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Mycochemical Profiling of *Hypsizygus ulmarius* (Bull. Ex Fr.) Redhead Fruit Bodies Cultivated on Local Nigerian Lignocellulosic Substrates

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ABSTRACT

Background and Objective: The demand for organic raw materials has increased significantly in the development of natural products, particularly following the COVID-19 pandemic, which intensified interest in the pharmaceutical, beverage, and cosmetic industries. This study aimed to profile aromatic myco-chemical compounds in *Hypsizygus ulmarius* sporophores cultivated on locally available Nigerian substrates. **Materials and Methods:** Mycelium of *Hypsizygus ulmarius* was cultured and maintained on Potato Dextrose Agar (PDA) and sorghum grain spawn, then inoculated onto sugarcane bagasse (SB), *Panicum maximum* (PM) straw, and their combination (SB+PM; 1:1). Aromatic compounds were extracted using cold and hot extraction techniques and analyzed via Gas Chromatography-Mass Spectrometry (GC-MS). Proximate composition and heavy metals were determined using Association of Official Analytical Chemists methods and Energy Dispersive X-ray Fluorescence (EDXRF), respectively. All experimental data were analyzed using Analysis of Variance (ANOVA) of the SPSS v25, and *post hoc* by Duncan's Multiple Range Test (DMRT) at $p<0.05$ while results were presented as mean±SD. **Results:** Sporophores cultivated on SB exhibited the largest cap size (7.14±0.84 cm) and weight (7.11±1.94 g), while those grown on PM had the longest stipe. Hot extraction yielded more volatile compounds than cold extraction. Octadecanoic acid methyl ester and Azulene were the most abundant compounds detected under cold and hot extraction methods, respectively. Heavy metals (Cr, Cd, Pb) were below permissible limits, whereas essential elements such as Fe and Zn showed significant variation ($p\leq0.05$). **Conclusion:** The findings highlight the potential of *H. ulmarius* as a valuable source of aromatic and nutritionally relevant compounds. Its commercial cultivation on local substrates could enhance the mushroom value chain and contribute to employment generation among Nigerian youth.

KEYWORDS

Hypsizygus ulmarius, aromatic compounds, sporophores, substrate

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INTRODUCTION

Molecular compounds in mushrooms occur as flavours, aromas and fragrances which are essential in the food, cosmetic and pharmaceutical industries. Flavours are volatile compounds in food stuff, whose aromatic properties are detected by smelling. They may be naturally present or synthesized during their processing by chemical reactions or microbial action. These compounds are usually present in very low concentrations[1,2].

Mushrooms have traditionally been relished as food or food flavouring agents because of their unique and subtle aroma[1]. The main odorants of mushroom aroma are eight-carbon compounds (C8). The 1-octen-3-ol is a characteristic aromatic compound of mushrooms; the profile of these essential aromatic molecular compounds varies with species and culture condition such as substrates. Cultivation conditions may also affect other mushroom properties, especially productivity, edibility and nutritional composition[3,4].

Mushroom flavours are mostly the unsaturated ketones, which contribute significantly to its characteristic aroma, largely responsible for their culinary umami taste appeal[2]. The main aromatic compounds in mushrooms have been investigated and identified mostly as the C8 group, which are mostly volatile organic compounds (VOCs), such as the 3-octanone, 3-octanol, 1-octen-3-ol, 2-octen1-ol and 1-octen-3-one. Among these aroma compounds, 1-octen-3-ol, also known as mushroom alcohol, is naturally found in all species of basidiomycetes and is considered to be the most important aroma compound for the culinary purposes[1]. This compound is present in most edible mushrooms in the amounts ranging from 1-4 mg/100 g of fresh fruit bodies and its bio-generation is due to oxygenation of the enzyme lipoxygenase in linoleic acid[5]. Lipoxygenase (LOX; linoleate: Oxygen oxidoreductase, EC 1.13.11.12) comprised a class of non-heme iron-containing dioxygenases, which is considered as a key enzyme in the oxidative degradation of lipid[4]. Apart from mushrooms, LOX is widely distributed in bacteria, algae, mammals and higher plants[1]. This enzyme acts on polyunsaturated fatty acid with cis,cis-1,4-pentadiene system, but geometrical configuration with cis-trans and trans-trans, which render it inactive or act as inhibitors to lipoxygenase catalysis[2]. Abdullah *et al.*[6] reported that the 13-hydroperoxy-9Z and 11-Ectadecadienoic acid [13-Z, E-HPOD] first result from the hydroperoxidation of linoleic acid by LOX, which is subsequently converted to 1-octen-3-one and 10-carbon compounds, and the former, finally reduced by alcohol oxidoreductase to 1-octen-3-ol[7,8].

