Gas Chromatography-Flame Ionization Detector Analysis of the Phytochemical Composition of *Pleurotus tuberregium* (Fr) Sing's Sclerotia: Potential Benefits.

Catherine C. Ikewuchi[1]; Jude C. Ikewuchi[1]*; Mercy O. Ifeanacho[1]; Ngozi M. Igboh[2]; and Ifeoma I. Ijeh[3]

[1]Department of Biochemistry, Faculty of Chemical Sciences, College of Natural and Applied Sciences, University of Port Harcourt, PMB 5323, Port Harcourt, Nigeria.
[2]Department of Biochemistry, Faculty of Science, Abia State University, PMB 2000, Uturu, Nigeria.
[3]Department of Biochemistry, College of Natural and Applied Sciences, Michael Okpara University of Agriculture, Umudike, PMB 7267, Umuahia, Nigeria.

E-mail: ecoli240733@yahoo.com*

ABSTRACT

The phytochemical composition of the Sclerotia of *Pleurotus tuberregium* (Fr) Sing's was investigated by gas chromatography using a flame ionization detector. The Sclerotia have very high contents of alkaloids (68.759 g/kg dry weight and 62.089 g/kg wet weight), tannins (62.284 g/kg dry weight and 56.242 g/kg wet weight), flavonoid (32.425 g/kg dry weight and 29.279 g/kg wet weight) and saponins (10.477 g/kg dry weight and 9.461 g/kg wet weight); high contents of hydroxycinnamic acids derivatives (3.633 g/kg dry weight and 3.280 g/kg wet weight) and lignans (1.323 g/kg wet weight and 1.194 g/kg dry weight); moderate contents of benzoic acid derivatives (0.761 g/kg dry weight and 0.687 g/kg wet weight), phytosterols (0.481 g/kg dry weight and 0.435 g/kg wet weight) and terpenes (0.108 g/kg dry weight and 0.097 g/kg wet weight); and low carotenoids (0.004 g/kg dry weight and 0.003 g/kg wet weight). Thirty eight known alkaloids were detected, consisting mainly of akuammidine (70.4%) and voacangine (25.2%). Five known carotenoids were detected, including anthera-xanthin (37.6%), lutein (21.3%), neoxanthin (18.7%), carotene (14.2%) and viola-xanthin (8.2%). The phytosterol extract consisted 100% of sitosterol, while the tannin extract consisted 100% of tannic acid. Twenty three known flavonoids were detected, consisting mainly of (-)-epicatechin (18.5%), kaempferol (18.2%), biochanin (8.7%), (+)-catechin (8.5%), naringenin (8.1%), daidzein (7.6%) and butein (5.0%). Four benzoic acid derivatives, vanillic acid (35.3%), ferulic acid (29.6%), 4-hydroxybenzaldehyde (23.7%) and 4-hydroxybenzoic acid (11.5%) were detected. Two hydroxycinnamic acid derivatives, p-coumaric acid (58.2%) and caffeic acid (41.8%) were detected. Eight lignans were detected, consisting mainly of galgravin (79.3%), retusin (11.2%) and dehydroabietic acid (9.5%). Four saponins (mainly 71.1% avenacin A1, and 26.6% avenacin B1), and five terpenes (including α-amyrin, 47.6%; lupeol, 17.9%; β-amyrin, 15.5%; bauerenol acetate, 13.3%; and taraxerol, 5.7%) were detected. These results show that the sclerotia are rich sources of nutraceuticals such as alkaloids, tannins, flavonoid, saponins, hydroxycinnamic acid derivatives and lignans.

(Keywords: alkaloids, β-sitosterol, flavonoids, tannic acid, *Pleurotus tuberregium* (Fr) Sing's Sclerotia)

INTRODUCTION

Mushrooms have continued to generate a lot of interest, especially with respect to their use as important items of commerce, cures for diseases, foods, and agents of bioremediation. *Pleurotus tuberregium* (Fr) Sing is an edible mushroom that is found in both tropical and subtropical regions of the world (Okhuoya and Okogbo, 1991), especially in southern Nigeria. It looks somewhat like an oyster mushroom (*Pleurotus ostreatus*) except that, when mature, the cap curves upward to form a cup-like shape. It forms large spherical to ovoid, subterranean sclerotia (or underground tuber) which sometimes measure up to 30 cm in diameter, in addition to a mushroom (Okhuoya and Okogbo, 1991; Fasidi and Olorunmaiye, 1994).

The fungus infects dry wood, where it produces the sclerotium, usually buried within the wood

tissues or between the wood and the bark. In Nigeria both the sclerotium and the mushroom are eaten. The sclerotium, which is hard, dark brown outside and white inside, is peeled and ground for use as soup thickener (Fasidi and Olorunmaiye, 1994). It is used in African traditional health care practice, in preparations for the treatment of childhood malnutrition, skin diseases, inflammation, headache, stomach ailments, colds, fever, asthma, smallpox, diabetes and high blood pressure (Okhuoya and Okogbo, 1991; Fasidi and Olorunmaiye, 1994; Okhuoya et al., 1998; Alobo, 2003; Chen and Huang, 2004). Its antifungal activity (Badalyan et al., 2008), ability to bioremediate crude oil polluted soils (Isikhuemhen et al., 2003; Adenipekun, 2008), and coagulate and disinfect natural and waste water (Yongabi, 2004), have been reported.

