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Phytochemical Confinement in Sections of *Pleurotus Tuber-regium* (King Tuber Mushroom) Basidocarp

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Abstract

Cellular compartmentation is responsible for the restriction of most products of metabolic processes to specified regions of the organism. In this study, a quantity of 10.0 kg of fresh sclerotia of *Pleurotus tuber-regium* purchased from Zarama market in Bayelsa State, Nigeria were washed, peeled and sliced. Eight kilograms of the sliced sclerotia were packed in a transparent cellophane bag and a temperature range of 33 – 35 °C was achieved at the 3rd day. Stipes of *P. tuber-regium* basidiocarps were observed at the 8th day and at the 10th day, the stipes sprouted out from the perforated openings. At the 14th day, fresh basidiocarps of *P. tuber-regium* were harvested and the stipe, cap-base and pileus were immediately separated and allowed to dry at room temperature for fourteen days. The dried samples were ground and the oils were extracted by standing a mixture of 10 g of sample in 20 ml of dichloromethane for 3 days. The process was repeated twice and the combined aliquot obtained were concentrated to 5 ml and purified. Two milliliters of the extracted oil were used for gas chromatographic and mass spectroscopy analysis. The highest peaks on the chromatogram of volatile components of the oils from the pileus, cap-base and stipe were observed at 15.663 min., 15.693 min. and 15.819 min. respectively. Propanoic acid was the predominant compound observed in the pilus with a percentage value of 29.763 %, while Hexadecanoic acid methyl ester was predominant in both the cap-base and stipe with values of 23.638 % and 26.677 % respectively.

Keywords: Phytochemical, Compartmentation, Confinement, Sclerotia, Stipe, Pilus and cap-base

1. Introduction

In trees and other plants, the above ground (visible) parts have remained their largest part. But in the case of macro-fungi, the visible basidiocarp is only the fruiting body of a bigger organism that is hidden either within a decomposing wood material or beneath the soil surface. One macro fungus with distinct characteristics is the *Pleurotus tuber-regium*. *P. tuber-regium* is amongst the numerous mushrooms of the tropical rain forest of Africa. It is one mushroom that both its basidiocarp and sclerotium are of economic importance due to their nutritive and medicinal properties. Aside Africa, P. tuber-regium also grows in Asia and Australia [1]. It is a saprotroph, that produces a food storage sclerotiun upon its consumption of decaying wood. The dimitic tissue of this mushroom is composed of generative thick-walled hyphae, while its spores are cylindrical, smooth and hyaline-like in appearance [2]. The formation of infundibuliform pilus, decurrent gills and a well-developed stipe is a known characteristic of its basidiocarp [3]. The junction between the stipe and the pilus which is referred to as the cap-base (reticulum) shows an irregular netted gilled formation that transcends to the gills beneath the cap.

In mushroom and other living cells, there is always a state of metabolism, which may be either catabolic or anabolic. Though the anabolic process always surpass the catabolic process during growth, cellular compartmentation is responsible for the restriction of most products of these metabolic processes to certain regions of the organism. These compounds, which may include waste products and toxic compounds are kept out of the cytoplasmic components by their confinement in the cellular vacuoles [4]. This confinement or localization of compounds may also be affected by the site of production of such compounds. The aim of this research is to ascertain the possible confinement or localization of compounds in the different structural compartments of the basidiocarp of P. tuber-regium.