Another important compound obtainable from mushrooms is azulene. It is a nonalternant 10 π-electron nonbenzenoid aromatic hydrocarbon, which has a fused structure of five and seven-membered rings[2]. It is used as a moisturizer for the treatment and prevention of skin diseases and other blemishes such as scaly skin, itchy skin, diaper rash, skin burns[7].

Similarly, mushrooms are considered a rich source of proteins, which make up 5% of the weight of a fresh fungus[1]. This amount is equivalent to 20-40% by weight of dry matter, with high contents of vitamins B and C complex[8,9]. Mushrooms are also rich in elements: Ca, P, Fe, K and low in Na, and is recommended for people with diabetes, high blood pressure and cardiac problems[7]. Chen *et al.*[9] studied the proximate content of *P. sajor-caju* and revealed that using different agro-wastes such as: Soybean, paddy straw, wheat, sunflower and Pigeon pea stalks, Soybean straws showed the maximum protein (25.80%), fat 92.82%) and ash (7.30%) contents[9]. He also reported that the variation in these nutrients could be due to their quality and quantity in the substrates[1].

Unlike other microbial sources of single cell proteins (SCPs), mushrooms have high consumer preference due to their unique taste[2]. They possess nutritional and medicinal properties, often referred to as neutraceuticals[10]. Mushrooms are considered to be good source of digestible proteins (10-40%), carbohydrates (3-21%) and dietary fibre (3-35%) on dry weight basis[9]. Edible species are limiting in sulphur-containing amino acids like cysteine and methionine[11]. Essential minerals such as iron, copper, zinc and manganese, which play important role in biological systems, are naturally found in mushrooms[2].

While fungi have long been utilized in the food industry, their potential for the industrial production of natural compounds has been overlooked. The general economic benefits of mushrooms should not be restricted to dietary, nutritional, pharmaceutical, or environmental, but could be extended to cosmetics or even pests and insects control. This study aimed to profile the aromatic molecular and mycochemical compounds of *H. ulmarius* fruit bodies cultivated on locally available Nigerian substrates.

MATERIALS AND METHODS

Study area and duration: The study was conducted at the Mushroom Research Laboratory of the Department of Biology, Federal University of Technology, Owerri (FUTO). The area is geographically located between Latitudes 5°396'N and 5°397'N and Longitudes 6°983'E and 6°987'E between April and September, 2025.

Spawn production/multiplication: Spawn of *H. ulmarius* was prepared using sorghum grains. The grains were subsequently boiled in tap water for 10-15 min using a gas-burning flame and completely drained of water before mixing with 2% (w/w) $CaCo_3$ and 4% $CaSO_4$ to optimize pH and prevent grain clumping, respectively; as recommended by Rudolph *et al.*[2]. They were further sterilized in an autoclave at 121°C for 30 min and allowed to cool at room temperature before they were aseptically inoculated with actively growing mother mycelial culture of *H. ulmarius,* by grain-to-grain transfer; and were subsequently incubated in the dark (0.25-180 Lux) at 272°C) until grains were fully colonized by *H. ulmarius* mycelia[12]. The completely colonized grain (spawn) was used to inoculate the sugarcane bagasse or final substrate during fruit body production[2-5].

Morphological measurements

Measurement of fruit body morphology: The effect of substrate on Pileus and stipe sizes of fruit bodies was determined at maturity. The mushrooms were harvested accordingly while Pileus and stipe sizes were measured in cm using meter rule[13].

Cap diameter: This was obtained by placing a transparent ruler across the centre of the pileus and reading off the diameter[13].

Cold extraction of molecular compounds: Chilled mushrooms (10 g) were macerated in a prechilled mortar and pestle (kept in ice bath) by using 10 mL of chilled water[12]. Pentadecane (SD fine chemicals, Mumbai) at a concentration of 5000 kg per 100 g to this paste and filled in a stoppered glass bottle. Chilled diethyl ether (10 mL) was added to it. The stoppered bottle was shaken to mix well all the contents and kept in a refrigerator (5°C) for 2 hrs. The contents were extracted with 10 mL of diethyl ether for another 2 times followed by 2 hrs of cold storage each time. After this, all the solvent was pooled and concentrated to 2 mL at room temperature[9]. This was analyzed for the presence of flavour components using Gas Chromatography and a Mass Spectrometer (GC-MS).