The sclerotia are rich in proteins (Ikewuchi and Ikewuchi, 2011), copper, iron, magnesium and manganese (Ikewuchi and Ikewuchi, 2009; Ijeh et al., 2009). They also contain phytochemicals like alkaloids, flavonoids, phytates, saponins and tannins (Ikewuchi and Ikewuchi, 2009; Ijeh et al., 2009). However, their detailed phytochemical composition is yet to be completely elucidated. So, in this study, the alkaloid, carotenoid, flavonoid, hydroxycinnamic acid, lignin, benzoic acid derivatives, phytosterol and tannin composition of *Pleurotus tuberregium* sclerotia, was determined by gas chromatography coupled to a flame ionization detector.

MATERIALS AND METHODS

Collection of Plant Samples

Samples of fresh Sclerotia of *Pleurotus tuberregium* were bought from Mile 3 Market, Port Harcourt, Nigeria. The identity of the sample was confirmed by Dr. Michael C. Dike of Taxonomy Unit, Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria; and Mr. John Ibe, the Herbarium Manager of the Forestry Department, National Root Crops Research Institute (NRCRI), Umuahia, Nigeria. They were rid of dirt, oven dried at 55 °C and ground into powder. The resultant powder was stored in an air tight container, for subsequent use.

Calibration, Identification and Quantification

Standard solutions were prepared in methanol for alkaloids, flavonoids and simple phenolics, acetone for carotenoids and lignans, methylene chloride for phytosterols, ethanol for hydroxycinnamic acids. The linearity of the dependence of response on concentration was verified by regression analysis. Identification was based on comparison of retention times and spectral data with standards. Quantification was performed by establishing calibration curves for each compound determined, using the standards. Sample chromatograms of the extracts are shown in Figures 1-5.

Determination of Alkaloid Composition

The extraction was carried out according to the method of Tram et al. (2002). The sample was extracted with methanol and subjected to gas chromatographic analysis. Chromatographic analyses were carried out on an HP 6890 (Hewlett Packard, Wilmington, DE, USA), GC apparatus, fitted with a flame ionization detector (FID), and powered with HP Chemstation Rev. A 09.01 [1206] software, to quantify and identify compounds. The column was a capillary DB-5MS (30 m × 0.25 mm × 0.25 µm film thickness). The inlet and detection temperatures were 250 and 320 °C. Split injection was adopted with a split ratio of 20:1. The carrier gas was nitrogen. The hydrogen and compressed air pressures were 28 psi and 38 psi. The oven program was: initial temperature at 60 °C for 5 min. First ramping at 10 °C/min for 20 min was followed by a second ramping at 15 °C/min for 4 min.

Determination of Carotenoid Composition

The extraction was carried out by a modification of the method of Rodriguez-Amaya and Kimura (2004). The carotenoids were successively extracted with acetone and a (1:1) mixture of diethyl ether and petroleum ether, then concentrated and re-dissolved in acetone before saponifying and re-extracting with a (1: 1) mixture of diethyl ether and petroleum ether. The resultant extracts were dried and re-dissolved in petroleum ether and subjected to gas chromatography analysis. Flame ionization detector (FID; range scanned, 300 to 600 nm) and capillary column, ZP-5 Column (30m × 0.32 mm × 0.25 µm film thickness) was used.

Temperature was programmed at 45 °C, held for 6 min, before programming at 38 °C/min to 250 °C. Initial column head pressure 3.47 psi.

Determination of Flavonoid Composition

The extraction was carried out according to the method of Millogo-Kone et al. (2009). The sample was extracted with methanol and the resultant extract was subjected to gas chromatographic analysis. Flame ionization detector (FID) and capillary column, HP INNOWax Column (30 m × 0.25 mm × 0.25 µm film thickness) were used. The inlet and detection temperatures were 250 and 320 °C. Split injection was adopted with a split ratio of 20:1. The carrier gas was nitrogen. The hydrogen and compressed air pressures were 22 psi and 35 psi. The oven program was: initial temperature at 50 °C, first ramping at 8 °C/min for 20 min, maintained for 4 min, followed by a second ramping at 12 °C/min for 4 min, maintained for 4 min.

Determination of Hydroxycinnamic Acid Derivatives' Composition

The extraction was carried according to method of Ortan et al. (2009). The sample was extracted thrice with methanol, and the extracts were pooled, concentrated and subjected to gas chromatographic analysis. Flame ionization detector (FID) and column, HP-5 (30m×0.32mm × 0.25 µm film thickness) was used. The samples were introduced via an all-glass injector working in the split mode, with nitrogen as the carrier gas, at a flow rate of 1mL/min. The injection and detector temperatures were 260 °C and 300 °C, respectively. The oven temperature was programmed at the start of the run from 170 °C to 250 °C at 5 °C/min.

Determination of the Lignan Composition

The extraction was carried out according to the method of Chapman et al. (2006). The sample was extracted with methanol, and a hexane/dichloromethane mixture, the resultant extract was rid of water and subjected to gas chromatography. Flame ionization detector (FID; range scanned, 350 to 400 nm) and column, ZP-5 (30m×0.32mm×0.25µm film thickness), detected at 300 nm were used. One microliter of sample was injected. The conditions for the GC were initial oven temperature of 40 °C, injector 250 °C, transfer line 280 °C, a solvent delay of 2.00 min, the temperature was ramped at 10 °C/min to a final temperature of 230 °C and held for 1.00 min.

Determination of Benzoic Acid Derivatives' Composition

The extraction was carried out according to the method of Ndoumou et al. (1996). The sample was extracted with methanol, and after removing the pigments with petroleum ether, were re-extracted with ethyl acetate, before gas chromatography analysis. Detection was achieved with a flame ionization detector (FID). The column was a capillary HP 1 Column (30 m × 0.25 mm × 0.25 µm film thickness). The inlet and detection temperatures were 250 and 320 °C. Split injection was adopted with a split ratio of 20:1. The carrier gas was nitrogen, at a pressure of 30 psi. The hydrogen and compressed air pressures were 28 psi and 32 psi. The oven program was: initial temperature at 60 °C for 5 min, first ramping at 15 °C/min for 15 min, maintained for 1 min, followed by a second ramping at 10 °C/min for 4 min.