2. Materials and Methods

2.1 Sample Collection, Growth, Preparation and Extraction

A quantity of 10.0 kg of fresh sclerotia of *P. tuber-regium* were purchased at Zarama market in Yenagoa Local Government area of Bayelsa State, South-South Nigeria and was taken to the University of Port Harcourt. The sclerotia were washed, peeled and the white inner part were sliced using a sterilized knife. A quantity of 8 kg of the sliced sclerotia was packed in a transparent cellophane bag and a thermometer was inserted. This set up was allowed to stand and a temperature range of 33 – 35 °C was achieved at the 3rd day. This temperature range was maintained and at the 8th day, sprouts of *P. tuber-regium* basidiocarps were observed and the cellophane bag was randomly perforated. Stipes of the basidiocarps sprouted out from the perforated openings at the 10th day and fully grown *P. tuberregium* were harvested at the 14th day. The stipe, reticulum and pileus of the harvested mushrooms were immediately separated using a clean and sterilized knife. The different parts were allowed to dry at room temperature in a dust free environment for a period of fourteen days and the dried samples were separately ground into fine powders. The ground samples were extracted by weighing 10 g of the sample into a well stopper bottle and 20 ml of dichloromethane was added. The mixtures were vigorously agitated and were left to stand for 3 days. The process was repeatedly carried out for two more times. The combined aliquot collected were concentrated on a steam berth to about 5 ml and purified by passing through a pasture pipette packed with silica gel and anhydrous sodium sulphate on a membrane and air dried to about 2 ml for gas chromatographic analysis.

2.2 GC-MS Analysis of Extracts

The mushroom extract was analysed using a combined gas chromatograph model HP 6890 and mass spectrometer model 5973 (Agilent Tech.) fitted with a capillary column HP-5 MS (5% phenylmethylsiloxane) 30.0 m x 250µm x 0.25µm, using Helium as a carrier gas at initial column temperature 120°C for 5 minutes. Thereafter, the column temperature was increased at 5°C per minutes to 320°C and held for 5 minutes. Electron impact ionization for mass spectroscopy was done at ionization energy of 70eV. The oil was diluted with 98% hexane and 2µl of the diluted sample was automatically injected into Agilent Tech. model 5973 mass spectrometer. The constituent compounds were identified using the Chem-Office

software attached to the MS library. The names and structures of the component oils were confirmed using the database of National Institute of Standard and Technology (NIST).

3. Results

The retention time, percentage concentration, molecular formula, molecular weight and structures of the essential oils in the extracts of the Pileus (Cap), Reticulum (Cap-base) and Stipe (Stem) of *P. tuber-regium* are shown in Tables 1, 2, and 3 respectively. Propanoic acid had the highest percentage concentration in the extract obtained from the pileus, with a value of 29.763 % followed by Propanamide with a value of 23.641 %. Extract obtained from the reticulum has Hexadecanoic acid, methyl ester as its predominant compound with a value of 23.638 % followed by Eicosane, 2-methyl- with a value of 20.435 %. The highest compound observed in the extract obtained from stipe was Hexadecanoic acid, methyl ester with a value of 26.677 % followed by trans-13-Octadecenoic acid, methyl ester with a value of 25.837 %.

Table 1: Essential oil in Pileus (Cap) of P. tuber-regium

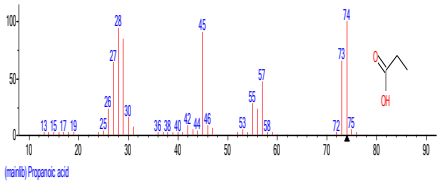
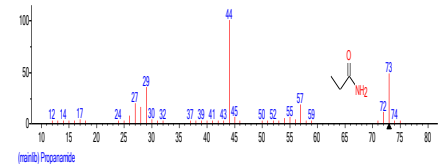
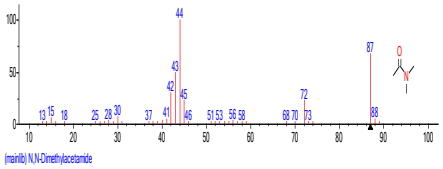
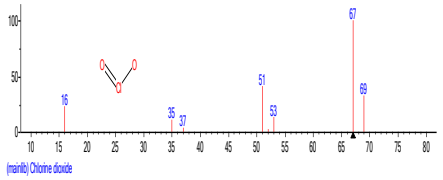
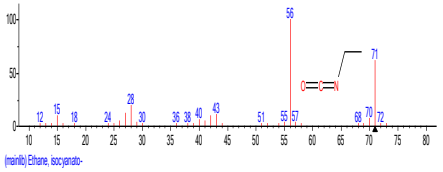
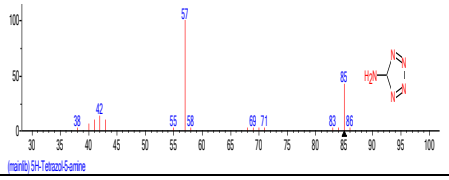
S/N	Compound	Retention Time (min)	Percentage concentration	Molecular formula	Molecular weight	Structure
1	Propanoic acid	15.663	29.763	$C_3H_6O_2$	74.0785	
2	Propanamide	16.270	23.641	C_3H_7NO	73.0938	
3	N,N-Dimethyl acetamide	18.295	5.044	C_4H_9NO	87.120	
4	Chlorine dioxide	18.648	13.160	ClO_2	67.45	
5	Ethane, isocyanato-	28.167	8.050	C_3H_5NO	71.0779	
6	5H-Tetrazol-5-amine	29.750	20.341	CH_3N_5	85.068	