Hot extraction of molecular compounds: The extract for analysis of volatiles was prepared by using the method of Zawirska-Wojtasiak[14]. Ten grams of mushroom sample was macerated by using a porcelain mortar and pestle in 100 mL distilled water and pentadecane at the concentration of 500 µg per 100 g of mushroom was added to it as an inner standard. This was allowed to stand for15-20 min for enzyme activation and then steam distilled at 50°C for 2 hrs and condensed fraction was collected into a well stoppered flask. This condensate (about 50 mL) was extracted 3 times with equal concentration of dichloromethane and the pooled solvent was dried over anhydrous Na_2SO_4 It was further concentrated to 2 mL at room temperature and analyzed for flavour components by using GC-MS analytical protocol[12,14].

Proximate analysis: Proximate analysis was conducted on each of the 3 fruit body samples. The protein, ash, fat, dry matter and crude fibres were determined according to the modified method by Stihi *et al.*[15] while Carbohydrate contents were determined by difference i.e:

$$CHO\ (\%) = 100-(Ash\ (\%)+Protein\ (\%)+Fat\ (\%)+Moisture\ (\%)$$

Determination of heavy metals: The amount of Fe, Cu, and Zn in the sample was estimated by Energy Dispersive X-ray Fluorescence (EDXRF) technique according to the method of Stihi *et al.*[15]. Cadmium (Cd) and Lead (Pb) were determined by the method of the calibration curve according to the absorption concentration. Several standard solutions of different known concentrations were prepared and the elemental concentration in the unknown sample was determined by extrapolation from the calibration curve. All fruit body sample concentrations were reported as mg/kg dry weight of material.

Statistical analysis: All experimental data were analyzed using Analysis of Variance (ANOVA) with SPSS version 25. Treatment means were separated using Duncan's Multiple Range Test (DMRT) at a 95% confidence level ($p<0.05$). Results were expressed as mean±standard deviation.

RESULTS AND DISCUSSION

Results of morphological features in Table 1 showed that fruit bodies from SB had the largest cap (7.14±0.84 cm) and WT (7.11±1.94 g) while those cultured on PM had the longest stipe compared to SB and SB+PM, as shown in Table 1. These mean values generally represent considerably large fruit body sizes. It has been generally noted that substrate and genetic characteristics are the two main factors that determine the morphological features of mushrooms[16,17]. Stihi *et al.*[15] reported that cap diameter and stipe length of fruiting bodies of mushrooms are dependent on the amount of oxygen circulation at the onset of primordial initiation and fruit body development[16]. Therefore, the large sizes of fruiting bodies observed in this study could be due to ample supply of oxygen in the cropping room during fruitbody development. These results significantly relate to those published by Ekanayake *et al.*[13] in an experiment involving the use of local substrates for the production of oyster mushrooms.

Azulene is a very important organic compound and an isomer of naphthalene widely used as a moisturizer to treat or prevent dry rough, scaly, itchy skin and other minor skin irritations[12].

Table 1: Macroscopic characteristics of *H. ulmarius*

Substrate	SB	PM	SB+PM
CD cm	7.14±0.84^{a}	5.10±0.57^{b}	4.90±0.23^{a}
SL cm	2.78±0.16^{a}	3.14±0.36^{a}	2.89±0.10^{a}
WT g	7.11±1.94^{a}	3.91±0.74^{b}	3.09±0.38^{a}

Means followed by the same alphabet within column are not significantly different by DMRT ($p\geq0.05$), means±SEM (n = 3), SB: Sugarcane bagasse, PM: *Panicum maximum*, CD: Cap diameter, SL: Stipe length and WT: Weight

Table 2: Nutritional Information of *H. ulmarius*

Substrate nutritional info.	SB	PM	SB+PM
MC	10.29±0.01^{g}	10.13±0.03^{f}	10.19±0.08^{f}
DM	89.72±0.00^{f}	89.87±0.03^{e}	89.81±0.08^{e}
ASH	2.18±0.02^{e}	2.44±0.02^{c}	2.51±0.06^{d}
CP	22.07±0.53^{d}	17.61±0.23^{b}	19.61±0.09^{c}
EE	2.14±0.02^{c}	2.31±0.11^{b}	2.16±0.05^{c}
CF	3.64±2.99^{b}	0.79±0.06^{b}	0.77±.040^{b}
CHO	30.03±2.47^{a}	23.14±0.42^{a}	25.04±0.13^{a}