Determination of Phytosterol Composition

Extraction of oil was carried out according to AOAC method 999.02 (Association of Official Analytical Chemists, 2002), while the analysis of sterols was carried out according to AOAC method 994.10 (Association of Official Analytical Chemists, 2000). This involved extraction of the lipid fraction from homogenized sample material, followed by alkaline hydrolysis (saponification), extraction of the non-saponifiables, clean-up of the extract, derivatisation of the sterols, and separation and quantification of the sterol derivatives by gas chromatography (GC) using a capillary column. Flame ionization detector (FID) and column, HP INNOWax Column (30m×0.25mm×0.25µm film thickness) was used. The inlet and detection temperatures were 250 and 320 °C. Split injection was adopted with a split ratio of 20:1. The carrier gas was nitrogen. The hydrogen and compressed air pressures were 22 psi and 35 psi. The oven program was: initial temperature at 60 °C, first ramping at 10 °C/min for 20 min, maintained for 4 min, followed by a second ramping at 15 °C/min for 4 min, maintained for 10 min.

Determination of Tannin Composition

Extraction was carried out according to the method of Luthar (1992). The sample was extracted with methanol followed by gas chromatographic analysis. Flame ionization detector (FID) and column, HP 5 Column (30 m × 0.25 mm × 0.25 µm film thickness) was used. The inlet and detection temperatures were 250 and 320 °C. Split injection was adopted with a split ratio of 20:1. He carrier gas was nitrogen. The hydrogen and compressed air pressures were 28 psi and 40 psi. The oven program was: initial temperature at 120 °C, followed by ramping at 10 °C/min for 20 min.

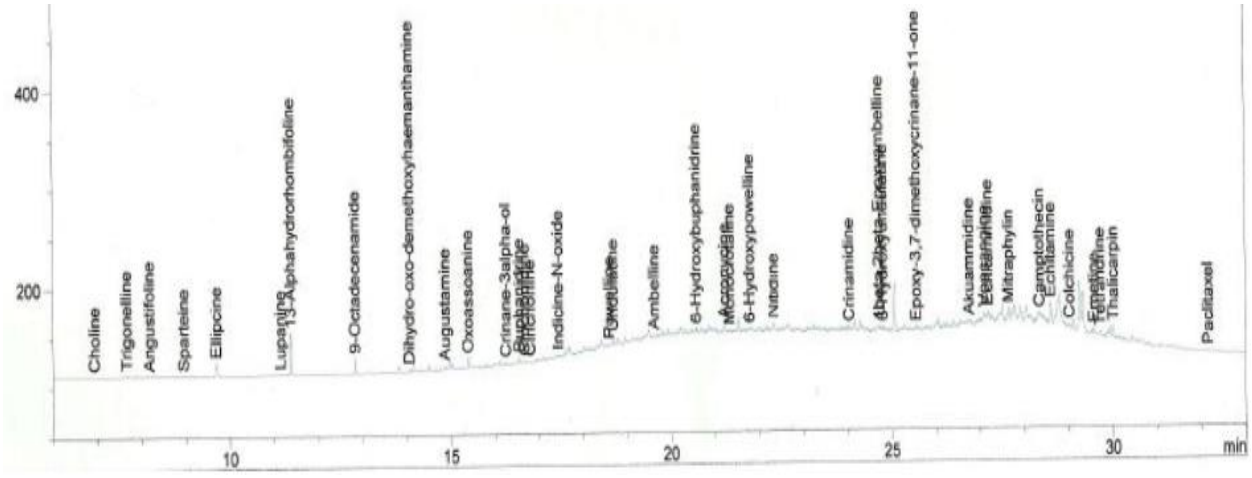

Figure 1: Chromatogram of the Alkaloids Fraction of *Pleurotus tuberregium* Sclerotia.

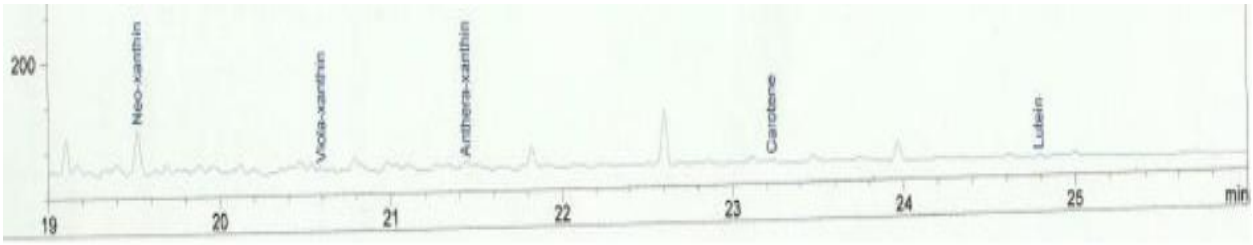

Figure 2: Chromatogram of the Carotenoids Fraction of *Pleurotus tuberregium* Sclerotia.

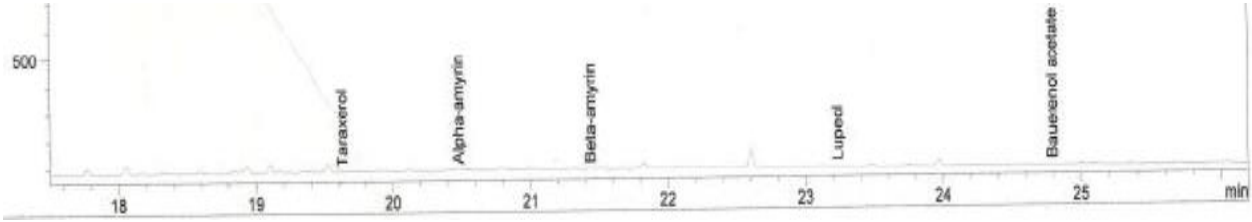

Figure 3: Chromatogram of the Terpenes Fraction of *Pleurotus tuberregium* Sclerotia.