Table 2: Essential oil in the Cap-base (Reticulum) of P. tuber-regium

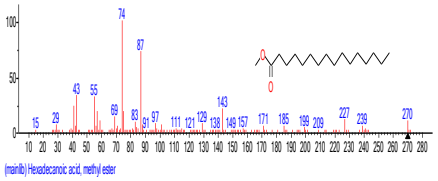
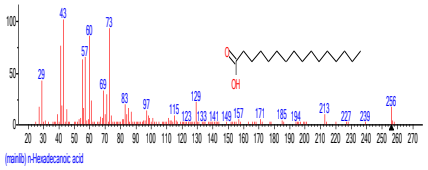
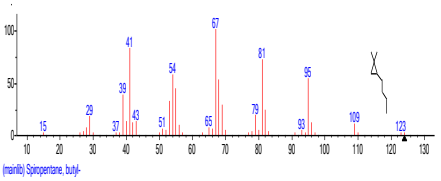
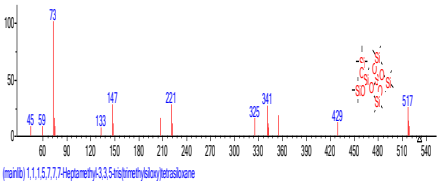
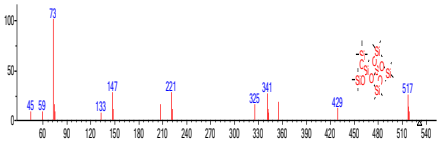
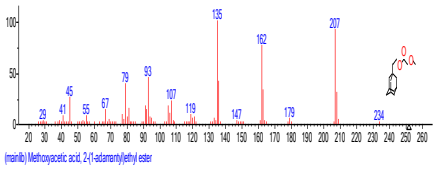
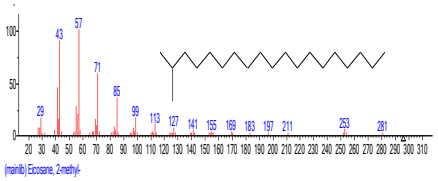
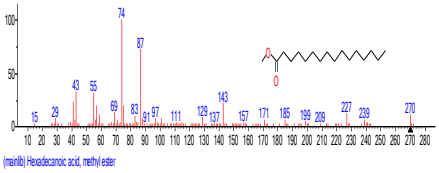
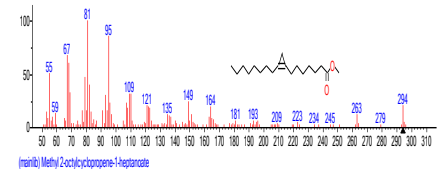
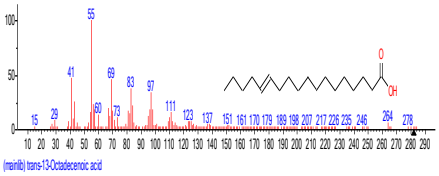
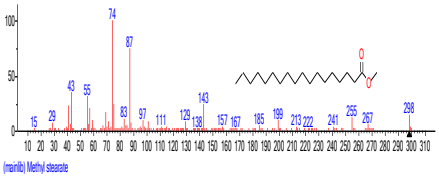
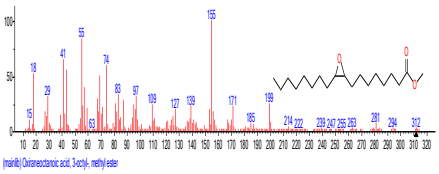
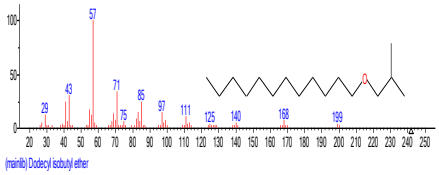
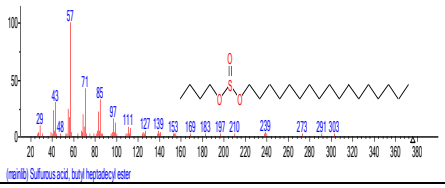
S/N	Compound	Retention Time (min)	Percentage concentration	Molecular formula	Molecular weight	Structure
1	Hexadecanoic acid, methyl ester	15.693	23.638	$C_{17}H_{34}O_2$	270.4507	
2	n-Hexadecanoic acid	16.289	20.170	$C_{16}H_{32}O_2$	256.4241	
3	Spiropentane, butyl-	18.641	4.584	C_9H_{16}	124.2233	
4	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	26.565	9.199	$C_{13}H_{40}O_5Si_6$	444.967	
5	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	27.898	10.578	$C_{13}H_{40}O_5Si_6$	444.967	
6	Methoxyacetic acid, 2-tridecyl ester	28.187	11.396	$C_{16}H_{32}O_3$	272.429	
7	Eicosane, 2-methyl-	29.768	20.435	$C_{21}H_{44}$	296.5741	

