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Research Article

ANTITUBERCULOSIS AND PHYTOCHEMICAL INVESTIGATION OF THE DICHLOROMETHANE EXTRACT *PLEUROTUS TUBER-REGIUM* (FRIES) SINGER SCLEROTIUM

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ABSTRACT
Incidence of tuberculosis infections is on the rise. The cost and length of orthodox treatment regimen coupled with the rise in multidrug resistance cases make the development of more effective and relatively cheaper alternative therapies imperative. The cold percolation method was used with dichloromethane as an extraction solvent to obtain dichloromethane extract of the edible mushroom *Pleurotus tuber-regium*. The extract was soluble in n-hexane and labeled PTHS. Antituberculosis screening was by using the colorimetric BACTEC MGIT 960 SIRES method. Phyto-constituents determination was by using standard phytochemical test reagents.The PTHS of *Pleurotus tuber-regium* inhibited the growth of clinical isolates of *Mycobacterium tuberculosis* at the test concentration of 32.5 µg/ml. Terpenoids and steroids were detected as the phytochemical constituents. This study confirmed the nutraceutical benefits of some edible mushrooms
Keywords: *Pleurotus tuber-regium,* tritepenoids, steroids, nutraceutical, tuberculosis.

INTRODUCTION

Tuberculosis (TB) is a common and often deadly infectious disease caused by *Mycobacteria tuberculosis*. Tuberculosis infection if left untreated kills over 50 % of its sufferers [1].The World Health Organization (WHO) reported 8.7 million new cases of TB in 2011 with a recorded deaths due to the disease put at 1.4 million worldwide[2]..Less developed countries, mostly in Africa and Asia are worst affected with India and China together accounting for almost 40 % while the African Region accounted for 24 % of the world's TB cases. Almost 80 % of TB cases among people living with HIV reside in Africa.[2]. In tropical Africa, the use of the rich forest resources in the treatment of diseases like malaria fever, asthma, tuberculosis, infertility, typhoid, psychosis, poison, and even in warding off mystical spells and witchcraft, are documented [3].

Pleurotus tuber-regium (Fries) Singer, commonly called the king tuber mushroom is an edible gilled fungus of the Agaricomycetes class. *Pleurotus tuber-regium* grows wild in both tropical and subtropical regions of the world. It is a common mushroom in the southern part of Nigeria and forms large spherical to ovoid, subterranean sclerotia (or underground tuber) which sometimes measure up to 30 cm in diameter [4-5]. The mushroom looks somewhat like an oyster mushroom (*Pleurotus ostreatus*) except that, when mature, the cap curves upward to form a cup-like shape. The sclerotium is dark brown on the outside and white on the inside. The fungus infects dry wood, where it produces the sclerotium, usually buried within the wood tissues but also found between the wood and the bark. Locally, it is called 'Katala' in Hausa, 'ike usu' or 'ero usu' in Igbo, 'Awu' in Igala and 'Umoho'usu' in Igede (Nigeria). Edible mushrooms are a popular and valuable food, low in calories and high in minerals, essential amino acids, vitamins and fibres[4]. Some of them produce substances having potential medicinal effects. In Nigerians, edible mushrooms are used mainly as food as a result of their good taste, appetizing aroma and nutrient contents[4,-5]. They are widely used today in Oriental countries as functional foods and are prescribed for prevention and treatment of diseases such as gastro-intestinal disorder, bleeding, high blood pressure and various bacterial infections[7]. The inhibitory activities of some selected mushroom metabolites on some bacteria including the gram positive acid fast bacterium (*M. smegnatis*) have been reported[8]. In traditional medical practice in Nigeria, *Pleurotus tuber-regium* is used in preparation of traditional medications for headache, stomach ailments, colds and fever, asthma, smallpox and high blood pressure as well as for weight gain and malnourished babies[4-6, 9]. Among *Pleurotus species,* several medicinal properties have been reported for extracts. They include anticancer properties attributable to their polysaccharides[10], anti-genotoxic, bio-antimutagenic activities[11], anti-inflammatory activity, anti-lipidaemic, antihypertensive, and anti-hyperglycaemic activites[12], antibacterial and antifungal activities[13] and as immunomodulators[14-15]. Nutritionally, *Pleurotus tuber-regium* (Fr) sclerotia is rich in protein (22.10 % w/w) and carbohydrate (63.03 % w/w), crude fibre (10.86 % w/w), and moderate contents of ash (2.97 % w/w) due to varying levels of essential mineral elements like; Ca, Mg, K , Na , Cu, Zn, Fe, Mn and Se[16]. Moderate phytate content and low alkaloids, saponins, flavonoids and tannins but high polysaccharide contents have been reported[16].

This study is aimed at a preliminary antituberculosis and phytochemical investigation of sclerotium of *Pleurotus tuber-regium*

MATERIAL AND METHODS

Sample collection and identification

The sclerotia used for this study were substrates collected from the Bezaleel Mushrooms in Port Harcourt, Nigeria and authenticated by a mycologist, Olutayo M. Adedokun of the Department of Crop and Soil Science, University of Port Harcourt. The plant materials were sorted out to remove humus, washed and dried under ambient condition. The dried materials were then pulverized into fine powder.