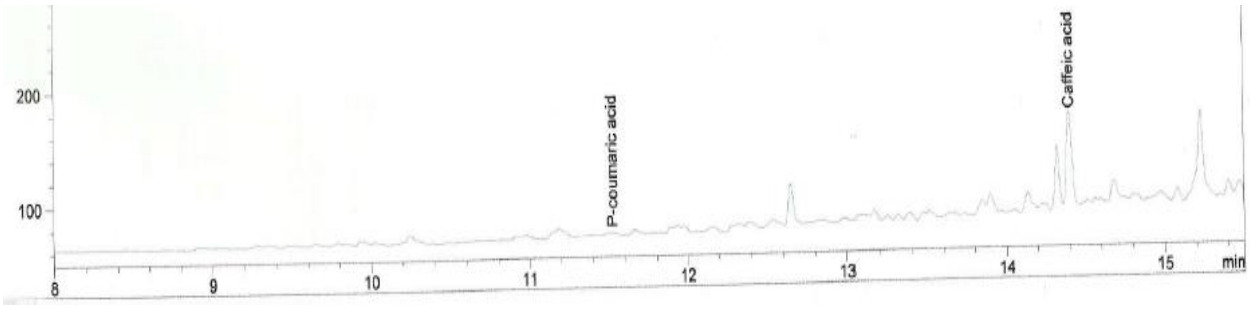

Figure 4: Chromatogram of the Hydroxycinnamic Acid Derivatives Fraction of *Pleurotus tuberregium* Sclerotia.

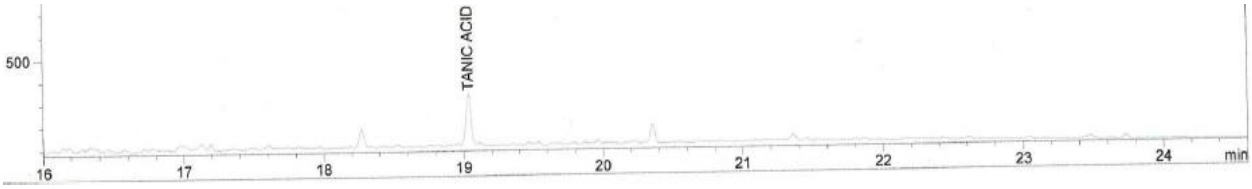

Figure 5: Chromatogram of the Tannins Fraction of *Pleurotus tuberregium* Sclerotia.

Derivation of Composition Per Wet Weight from the Composition Per Dry Weight

The compositions per dry weight of the determined parameters were derived from the compositions per wet weight and vice versa, using the following formula, adapted from Health Canada Official Methods (1999).

$$Composition\ per\ wet\ weight\ (\%) = \frac{Composition\ per\ dry\ weight\ (\%) \times Dry\ matter\ content(\%)}{100}$$

RESULTS AND DISCUSSION

As shown in Table 1, thirty eight known alkaloids were detected, consisting mainly of akuammidine (70.37%), voacangine (25.24%), echitamine (2.25%), angustifoline (0.75%) and echitamidine (0.57%). In animal experiments voacangine has shown activities such as cardiovascular effects, depressant action on the central nervous system (De Smet, 1996), retardation of the appearance and severity of convulsions (Carroll and Starmer, 1967), lowering of body temperature and blockade of the thermoregulator system (Carroll and Starmer, 1967), analgesic, local anaesthetic and anti-leishmanial activities, decreasing voluntary activity (Carroll and Starmer, 1967; Okuyama et al., 1992; Pratchayasakul et al., 2008; Pereira et al., 2011), potentiation of hypnotic effects of barbiturates (Okuyama et al., 1992) and acetyl cholinesterase inhibitory effect (Andrade et al., 2005).

Echitamine possesses a battery of pharmacological activities which include lowering of systemic arterial pressure (i.e. hypotensive activity) in normotensive anaesthetized animals, inductions of negative chronotropic and inotropic responses in isolated atrial muscle strips, relaxation of isolated vascular and extra-vascular smooth muscles, inhibition of electrically-provoked and agonist-induced contractions or relaxations of isolated smooth muscle preparations, paralysis of electrically-evoked skeletal muscle twitches, induction of diuresis and anti-tumor activity (Chandrasekaran and Nagarajan, 1981; Ojewole, 1984; Kamarajan et al., 1991; Jagetia et al., 2005).

Akuammidine has hypotensive, anti-plasmodial, anti-depressant, skeletal muscle relaxant and local analgesic activities (Dhingra and Sharma, 2006; Hirasawa et al., 2009). Its local analgesic activity is about 3 times as potent as cocaine. It acts selectively as a sympatholytic,

unaccompanied by para-sympatholytic effects. It inhibits the irritability of the sympathetic nervous system and opposes akuammine (Nyunaï and Njifutié, 2006). Epoxyambelline both alone and in 1:1 combination with ambelline, produces moderate to pronounced activation of mouse spleen lymphocytes (Ghosal et al., 1984). This probably may be the basis of the immunomodulatory activity of the sclerotia.

Table 1: Alkaloids Composition of *Pleurotus tuberregium* Sclerotia.