MC: Moisture content, DM: Dry matter, ASH: Ash, CP: Crude protein, EE: Ether extract, CF: Crude fiber, CHO: Carbohydrates, Values are expressed as g/100 g (%, dry-weight basis) and presented as mean±SE. Different superscript lowercase letters indicate significant differences ($p<0.05$)

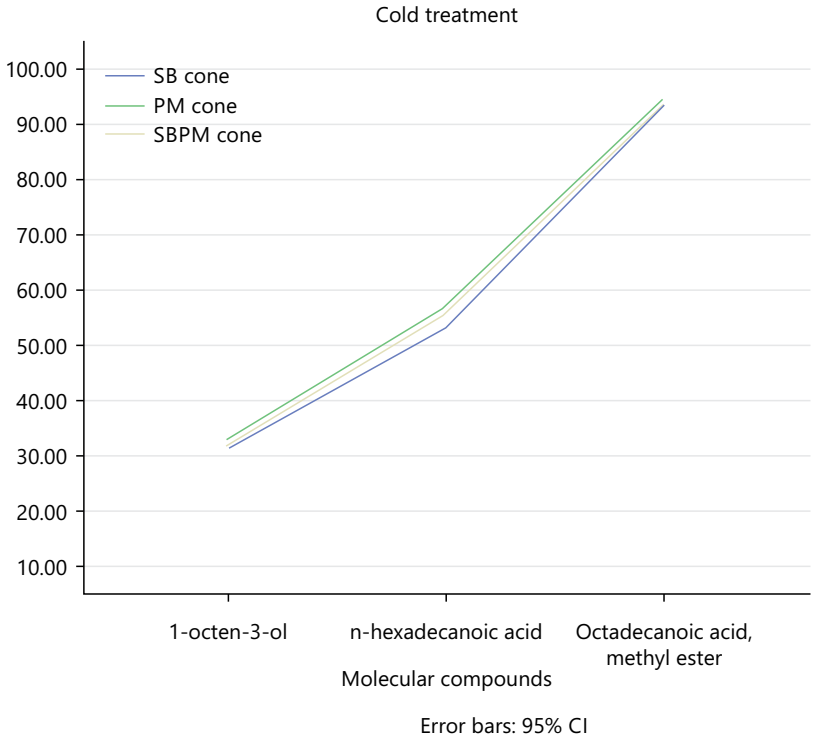

Fig. 1: Cold extraction of molecular compounds
y-axis: % abundance

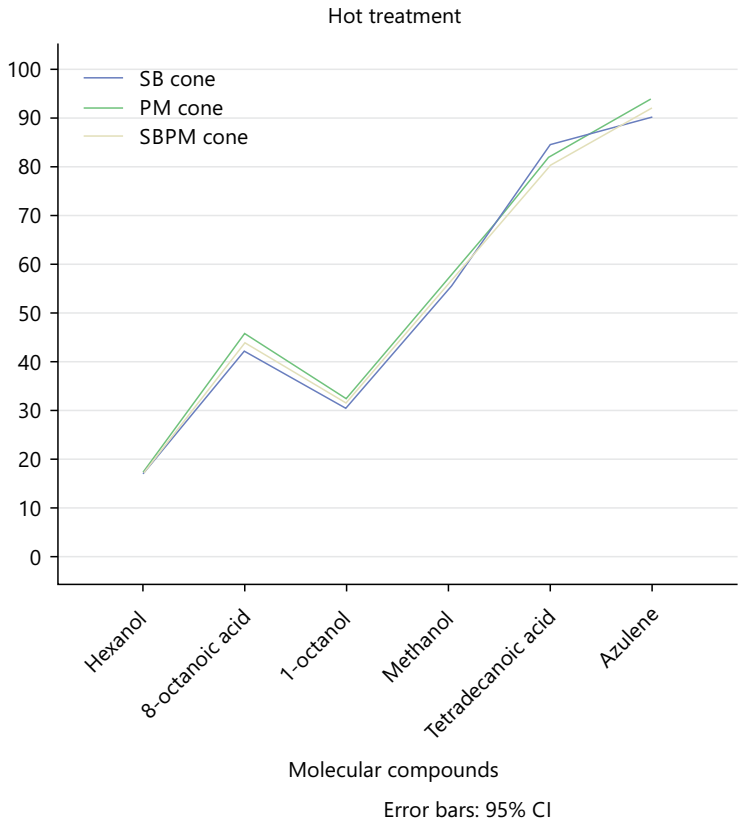

Fig. 2: Hot extraction of molecular compounds
y-axis: % abundance

Aromatic compounds are the most important organoleptic characteristic of mushroom products[15]. Misharina *et al.*[12] stated that the principal compounds producing the aroma of mushrooms are aliphatic alcohols and ketones and the qualitative and quantitative compositions of volatile substances significantly depend on extraction method and substrate.