Table 3: Essential oil in the Stipe (Stem) of P. tuber-regium

S/N	Compound	Retention Time (min)	Percentage concentration	Molecular formula	Molecular weight	Structure
1	Hexadecanoic acid, methyl ester	15.819	26.677	$C_{17}H_{34}O_2$	270.4507	
2	Methyl 2-octylcyclopropene-1-heptanoate	17.904	12.603	$C_{19}H_{34}O_2$	294.472	
3	trans-13-Octadecenoic acid, methyl ester	17.969	25.837	$C_{19}H_{36}O_2$	296.4879	
4	Methyl stearate	18.348	14.579	$C_{19}H_{38}O_2$	298.504	
5	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)-	27.582	5.587	$C_{30}H_{50}O$	426.7174	
6	Dodecyl isobutyl ether	28.209	9.944	$C_{16}H_{34}O$	242.4406	
7	Sulfurous acid, butyl hepta decyl ester	29.811	4.773	$C_{21}H_{44}O_3S$	376.636	

4. Discussion

The high concentration of propionic in the stipe of this mushroom may be of protective and preservative mechanism from animal predators and microbial attack. The unpleasant pungent smell of this carboxylic acid irritates animals from attacking and feeding on this mushroom. The ability of propionic acid to inhibit the growth of mold and bacteria not only serve as a protective mechanism, but also preserve the mushroom from spoilage due to microbial attack. This corroborates the work of [5], which reported the use of sodium and calcium salts of propionic acid in the preservation of baked product. Moreover, propionic acid has been in use

as a food additive in the European Union (EU) [6], USA [7], Australia and New Zealand [8], with E number E280. Although propionic acid is produced as propionyl-CoA through catabolic degradation of fatty acids with odd numbers of carbon atoms, it can also be produce during the breakdown of certain amino acids. The generation of succinyl-CoA (an intermediate of citric acid cycle) through propionic acid degradation in vertebrates shows that the high concentration of propionic acid in this mushroom will have no adverse effect to human consumers except in rare cases of propionic acidemia.