Compounds	Retention time (min)	Composition (mg/kg)	
		/dry weight	/wet weight
Choline	7.057	21.823	19.706
Trigonelline	7.641	0.000	0.000
Angustifoline	7.979	514.764	464.832
Sparteine	9.103	33.199	29.979
Ellipcine	9.671	3.679	3.322
Lupanine	11.195	57.744	52.143
13-α-Hydrorhombifoline	11.384	9.891	8.932
9-Octadecenamide	12.938	3.707	3.347
Dihydro-oxo-demethoxyhaemanthamine	14.059	2.705	2.443
Augustamine	14.931	96.120	86.796
Oxoassoanine	15.397	3.799	3.422
Cinchonidine	16.783	25.058	22.627
Cinchonine	16.247	8.767	7.916
Crinane-3α-ol	16.459	23.451	21.176
Buphanidrine	16.674	6.611	5.970
Indicine-N-oxide	17.471	5.063	4.572
Undulatine	18.765	2.613	2.359
Powelline	18.590	9.030	8.154
Ambelline	19.686	3.841	3.469
6-Hydroxybuphanidrine	20.473	13.652	12.327
Acronycine	21.266	9.890	8.912
Monocrotaline	21.328	10.301	9.302
6-Hydroxypowelline	21.822	31.999	28.895
Nitidine	22.365	15.017	13.561
Crinamidine	23.970	108.522	97.995
1β,2β-Epoxyambelline	24.722	12.412	11.208
6-Hydroxyundulatine	24.793	13.651	12.327
Epoxy-3,7-dimethoxycrinane-11-one	25.485	4.669	4.216
Echitamidine	26.957	391.975	353.954
Akuammidine	26.842	48385.918	43692.484
Voacangine	27.069	17354.585	15671.190
Mitraphylin	27.436	0.044	0.040
Camptothecin	28.247	12.388	11.186
Echitamine	28.640	1545.821	1395.877
Colchicine	28.935	6.451	5.825
Emetine	29.573	3.308	2.987
Tetrandrine	29.747	5.323	4.807
Thalicarpin	30.156	0.090	0.081
Paclitaxel	32.340	0.021	0.019
Total alkaloids		68758.867	62089.257

Table 2 shows the carotenoids, phytosterols and tannins composition of *Pleurotus tuberregium* Sclerotia. Five known carotenoids were detected, including anthera-xanthin (37.64%), lutein (21.31%), neoxanthin (18.66%), carotene (14.19%) and viola-xanthin (8.20%). Their sterol extract consisted 100% of sitosterol, while the tannin extract consisted 100% of tannic acid (Table 2).

Table 2: Carotenoids, Phytosterols and Tannins Composition of *Pleurotus tuberregium* Sclerotia.

Compounds	Retention time (min)	Composition (mg/kg)	
		/dry weight	/wet weight
Carotenoids			
Neoxanthin	19.521	0.704	0.636
Viola-xanthin	20.594	0.309	0.279
Anthera-xanthin	22.438	1.420	1.282
Carotene	23.232	0.535	0.483
Lutein	24.790	0.804	0.726
Total carotenoids		3.772	3.406
Phytosterols			
Cholesterol	20.587	0.000	0.000
Cholestanol	21.380	0.000	0.000
Ergosterol	22.401	0.000	0.000
Campesterol	23.001	0.000	0.000
Stigmasterol	23.236	0.000	0.000
5-Avenasterol	24.103	0.000	0.000
Sitosterol	24.774	481.414	434.717
Total sterols		481.414	434.717
Tannins			
Tannic acid	19.035	62283.687	56242.169
Total tannins		62283.687	56242.169

Lutein has antioxidant (Tinoi et al., 2006), photo-protective (Pintea et al., 2003) and anti-cancer (Sertie et al., 1990; Dillard and German, 2000; Tinoi et al., 2006) activities. Carotenes have pro-vitamin A, antioxidant (Tinoi et al., 2006) and anti-cancer (Dillard and German, 2000) activities.

Stigmasterol is a brassinosteroid, which are growth regulator and signaling molecules essential for normal plant growth (Rao et al., 2002; Ayad et al., 2009). As plant hormones, steroids have regulatory function in cell elongation and division, vascular differentiation and other diverse developmental processes (Priti, 2003; Sasse, 2003). Brassinosteroids have the ability to confer resistance to plants against various abiotic stresses (Priti, 2003). Plant sterols modulate the activity of ATPase (Piironen et al., 2000). Cholesterol and stigmasterol stimulate export of H^+ at low concentrations, whereas all

other sterols act as inhibitors. In animals, phytosterols have anti-inflammatory (Dillard and German, 2000), hypocholesterolemic and anti-cancer (Piironen et al., 2000; Tinoi et al., 2006) activities. β-Sitosterol has anti-inflammatory, anti-neoplastic, hypoglycemic, atheroprotective, hepatoprotective, immune-modulating and anti-pyretic and hypocholesterolemic activities (Ivorra et al., 1988; Nan-Lin and Pin Tome, 1988; Dillard and German, 2000; Piironen et al., 2000; Beta-sitosterol Monograph, 2001; Berger et al., 2004). It also has hypotensive properties with little effect on heart rate (Ogundaini et al., 1983, cited in Ogundaini et al., 2005). Tannic acid is an antioxidant, hypoglycemic, hepatoprotective and hypocholesterolemic agent (Liu et al., 2005; Basu et al., 2007; Mittal et al., 2010).

