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ELEMENTAL AND MYCOCHEMICAL PROFILE, ANTIMICROBIAL ACTIVITIES AND USES OF THE SCLEROTIUM OF *PLEUROTUS TUBER-REGIUM*, A MEDICINAL MUSHROOM IN GHANA

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ABSTRACT

Much of the interest in food and nutritional value of mushrooms has been concerned with the amount of nutritional quality of their protein, amino acids, carbohydrates, fats, essential vitamins, mineral element composition and medicinal therapeutic applications by traditional medicinal plant practitioners. This paper reports the elemental composition, mycochemical screening and *in vitro* inhibitory activities on three pathogens as well as uses of the sclerotium of medicinal mushroom *Pleurotus tuber-regium* (Fr.) Sing. (=*Lentinus tuber-regium*) in four regions of Ghana. The rapid appraisal method was used to document the diverse ways traditional medicinal practitioners use this fungus in therapeutic applications. The combination of the tuber (sclerotium) of *P. tuber-regium* with other plants such as *Euphorbia hirta, Eugenia carophylla*, *Solanum torvum* leaves, *Xylopia aethiopica* and *Chromolaena odorata* for various purposes are highlighted. Determination of the elemental composition of the sclerotium by Neutron Activation Analysis showed that it contained Aluminium (165.08±7.8mg/kg), Calcium (1.10%), Chlorine (564.04±12.7mg/kg), Cobalt (0.005±0.0mg/kg), Chromium (6.25±1.10mg/kg), Iron (101.38±25.4mg/kg), Potassium (0.4%), Manganese (35.5±2.26mg/kg), Sodium (75.41±2.1mg/kg), Rubidium (18.14±0.02mg/kg), Scandium (0.010±0.001mg/kg) and Zinc (56.01±8.0mg/kg). Mycochemical analysis showed the presence of saponins, alkaloids, flavonoids, glycosides, steroids and terpenoids in at least one of the three solvent extractions; hot water, cold water and ethanol. However, there were very little *in vitro* inhibitory activities of these extracts against *Samonella typhimurium*, *Escherichia coli* and *Candida albicans*. These may explain the therapeutic value of the sclerotium (tuber) used by traditional medicine practitioners.

Keywords: *Pleurotus tuber-regium*, sclerotium, mycochemicals, mineral elements.

INTRODUCTION

Pleurotus tuber-regium (Fr.) Sing. (=*Lentinus tuber-regium*) is found in many parts of tropical Africa and part of the local people's food system. It is known to produce mushroom fruiting body from a unique globose sclerotium that is more or less like a giant truffle (Nkwokolo, 1987). The subterranean sclerotia are usually of various sizes ranging from a few centimeters to several centimeters in diameter. They vary in shape from oval to spherical, dark brown on the outside and whitish on the inside (Okhuoya and Okogbo, 1991; Oso, 1977b).

The local people in Nigeria often collect these sclerotia from the forests, induce sporophores from them by burying them in warm humid soil or dry and store them for future use as food (Isikhuemhen and Okhuoya, 1995). The tuberous sclerotium can be used as partial replacement for melon seed (*Citrulus lanatus*) or groundnut (*Arachis hypogea*) cake in traditional preparation of sauce and soup (Isikhuemhen and Okhuoya, 1995). The use of the tuberous sclerotium as food is not widespread in Ghana. However, in many parts of tropical Africa, the sclerotium is milled with melon or groundnut seeds, seasoned and the moist mix is moulded into patties for cooking or baking. The groundnut cake patties are consumed as snacks while sporophores are cooked as soup and the melon seed patties are eaten as meat substitutes providing a cheap source of protein in tropical Africa (Nkwokolo, 1987). Ikewuchi and Ikewuchi (2009) found that the sclerotia was rich in protein, fibers and carbohydrates.

In some parts of Africa including Ghana, *Pleurotus tuber-regium* is used for medicinal purpose by traditional medicinal practitioners (Zoberi, 1973; Oso, 1977a,b; Okhuoya and Okogbo, 1990). There are records of how the local people prepare and use *P. tuber-regium* for

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medicines in Nigeria (Oso, 1977a,b; Isikhuemhen and Okhuoya, 1995). Dzomeku (unpublished data, 2009) reports of its use by people in the Brong Ahafo and Volta Regions of Ghana in treating asthma, underweight in babies, boils and so on. Isikhuemhen and LeBauer (2004) and Okhuoya *et al*. (1998) mentioned their use in therapies for treating malnutrition and anaemic conditions as well as embalming corpses among rural dwellers in Ghana. The objectives of these investigations was to survey five selected regions in the southern sector of the country to collect information on the uses of the sclerotium of *P. tuber-regium* by the traditional herbal medicine practitioners. Analysis of the elemental and mycochemical composition of the sclerotium was also carried out to help explain the therapeutic value of the tuber (sclerotium). Again, *in vitro* inhibitory activities of three crude extracts of the sclerotium were carried out on three pathogens to ascertain the antimicrobial potential.