The high concentration of chlorine dioxide in the stipe of this mushroom also adds to its protective arsenal. Chlorine dioxide has many applications both as an oxidizer and a disinfectant even at low concentrations [9]. It is one EPA registered biocide that is not negatively affect by pH, silica and phosphate and it does not lose potent over time. Chlorine dioxide can also be used in the treatment of blueberries, raspberries and strawberries against yeast and molds. This indicates that its high concentration in this mushroom serves as a broad spectrum antimicrobial agent that also helps to protect the mushroom against bacterial and fungal infection. Propanamide and Tetrazole presence of in the pilus of this mushroom may not be of defense or protective importance but rather of medicinal significance. Propanamide is a mono-substituted amide which reacts in different organic processes to form compounds which may be of pharmaceutical use to humans. For example, the generation of propanamides group via the insertion of methylene group to a 3-nitro-1H-1,2,4-triazole-based acetamides enhances its potency against chagasic and human African trypanosomiasis [10]. Tetrazole and its derivatives have also enjoyed pharmaceutical recognition not only because of their unique structure, but also because of their applications as antihypertensive, anticonvulsant, antialergic and antibiotic agents [11]. Moreover, antimicrobial, antifungal, anticancer, antinociceptive, antidiabetic, anti-inflammatory, analgesic as well as cyclooxygenase inhibitory activities of tetrazole have also been reported [11].

The seven compounds observed in the cap-base of this mushroom were different from those observed in the pilus. Though this variation shows a possible localization or confinement of compounds in the different parts of the mushroom, it also reflects the relevance and potentials of the different parts of this mushroom. For instance, the methyl ester of Hexadecanoic acid observed in the cap-base and the stipe of this mushroom not only induce autolysis of membranous structures but also inhibits both cellular phagocytic activity and nitric oxide (NO) production. It also reduces tumor necrosis factor alpha (TNFα), Prostaglandin E2 (PGE2), and Interleukin 10 (IL-10) without altering the ATP levels. Though the high concentration of eicosane in the cap-base of this mushroom may neither be of protective nor medicinal value, it may be responsible for the waxy nature of the cap-base. Moreover, its near unreactive nature may be responsible for the bridge in the movement of compounds form the pilus to the stipe and vice versa. Eicosane is a colourless, nonpolar molecule that is nearly unreactive except when ignited. It is practically insoluble in water and its phase transition at moderate temperature makes it a viable compound for phase change material [12].

Though the pesticidal, insectidal and nematicidal properties of n-Hexadecanoic acid acid, whose high percentage concentration was also observed in the cap-base of this mushroom, serves as a protective mechanism and may be responsible for the nemato-phagocytic potential of this mushroom, it also has some relevant medicinal properties. n-Hexadecanoic acid has shown some medicinal properties such as cancer preventive, antioxidant, hypochloesterolemic, antiandrogenic, haemolytic, and 5-Alpha reductase inhibitory properties [13]. The use of oils reach in n-hexadecanoic in traditional medicine for the treatment of rheumatoid arthritis [14], is attributed to the competitive inhibitory activity of n-hexadecanoic on phospholipase A2 [15]. Aside hexadecanoic acid, methyl ester, whose high percentage was observed in the pilus and stipe of this mushroom, high concentration of trans-13-octadecanoic acid, methyl ester was also observed in its stipe. Scientific reports on trans-13-octadecanoic acid indicates that its presence in this mushroom may not be of either preservative or protective significance but rather of medicinal importance. Though Krishnamoorthy and Subramaniam, (2014), reported the flavoring potentials of trans-13-octadecanoic acid, its medicinal role as an anti-inflammatory,

antiandrogenic, cancer preventive, dermatitigenic, antileukotriene-D4, hypocholesterolemic, 5-alphareductase inhibitor, anemiagenic roles out-weighs its protective potentials as an insectifugal agent [16].

5. Conclusion

The localization of phytochemicals in the different parts of the basidiocarp of this mushroom shows that each part of the basidiocarp has the potentials to synthesize its required compounds which maybe of relevance as either a protective or presevative compound to the mushroom.

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