The flavonoid composition of *Pleurotus tuberregium* Sclerotia, investigated is presented in Table 3. Twenty three known flavonoids were detected, consisting mainly of (+)-catechin (8.45%), apigenin (3.81%), butein (5.04%), naringenin (8.11%), biochanin (8.69%), kaempferol (18.22%), (-)-epicatechin (18.50%), daidzein (7.60%), quercetin (3.51%), (+)-gallocatechin (3.38%), genistein (2.47%) and nobiletin (2.88%). The sclerotia has higher quercetin (73 mg/kg, US Highbush Blueberry Council, Summer 2005; 17-24 mg/kg, Hakkinen et al., 1999), myricetin (26 mg/kg, US Highbush Blueberry Council, Summer 2005; 23-26 mg/kg, Hakkinen et al., 1999) and epicatechin (11.1 mg/kg, US Highbush Blueberry Council, Summer 2005) contents than blueberry.

Luteolin has antibacterial, anti-inflammatory, anti-mutagenic, antioxidant and immunomodulating activities (Dillard and German, 2000). Catechins have anti-carcinogenic (Tapas et al., 2008), antimicrobial, antioxidant (Tapas et al., 2008), and hypocholesterolemic activities. They protect LDLs from oxidation (Chu et al., 2004) and prevent cardiovascular disease (Kang et al., 1999; Demeule et al., 2000). They also protect neurons, enhance resistance of red blood cells to oxidative stress and inhibit ultraviolet radiation-induced oxidative stress in the skin (Chu et al., 2004). Kaempferol is known for its strong antioxidant and anti-inflammatory properties (Lau, 2008). It also has antibacterial, anti-cancer, anti-fungal, antioxidant, hypocholesterolemic, cardio-protective, hepatoprotective, hypoglycemic, hypotensive and immunomodulatory activities (Ahmad et al., 1993; Song et al., 2003; De-Sousa et al., 2004; Oh et al., 2004; Lim, et al., 2007; Lau, 2008; Lin et al., 2011). Over the years, studies have shown that it can help in the prevention and treatment of cancers, neuron disorder, and cardiovascular, heart, spinal cord, and brain disease (Lau, 2008). It inhibits both oxidative susceptibility of LDL in vitro, and platelet aggregation (Kowalski et al., 2005; Lau, 2008). Its rutinoside causes remarkable decrease in systolic, diastolic, mean arterial blood pressure and heart rate (Ahmad et al., 1993).

Table 3: Flavonoids Composition of the Sclerotia of *Pleurotus tuberregium.*

Compounds	Retention time (min)	Composition (mg/kg)	
		/dry weight	/wet weight
(+)-Catechin	13.744	2739.704	2473.953
(+)-Gallocatechin	15.020	1097.503	991.045
Genistein	15.618	800.298	722.670
Diadzein	16.037	2462.852	2223.955
Apigenin	16.371	1235.831	1115.955
Butein	16.672	1634.761	1476.190
Naringenin	16.782	2629.111	2374.088
Biochanin	17.092	2817.958	2544.616
Luteolin	17.362	572.321	516.806
Kaempferol	18.493	5906.662	5333.716
(-)-Epicatechin	19.518	5998.014	5416.207
(-)-Epigallocatechin	20.601	226.118	204.185
Quercetin	21.436	1137.698	1027.341
(-)-Epicatechin-3-gallate	22.518	58.468	52.797
(-)-Epigallocatechin-3-gallate	23.230	80.158	72.383
Isorhamnetin	24.101	21.838	19.720
Robinetin	24.186	176.871	159.714
Ellagic acid	24.622	420.136	379.383
Myricetin	24.730	331.516	299.359
Baicalein	25.697	632.908	571.516
Nobiletin	26.288	926.457	836.590
Baicalin	27.065	396.951	358.447
Silymarin	27.810	120.539	108.847
Total flavonoids		32424.629	29279.440

Apigenins have antioxidant (Skerget et al., 2005), hypoglycemic, hepatoprotective (Zheng et al., 2005; Panda and Kar, 2007), diuretic, hypotensive, smooth muscle relaxation enhancing (Dillard and German, 2000), anti-tumor (Wang et al., 2005), anti-phlogistic, anti-spasmodic (National Cancer Institute, n.d.), anti-inflammatory (Hougeea et al., 2005), antibacterial (Martini et al., 2004), anti-proliferative, oxygenase

inhibitory (Abate et al., 2005), and apoptosis inducing (Saeed et al., 2006; Tajdar and Sarwat, 2006) activities. The monomer apigenin is fit into a pharmacophore model for ligands binding to the GABA receptor benzodiazepine site (Svenningsen et al., 2006). It acts as an inhibitor of IL-4 synthesis and CD40 ligand expression by basophils. It also has protective effect on radiation-induced chromosomal damage in human lymphocytes (Kanokporn Noy et al., 2005; Saeed et al., 2006). It inhibits motility and invasion of prostate carcinoma (Franzen et al., 2009) and melanoma (Caltagirone et al., 2000) cells, disrupts actin cytoskeleton organization, and inhibits FAK/Src signaling (Franzen et al., 2009).

Naringenin has antioxidant, hepatoprotective (Lee et al., 2004; Pari and Gnanasoundari, 2006), hypocholesterolemic (Lee et al., 1999), gastroprotective (Raj Narayana et al., 2001; Ganapathy et al., 2008), cardioprotective, anti-cancer (Wei-wei et al., 2009) and anti-ulcer (Parmar and Parmar, 1998) activities. Naringin has hypoglycemic activities (Jung et al., 2004), while epicatechin has antioxidant, hypoglycemic and immunomodulatory activities (Chakravarthy et al., 1982; Vinardell and Mitjans, 2008). Diadzein and genistein, lower total cholesterol, LDL-cholesterol and blood pressure, reduces coronary and generalized thrombosis (Houston, 2002, 2005, 2007), and exhibits oestrogen-like properties (Dixon and Ferreira, 2002; Pereira et al., 2009). They inhibit tyrosine kinase activity, which decreases vascular smooth muscle contraction and lowers blood pressure, and inhibit oxidation activity in the blood vessel (Houston, 2002, 2005, 2007). Genistein also has hypoglycemic activity (Lee, 2006). Nobiletin has anti-fungal activity (Tapas et al., 2008), while silymarin has antioxidant and hepatoprotective activities (Suja et al., 2004). Luteolin has antibacterial, anti-inflammatory, anti-mutagenic and antioxidant activities (Dillard and German, 2000). Myricetin exhibits hypoglycemic (Ong and Khoo, 1996), antibacterial, anti-gonadotropic and antioxidant activities (Dillard and German, 2000).