MATERIALS AND METHODS

Four regions (Eastern, Brong Ahafo, Western and Volta) were selected for the distribution on the basis of preponderance of herbal medicine practitioners. The rapid appraisal method using structured questionnaires was employed to document information on therapeutic applications of traditional medicinal practitioners in the four regions.

Preparation of samples of sclerotia for elemental analysis

Sample was dried and homogenized in a Retch RS 100 pulverizer for ten minutes. Six replicate samples, 200mg of the sample and standard reference materials (National Institute Standards and Technology 1566b Oyster Tissue and National Bureau of Standards of 1571 Orchard Leave) were weighed into a clean polyethylene foil, wrapped and heat-sealed with a hair dryer. The sub-samples were packed into a bigger polyethylene capsule and heat-sealed. The standards were treated in the same manner as the samples. Orchard leave was used for the validation.

Sample irradiation and measurements

All samples were irradiated at the inner site of the Ghana Research Reactor-1 (GHARR-1) facility operating at 15KW and at thermal flux of $5 \times 10^{11} ncm^{-2}s^{-1}$. The sample transfer was done by means of a pneumatic transfer system, which operates at 0.6MPa.

Samples and standards for short-lived elements were irradiated for one hundred and twenty seconds and counted for six hundred seconds. For medium-lived elements, samples and standards were irradiated for one hour, delayed for twenty fours and counted for six hundred seconds and for long-lived elements, samples and standards were irradiated for four hours, delayed for fourteen days and counted for fifty four thousand seconds on a gamma spectroscopy system. The spectroscopy system consists of a high purity germanium (HPGe) N-type coaxial detector, multichannel analyser (MCA), emulation software card and a 486 microcomputer was used for data evaluation and analysis. The detector has resolution for Co^{60} gamma-ray energy of 1332 KeV. The detector has a relative efficiency of 25%. By means of the MCA card, spectral intensities of the samples were accumulated and analysed qualitatively to identify the elements present. The area under the photo peak of each identified element was converted into concentrations by means of gamma-spectra analysis software.

Crude Extraction of Mycochemicals from sclerotium

About 50g of each pulverised sclerotium was soaked in 200mL of the solvents [cold water (10^oC), hot water (80^oC) and ethanol] for thirty-six hours. The crude extracts were gravity filtered through Whatmann's No. 2 filter paper. The filtrates were concentrated by evaporating excess solvent in a hot water bath and stored in the dark.

Mycochemical screening of crude extract of sclerotium

Detection of Alkaloids: One mL of the extract was shaken with 0.5mL of picric acid in a test tube and observation made for the presence of brick red precipitate which indicated a positive result (Latha *et al*., 2013)

Test for Saponin (frothing test): One mL of the extract was put into a test tube and shaken vigorously. This was then allowed to stand on the bench for a minute and then observed for the formation of stable froths which indicated positive result.(Usman *et al*., 2009).

Test for Flavonoids: Few quantity of the extract was dissolve in water and filtered; to this 2ml of the 10% aqueous sodium hydroxide was later added to produce a yellow coloration. A change in color from yellow to colorless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids (Trease and Evans, 2002).

Test for Steroids: 2ml of acetic anhydride was added to 5ml of the extract. 2ml of sulphuric acid was later added. Violet to blue coloration in the sample indicated the presence of steroids (Sofowora, 1993).

Test for Glycosides: The extract was hydrolyzed with HCl solution and neutralized with NaOH solutions. A few drops of Fehling's solution A and B were added. Red precipitate indicates the presence of glycosides (Sofowora, 1993).

