activity in frozen and canned *Boletus edulis* mushroom. *Polish Journal of Natural Sciences* **4 (Suppl.)**, 43-48

Jong SC, Birmingham JM (1993) Mushrooms as a source of natural flavour and aroma compounds. In: Chang ST, Buswell JA, Chiu S (Eds) *Mushroom Biology and Mushroom Products*, The Chinese University Press, Hong Kong, pp 345-366

Kang H, Hwang S, Lee H, Park W (2002) Effects of high concentrations of plant oils and fatty acids for mycelial growth and pinhead formation of *Hericium erinaceum*. *Transactions of the American Society of Agricultural Engineers* **45 (1)**, 257-260

Kawagishi H, Ando M (1990) Hericeone, A and B as cytotoxic principle from the mushroom *Hericium erinaceum*. *Tetrahedrom Letters* **31 (3)**, 373-376

Lakshmi B, Tilak JC, Adhikari S, Davasagayam TPA, Janardhanan KK (2004) Evaluation of antioxidant activity of selected Indian mushrooms. *Pharmaceutical Biology* **42 (3)**, 179-185

Lucas EH, Rigler RL, Byrrum RU, Stock OO (1957) Tumour inhibition in *Boletus edulis* and other Holobasidiomycetes. *Antibiol. Chemotherapy* **7**, 1-4

Mitchell C (2003 *Vibrant Health in the 21st Century*, The Stanborough Press, UK, pp 127-128

Mirko S (1976) Mushrooms and fungi. *Mycologia* **48**, 10-12

Okwu DE (2001) Evaluation of the chemical composition of indigenous species and flavouring agents. *Global Journal of Pure and Applied Science* **7**, 455-459

Okwu DE, Okwu ME (2004) Chemical composition of *Spondias mombin*. Linn. Plant parts. *Journal of Sustainable Agriculture and Environment* **6 (2)**, 140-147

Okwulehie IC, Odunze E (2004b) Evaluation of the myco chemical and mineral composition of some tropical edible mushrooms. *Journal of Sustainable Agriculture and Environment* **6 (2)**, 157-162

Peng JP, Kobayashi H (1995) Novel Furastonol glycoside from *Allium macrosternon*. *Planta Medica* **6**, 58-61

Pearson D (976) *Chemical Analysis of Foods* (7th Edn), Chard 111, Edinburgh, pp 422-511

Rambelli A, Menini UG (1983) *Manual on Mushroom Cultivation*, F.A.O. Italy, 165 pp

Rodriguez KF, Hesses M (2000) Antimicrobial activities of secondary metabolites produced by endophytic fungi from *S. mombin*. *Journal of Basic Microbiology* **40 (4)**, 261-267

Stamets PS (1993) *Growing Gourmet and Medicinal Mushrooms*, Ten Speed Press, Berkeley, USA, 554 pp

Stray F (1998) *The Natural Guide to Medicinal Herbs and Plants*, Tigers Books International, London, pp 12-16

Suzuki S, Oshima S (1976) Influence of shii-ta-ke (*Lentinus edodes*) on human serum cholesterol. *Mushroom Sciences* **1**, 463-467

Valazquez-Cedeno MA, Farmet AM, Ferre E, Savoie JM (2004) Variation of lignocellulose activities in dual cultures of *Pleurotus ostreatus* and *Trichoderma longibranchiatum* on unsterilized wheat straw. *Mycologia* **9 (94)**, 712-719