Quercetin is frequently used therapeutically in allergic conditions, including asthma and hay fever, eczema, and hives (Thorne Research Inc., 1998; Thornhill and Kelly, 2000; Lakhanpal and Kumar, 2007), due to its ability to mediate production and manufacture of pro-inflammatory compounds. Additional clinical uses include treatment of gout (Yoshimoto et al., 1983; Kim et al., 1998), pancreatitis and prostatitis, which are also, in part, inflammatory conditions (Lakhanpal and Kumar, 2007). It has antibacterial (Lakhanpal and Kumar, 2007; Rigano et al., 2007), hypocholesterolemic (Arai et al., 2000), anti-anemic (Sen et al., 2005), gastro-protective, antiviral (Thorne Research Inc., 1998; Lim et al., 2007; Tapas et al., 2008), anti-carcinogenic (Thorne Research Inc., 1998; Caltagirone et al., 2000; Lamson and Brignall, 2000; Tapas et al., 2008), anti-cataractogenic (Thorne Research Inc., 1998; Lakhanpal and Kumar, 2007), anti-diabetic (Vessal et al., 2003; Lakhanpal and Kumar, 2007), antioxidant (Houston, 2002, 2005, 2007), anti-hypertensive (Duarte et al., 2001), anti-inflammatory (Yoshimoto et al., 1983; Kim et al., 1998), anti-allergic (Thorne Research Inc., 1998; Thornhill and Kelly, 2000; Lakhanpal and Kumar, 2007), and hepatoprotective (Peres et al., 2000; Lee et al., 2003; Pavanato et al., 2003; Chen, 2010) activities. It inhibits calmodulin dependent enzymes present in cell membrane such as ATPases and phospholipases thereby influencing membrane permeability (Havsteen, 1983). It affects other calmodulin dependent enzymes that control various cellular functions, including the secretions of histamine from mast cells (Bennett et al., 1981; Middleton et al., 1981; Pearce et al., 1984; Middleton and Drzewiecki, 1985; Fox et al., 1988; Otsuka et al., 1995; Thornhill and Kelly, 2000). It has also been shown to limit the function of adhesion molecules on endothelial cells (Middleton and Anne, 1995); chelate ions of transition metals such as iron, thereby preventing them from initiating the formation of oxygen free radicals (Afanas'ev et al., 1989; Ferrali et al., 1997); directly inhibit lipid peroxidation (Sorata et al., 1984); and inhibit platelets aggregation (Osman et al., 1998). Its intake protects against coronary heart disease, caused by oxidized LDL (bad cholesterol) (Thorne Research Inc., 1998; Hertog et al., 1993, 1995).

Quercetin has been shown to inhibit aldose reductase, the first enzyme in the polyol pathway, and therefore, may be beneficial in the nutritional management of diabetes, and the prevention of long-term diabetic complications such as cataracts, nephropathy, retinopathy and neuropathy (Lakhanpal and Kumar, 2007). It may also provide beneficial effects in people with diabetes by decreasing oxidative stress and preserving pancreatic beta-cell integrity. Quercetin also spares vitamin C, stabilizes cell membranes including those of mast cells, and

helps in maintaining lens transparency after an oxidative insult.

The benzoic acid derivatives' composition of the Sclerotia of *Pleurotus tuberregium* is presented in Table 4. Four benzoic acid derivatives were detected. They include vanillic acid (35.30%), ferulic acid (29.59%), 4-hydroxybenzaldehyde (23.66%) and 4-hydroxybenzoic acid (11.46%). Two known hydroxycinnamic acids, p-coumaric acid (58.20%) and caffeic acid (41.80%) were detected. Eight known lignans were detected, consisting mainly of galgravin (79.34%), retusin (11.15%) and dehydroabietic acid (9.47%).

Table 4: Benzoic Acid Derivatives Composition of *Pleurotus tuberregium* Sclerotia.

Compounds	Retention time (min)	Composition (mg/kg)	
		/dry weight	/wet weight
Benzoic Acid Derivatives			
4-Hydroxybenzaldehyde	8.799	180.101	162.631
4-Hydroxybenzoic acid	12.367	87.243	78.780
Vanillic acid	15.238	268.709	242.645
Ferulic acid	18.053	225.253	203.404
Total benzoic acid derivatives		761.306	687.459
Hydroxycinnamic Acids Derivatives			
p-Coumaric acid	11.522	2114.310	1909.222
Caffeic acid	14.136	1518.521	1371.224
Total hydroxycinnamic acids		3632.831	3280.447
Lignans			
2-Allyl-5-ethoxy-4-methoxyphenol	11.377	0.000	0.000
(9E,12E,15E)-9,12,15-Octadecatrien-1-ol	14.096	0.087	0.078
Apigenin-4',7-dimethyl ether	16.753	0.396	0.358
Dehydroabietic acid	18.619	125.289	113.136
Retusin	19.460	147.480	133.175
Galgravin	20.448	1049.312	947.529
Epieudesmin	22.410	0.000	0.000
Sakuranin	24.015	0.000	0.000
Total lignans		1322.564	1194.275

The caffeic acid content is comparable to those reported for thyme, aniseed, caraway and rosemary (> 1000 mg/kg) (IARC, 1993). P-coumaric acid has antioxidant properties and is believed to reduce the risk of stomach cancer (Ferguson et al., 2005; Oksana et al., 2012) by reducing the formation of carcinogenic nitrosamines (Kikugawa et al., 1983; Oksana et al., 2012). It represses the expression of T3SS genes of the plant pathogen *Dickeya dadantii*, suggesting that plants can also defend against bacterial pathogens by manipulating the expression of the type III secretion system (Yan et al., 2009; Oksana et al., 2012). Lignans are one of the major classes of phytoestrogens and also act as antioxidants.