Test for Terpenoids: 0.2g of the extract was mixed with 2mL of chloroform ($CHCl_3$) and 3mL of concentrated H_2SO_4 were carefully added to form a layer. A reddish

Fig. 1. Morphology of the *Pleurotus tuber-regium.*

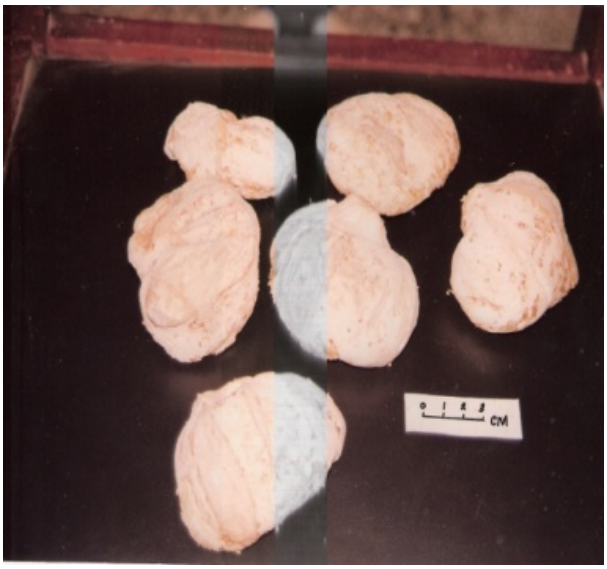

Fig. 2. Subterranean sclerotia (tubers) of varying sizes.

brown coloration at the interface was indicative of the presence of terpenoids (Sofowora, 1993).

Culturing of microorganisms and antimicrobial sensitivity testing

The test microorganisms: *Salmonella typhimurium*, *Escherichia coli* and *Candida albicans* were obtained from the University for Development Studies/DANIDA Microbiology Laboratory. They were further cultured in Mueller Hinton Broth and Malt Extract Broth respectively at 37^{o} C for 24 hours for *E. coli* and *S. typhimurium* and 5 days for *C. albicans*. The well diffusion method was used to determine the antimicrobial activity of the crude sclerotium extracts. Tetracycline (25µg/mL) and nystastin (20µg/mL) were used as standard antibiotic and antifungal agents respectively.

RESULTS AND DISCUSSION

The morphology of the *Pleurotus tuber-regium* is shown in figure 1. The subterranean sclerotia (tubers) of varying sizes are presented in figure 2.

Results of the structured questionnaire on the uses of *P. tuber-regium* were interesting but did not vary much from Region to Region and can be summarized as follows:

(a) Blended sclerotium applied in combination with; (i)'shire' (white clay) or (ii) *Euphorbia hirta*, 'pepre' (*Eugenia caryophyllata*) or (iii) blended rice and applied to premature babies to make them grow bigger, get smooth skin or even make them sleep well.
(b) Blended sclerotium is combined with; (i)'ayilo' (white clay), 'pepre' and smeared on breast or (ii) shea (*Butryospermum parkii*) butter or cocoa (*Theobroma cacao*) butter and applied to nipples, or (iii) blended sclerotium is mixed with 'atropo' (*Solanum torvum*) leaves, pepper leaves and oil palm soup in order to enhance production of breast milk in nursing mothers.
(c) Blended sclerotium mixed with agushie (*Cucumeropsis edulis* seed) or *Solanum macrocarpon* can be used in soup to enhance lactation.
(d) Blended sclerotium is mixed with honey or coconut juice or with Ethiopian pepper (*Xylopia aethiopica*) and then drank to cure heart diseases and palpitations.
(e) The blended sclerotium is mixed with *Chromolaena odorata* (=*Eupatorium odoratum*) and 'krobo' (white clay) for preservation of dead bodies.
(f) The sclerotium is blended and mixed with wasp nest soil and *Borassus aethiopium* fruit or palm fruit and applied as ointment on boils.
(g) The goldsmith also uses the pulverized sclerotium to clean precious metals like gold and silver in his trade.

There were other combinations of plant parts (fruit, roots, leaves) with blended sclerotia of *P. tuber-regium* used for herbal therapy. But the specific ailments these combinations cure were not clearly stated. The plants are *Kigelia africana, Terminalia ivorensis, Celtis mildbraedii, C. zenkeri, C. weightii, Colocasia esculenta, Xanthosoma mafaffa, Piper guineense, Tetrapleura tetraptera, Aframomum melagueta, Phoenix reclinata, Ficus capensis, Cyperus esculenta, Ficus exasperata, F. asperifolia, Kigelia angolensis and Ceiba pentandra.*

The medicinal use of this fungus is also well known among traditional herbal medicine practitioners in Ghana. Sawyerr (1993) stated that it is used orally in the

Table 1. Mycochemical screening of crude extracts of *P. tuber regium* sclerotium.

Mycochemical	Ethanolic extract	Hot water extract	Cold water extract
Alkaloids	-	+	+
Flavonoids	-	+	+
Glycosides	-	+	-
Reducing sugars	-	-	-
Saponins	-	+	+
Steroids	+	+	+
Terpenoids	+	+	+

Table 2. Antimicrobial Sensitivity Test of various extracts of *P. tuber regium* against three pathogens.