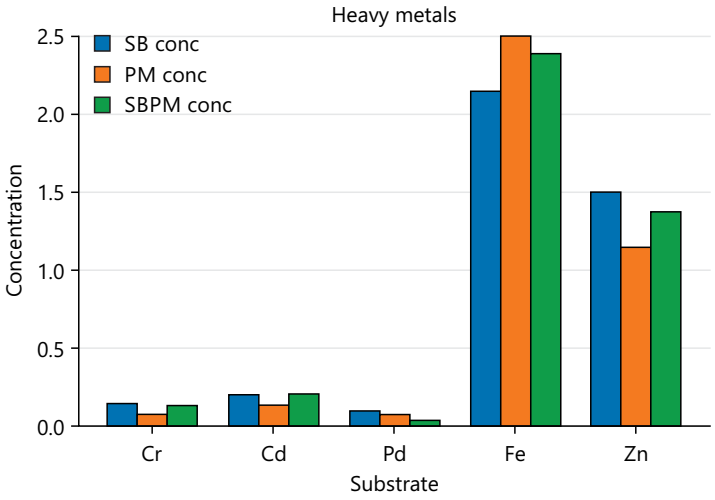

Fig. 3: Heavy metals concentration of mushroom fruitbodies
y-axis: conc µg/g

Results of proximate analysis of *H. ulmarius* (Table 2) show that crude protein content of fruitbodies cultivated on SB (22.07±0.53) was higher than those of PM and SB+PM. Mushroom protein has been identified as a valuable additive to the human diet[16]. Similarly, the results of the proximate content of *Pleurotus sajor-caju* revealed that using different agro-wastes such as Soybean, paddy straw, wheat or dowar straws, sunflower and Pigeon pea stalks showed maximum protein (25.80%), fat 92.82%) and ash (7.30%) content.

Carbohydrate (CHO) was the second highest nutrient in fruitbodies across all the substrates after dry mater. Ether extract content recorded in fruitbodies from all the substrate was low. This was in conformity with the reports by Stihi *et al.*[15]. Hausiku and Mupambwa[20] reported that mushrooms generally contain low-oil and fat, and because of the low content of oil and fat in mushrooms, they are recommended as good supplements for patients with cardiac problems.

Results of heavy metals content (Fig. 3) of the mushrooms show that some heavy metals were detected in relatively low concentrations in the fruitbodies harvested from the three substrate[21-25]. Unlike Fe and Zn, results showed that Cr, Cd and Pb contents were not significantly different ($p \geq 0.05$) in fruitbodies cultivated on the three substrates. The recorded Pb and Cd values fall below the European Communities (Commission Regulation [EC] No 466/2001) admitted maximum level at 2 and 3 mg/1 kg d.w, respectively, in cultivated mushrooms. Different studies[22,26-28] reported that mushrooms can uptake heavy metals from the substrate by means of mycelia or even from the atmosphere through their stipe and pileus tissues. The distribution of these heavy metals is usually uneven within the fruiting body[29-32].

CONCLUSION

This study demonstrated that locally available lignocellulosic substrates significantly influenced the mycochemical composition and nutritional quality of *Hypsizygus ulmarius* fruit bodies. Hot extraction proved more effective than cold extraction for recovering volatile aromatic compounds, with Azulene predominating in hot extracts and Octadecanoic acid methyl ester in cold extracts. Mushrooms cultivated on sugarcane bagasse exhibited superior morphological characteristics and higher protein content, while all samples contained heavy metals within permissible safety limits. These findings indicate that *H. ulmarius* cultivated on indigenous substrates represents a safe and valuable source of nutritionally important and industrially relevant bioactive compounds, supporting its potential application in the food, pharmaceutical, and cosmetic industries. Further studies should evaluate the biological activities and commercial-scale production of the identified aromatic compounds.

SIGNIFICANCE STATEMENT

This study provides novel information on the influence of locally available Nigerian lignocellulosic substrates on the aromatic compound profile, nutritional composition, and heavy metal content of *Hypsizygus ulmarius*. The findings identify sugarcane bagasse as a promising substrate for producing mushrooms with enhanced nutritional and bioactive properties while maintaining food safety standards. These results contribute to the sustainable utilization of agricultural residues, support value addition in mushroom cultivation, and provide a scientific basis for the development of mushroom-based products for the food, pharmaceutical, and cosmetic industries.

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