In general, phenolics are important for cell structure, signaling and pigmentation (Adyanthaya, 2007). Phenolic acids are known to act as allelochemicals (Yoshioka et al., 2004), protect plants against environmental and biological stress such as high energy radiation exposure, bacterial infection or fungal attacks (Tüzen and Özdemír, 2003), cold stress, hyperthermia (Adyanthaya, 2007) and oxidative stress (Dillard and German, 2000). Thus, the present result suggests a likely allelopathic potential of *Pleurotus tuberregium* Sclerotia.

4-Hydroxybenzoic acid esters (also called parabens), are widely used as antimicrobial agents in a large variety of food, pharmaceutical, and cosmetic products due to their excellent antimicrobial activities, low toxicity and stability over a wide pH range (Valkova et al., 2001). 4-hydroxybenzoic acid has antifungal, anti-mutagenic, anti-sickling, estrogenic (Pugazhendhi et al., 2005; Khadem and Marles, 2010; Oksana et al., 2012), and antimicrobial (Chong et al., 2009; Khadem and Marles, 2010; Oksana et al., 2012) activities. In plants, p-hydroxybenzoic acid increases the impermeability of the cell wall, leading to increased resistance against pathogen infection (Tan et al., 2004; Horváth et al., 2007). It is synthesized de novo in stems and petioles in response to a mobile signal (Smith-Becker et al., 1998). It also has a growth stimulation effect (Kamaya et al., 2006).

Vanillic acid (4-hydroxy-3-methoxybenzoic acid) is used as a flavoring agent. It has anti-sickling, anthelmintic, hepatoprotective (Itoh et al., 2009, 2010; Khadem and Marles, 2010; Oksana et al., 2012), immune-modulating and anti-inflammatory (Chiang et al., 2003) activities. It also inhibits snake venom 5'-nucleotidase (Dhananjaya et al., 2009; Khadem and Marles, 2010; Oksana et al., 2012). 4-Hydroxybenzaldehyde is used as a flavour and fragrance agent. Ferulic acid protects against coronary disease, increases sperm

viability and has hypocholesterolemic activity (Shiyi and Kin-Chor, 2004).

Table 5 shows the saponins and terpenes composition of *Pleurotus tuberregium* Sclerotia. Four known saponins were detected, and they include avenacin A1 (71.14%), avenacin B1 (26.59%), avenacin B2 (2.08%) and avenacin A2 (0.20%). Five known terpenes were detected, and they include α-amyrin (47.55%), lupeol (17.93%), β-amyrin (15.47%), bauerenol acetate (13.34%) and taraxerol (5.72%).

Table 5: Saponins and Terpenes Composition of *Pleurotus tuberregium* Sclerotia.

Compounds	Retention time (min)	Composition (mg/kg)	
		/dry weight	/wet weight
Saponins			
Avenacin A1	21.435	7453.413	6730.432
Avenacin B1	23.231	2785.401	2515.217
Avenacin A2	24.790	20.612	18.613
Avenacin B2	26.294	217.524	196.424
Total Saponins		10476.950	9460.686
Terpenes			
Taraxerol	19.626	6.153	5.556
α-Amyrin	20.473	51.191	46.226
β-Amyrin	21.440	16.649	15.034
Lupeol	23.235	19.299	17.427
Bauerenol acetate	24.793	14.362	12.968
Total Terpenes		107.654	97.212

Saponins are reported to have broad range of pharmacological properties (Soetan, 2008). They have hypoglycemic properties (Soetan, 2008). Avenacins have antimicrobial properties (Armah et al., 1999; Mert Türk et al., 2005; Soetan, 2008). Avenacin A-1 has potent anti-fungal activity (Armah et al., 1999), and is believed to confer resistance against a range of soil fungi (Field et al., 2006). Ouabain is a cardiotonic steroid (Dmitrieva and Doris, 2002).

Terpenes are used as flavor enhancers in food, fragrances in perfumery, and in traditional and alternative medicines such as aromatherapy (Kappers et al., 2005; Zwenger and Basu, 2008). They have anti-cancer (Dewick, 2004), antimicrobial (Islam et al., 2003), antioxidant (Dillard and German, 2000), cardiotonic and insecticidal (Soetan, 2008) activities. Limonoids have anti-cancer properties (Islam et al., 2003), providing protection to lung tissue, and inhibiting pancreatic and gastric carcinogenesis (Dillard and German, 2000). Limonene promotes glutathione-S-transferase activity and cancer cell apoptosis. In a bioremediative study by Suttinun et al. (2004), limonene and pinene were found to increase the uptake and subsequent degradation of trichloroethylene by bacteria.

CONCLUSION

These results show that the sclerotia are a rich source of alkaloids, tannins, flavonoid, saponins, hydroxycinnamic acids and lignans, lending credence to their medicinal uses. This also highlights their potential as sources of nutraceuticals.

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