Test organisms	Mean zones of inhibition (mm)				
	Cold water extract	Hot water extract	Ethanol extract	Control	Standard
E.coli	-	-	-	-	3.5
S. typhi	-	-	-	-	3.5
C. albicans	-	-	-	-	20.3

treatment of heart pains and persistent coughs; the ground and pulverized sclerotium is applied topically to underweight and premature babies to accelerate weight gain. This confirms findings in this paper. Indeed, one of the local names given to the sclerotium the Western Region of Ghana is 'Akeseduro' or 'Huhummofra' implying it bloats children or is a fattening drug (personal communication). The listed use of the sclerotium to prepare medicine extends the list of medicinal preparation made from this fungus in combination with other plant parts. However, owing to trade secrecy, many of the traditional herbal medicine practitioners are unwilling to disclose the entire recipe and formulation for therapeutic application. In mid-western Nigeria, the sclerotium is ground with herbs of undisclosed identity, fried with palm oil and administered orally to pregnant women to aid in the development of the foetus (Oso, 1977b). The Urhobo of mid-west Nigeria also use the sclerotium for the treatment of chest pains and small pox (Fasidi and Olurunmaiye, 1994). They grind the sclerotium with other herbs and use it for the treatment of boils. The Ibos of Nigeria (Ogun State) fry eggs with a mixture of powdered sclerotium, unripe palm fruits, maize and bark of *Garcinia kola* and *Fagara macrophylla* for the treatment of asthma, high blood pressure and nervous disorders (Oso, 1977b). Adejumo and Awosanya (2005), also found its use in the treatment of dysentery.

Mycochemical and Elemental analysis of sclerotium

Six mycochemicals were detected in the crude extracts of ethanolic, cold and hot water treatments of the sclerotium and are summarized in table 1.

Although several mycochemicals were present in the different extracts, there were no inhibitory activities against the test organisms as shown in table 2. This probably was due to low concentrations of these chemicals in the extracts. On other hand, elemental analysis revealed several elements as shown in table 3.

Table 3. Elemental composition of the sclerotium of *P. tuber-regium.*

Element	Concentration (mg/kg)
Aluminium (Al)	165.08 ± 1.76
Calcium (Ca(%))	1.10411 ± 0.04
Chloride (Cl^-)	567.04 ± 12.7
Cobalt (Co)	0.005 ± 0.0001
Chromium (Cr)	6.25 ± 1.01
Fe (Iron)	101.38 ± 25.4
Potassium (K(%))	0.3991 ± 0.004
Manganese (Mn)	35.50 ± 2.26
Sodium (Na)	75.41 ± 2.1
Rubidium (Rb)	18.14 ± 0.02
Scandiun (Sc)	0.01 ± 0.001
Zinc (Zn)	56.01 ± 8.0

Research has shown that many mushroom species accumulate several elements during their growth depending on the soil type and area where they grow in the wild. Several reviews of trace element content of mushroom have been published (Seeger, 1982; Michelot *et al*., 1998; Kalać and Svoboda, 2000; Kalać *et al*., 2004). High content of metals have been observed in mushroom growing in heavily polluted areas (Cuny *et al*., 2001), landfills of sewage sludge (Zabowski *et al*., 1990) and emission areas (Lepšová and Mejstrík, 1988) and cities (Kuusi *et al*., 1981; Falandysz *et al*., 1995). *P. tuber-regium* from the Volta Region contained high

amount of Aluminium, and traces of Cobalt, Scandium and Rubidium. Further studies are required to elucidate the potential toxicity. Again, to overcome possibly high toxicity levels the mushroom could be cultivated on known substrates such as sawdust. On the other hand, the fungus contained Sodium, Potassium and Chloride which are very essential in the maintenance of osmotic balance between cells whereas Calcium, Iron and Zinc are cofactors in the structure of some enzymes required for several biochemical pathways (Soetan *et al*., 2010). Similar elements were analyzed from the sclerotium obtained from Nigeria (Agomuo, 2011). These may explain the therapeutic value of the sclerotium in herbal portions applied for healing various ailments.

CONCLUSION

The sclerotium of *Pleurotus tuber regium* is used in many therapeutic formulations by traditional herbal practitioners. It contains very important elements and mycochemicals making it very useful in these therapeutic remedies. The solvents extracts of the sclerotium however, did not inhibit the test organisms *in vitro* probably due to low yield of the inhibitory principle in the extracts.

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