Reagents and Instruments

Reagents and solvents used in this study were of analytical grade and are products of BDH and Sigma-Aldrich. Standard control drugs used include rifampicin [99 %], ethambutol [99%], isoniazid [99 %], and dihydrostreptomycin [99 %] and were kindly supplied by the Tuberculosis Research laboratory of the Zankli Medical Centre, Abuja, Nigeria. Mycobacteria growth indication tube (MGIT), The pathogenic micro-organisms used for the study are clinical MTB strains and were cultured at the TB Research laboratory of the Zankli Medical Centre, Abuja, Nigeria

Extraction of *Pleurotus tuber-regium* sclerotia

Using the cold percolation method with dichloromethane as extraction solvent, 100g of the powdered dried sclerotia was transferred into a percolator and allowed to soak in 500 ml of dichloromethane as menstrum for 72 hours and drained off. Fresh dichloromethane was then added and allowed to soak for yet another 72 hours and then drained off. The sequence was repeated five times until a colourless extract was obtained. This is to achieve exhaustive extraction. The dichloromethane filtrates were pooled together and concentrated by evaporation to one-tenth using a rotary evaporator set at 40^{o}C. The concentrated dichloromethane extract was then transferred to a petri dish and allowed to air dry in a fume cupboard. Attempt at further fractionation of the dried extract with n-hexane resulted in the fraction dissolving in the n- hexane. This resulting n-hexane solution was labeled PTHS. The PTHS was then subjected to antimycobacteria assay using the high throughput colorimetric BACTEC MGT 960 SIRES method[17]

Antituberculosis susceptibility test

This was done using the BACTEC MGIT 960 SIRES system[17]. To the fluorescence Mycobacterium growth indicator tube (MGIT) containing 7 ml of midlebrook 7H9 broth was added 0.8 ml of OADC-PANTA growth supplement [Oleic acid-Albumin- Dextrose-Catalase (OADC) -polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin (PANTA)] antibiotics to inhibit the growth of non-tuberculosis microorganism contaminants, 0.5 ml of 0.5Mcfarland decontaminated clinical isolate of *mycobacterium tuberculosis,* and 0.1 ml of 0.273 mg/ml of PTHS. Streptomycin (1.0µg/ml), isoniazid (0.1µg/ml), rifampicin (1.0µg/ml), and ethambutol (5.0µg/ml) were used as standard control drugs. The MGIT tubes were placed inside the BACTEC 960 SIRES system programmed as per manufacturer's instruction and incubated for 14 days[17].

Phytochemical methods

Preliminary phytochemical tests for alkaloids, terpenoids, steroids, saponins, phenolics, and Flavonoids were carried out on the extracts. The methods were based on reported standard procedures[18.19]

Table 1: Antituberculosis activity of fractions from *P. tuber regium*

Fractions	PTHS	DHS	INH	RIF	EMB	GC
Test conc (µg/ml)	32.5	1.0	0.1	1.0	5.0	-
Colony Growth unit	0	0	0	0	0	400

Key: DHS-Dihydrostreptomycin, INH- Isoniazid, RIF- Rifampicin, EMB- Ethambutol

Table 2: Phytochemical Screening Results for fractions from *Pleurotus tuber-regium*

Screened extract	Alkaloids	Terpenoids/steroids		Phenolics	Carbohydrates/glycosides		Saponins
		Liebermann test	Salkowski test	$FeCl_3$ test	Molisch test	Fehling's test	
PTHS	-	+	+	-	-	-	-

Key: + present; - not present

RESULTS AND DISCUSSION

The *in vitro* antituberculosis activity spectrum of PTHS at the test concentration of 32.5 µg/ml in table 1 is similar to those of the standard drugs. The result of the phytochemical screening in table 2 showed the presence of triterpenoids and steroids as the only phyto-constituents in the bioactive PTHS (yield = 0.02 % w/w) of fresh *P. tuber-regium*. Several triterpenoid have been reported to have antituberculosis activity [20-24]. The presence of triterpenoids in the dichloromethane extract as observed from the result of the phytochemical screening in table 2 could be responsible for the observed antituberculosis activity. The absence of alkaloids and phenolic compounds in the scleretium is in contrast to what was reported for the fruit bodies[16]. This could be attributed to the morphological factor and stage of development. Some bioactive triterpenoids have been isolated from several species of mushroom. Pleuromutillin, a novel protein synthesis inhibitor with antituberculosis activity, have reportedly been isolated from some species of mushrooms[20]. 3,11-dioxolanosta-8,24(Z)-diene-26-oic acid from the mushroom specie *Jahnoporus hirtus,* and confluentin, grifolin, and neogrifolin from *Albaterllus flettii* have been isolated with antibacterial activity[21]. Lanostane –type triterpenes have been isolated from the mushroom *Astraeus pteridis* with good inhibitory activity against *M. tuberculosis*[22]. Also from the fruiting bodies of *Ganoderma collossum* have been isolated the lanostane-type triterpenes: colossolactone V colossolactone VI, colossolactone VII, colossolactone VIII and colossolactone E with anti-HIV protease activity[23]. Three triterpenoids, sublateriols A-C isolated from *Naematoloma sublateritium* have been reported[24].

Further Studies

Isolation, characterisation and Minimum inhibitory concentration determination of the active compound(s) and structure activity relationship investigation is on-going

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