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Research Article

Volatile and Non-polar Chemical Constituents of Cultivated Oyster Mushroom *Pleurotus ostreatus*

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ABSTRACT
Oyster mushrooms have been widely used as food because of their nutritional properties. Despite being regarded as good potentials in producing bioactive compounds without causing toxicity to humans, little phytochemical studies have been done on Kenyan widely cultivated Oyster mushroom. The present investigation was carried out to determine the possible chemical components from *P. ostreatus* by GC-MS Technique. This analysis revealed that the volatile non- polar compounds for *P. ostreatus* extract with the highest normalized amounts were 1, 3-dimethylbenzene (32.803%) and phenyl ethyl alcohol (21.557%). Other compounds like 1-octen-3-ol (0.864%), 1,2-Benzenedicarboxylic acid (0.783%), n-undecane (0.127%), cedrol (0.106%) and heptadecane (0.146%) were also obtained as minor components from the *P. ostreatus* crude extract of hexane.

Key words*:* Oyster mushroom, *P. ostreatus*, volatiles, GC-MS analysis

INTRODUCTION

Oyster mushrooms, scientifically known as *Pleurotus sp.* are saprophytic organisms which obtain nutrients through metabolizing non-living organic matter[1]. Oyster mushrooms grow wildly in Western part of Kenya and are also easily artificially cultivated. They belong to the class Basidiomycetes, family of Polyporaceae and the genus of *Pleurotus*[2]. To date, approximately as many as 70 species of *Pleurotus* have been recorded and new species are discovered more or less frequently although some of these are considered identical to previously recognised species[3,4]. *P. ostreatus* is among the most cultivated species among the oyster mushrooms and specially considered due to its high nutritional values and medicinal importance[5]. In addition, *P. ostreatus* is low in calories, sodium, fat and cholesterol, while rich in protein, carbohydrate, fibre, vitamins and minerals. These nutritional properties make this mushroom a good dietary food[2]. Reports show that *Pleurotus* sp. mushrooms have great potential for the production of non polar compounds of fungal aroma that stimulate the appetite and give mushroom dishes a characteristic flavour[6,7,8]. The main substances responsible for the aroma of most edible mushrooms like *P. ostreatus* are octavalent carbonate alcohols and carbonyl compounds, among them 1-octanol, 3-octanol, 3-octanon, 1-caprynol-3-ol, 1-octynol-3-ol, 2-octynol-3-ol and 1-caprynol-3-on[9]. Owing to the presence of 1-octynol-3-ol which dominates in *P. ostreatus*[10]. The aroma of the mushroom also depends on the content of amino acids, nucleotides, and some other elements such as nitrogen, phosphorus, potassium, sulphur, iron and zinc and components of the autoxidation of unsaturated fatty acids[11]. Oyster mushrooms are also known to be rich sources of different classes of compounds including; flavonoids, polysaccharides, triterpenoids, lentinan, lovastatin, pleuran, steroids, glycopeptides, saponins, xanthones, coumarins, alkaloid, ß-glucan, proteoglucan, lectin, purin, fenil, fatty acids and propanoid[12,13]. These metabolites have been used as antimicrobials with fewer side effects and are a prolific resource for drugs because of their antitumor, antibacterial, antifungal and reducing hypercholesterolemia activities[14,15]. We therefore report the results of the GC-MS analyses of volatile and non-polar compounds, identified from this species.

MATERIAL AND METHODS

Sample collection and identification

The fresh fruiting bodies *P. ostreatus* materials were collected from the Mushroom Project farm Masinde Muliro University, Kakamega County in Western Kenya. The mushroom fruiting bodies were characterized and identified as *P. ostreatus* according to Das *et al.*[4] and Selima[15]. Confirmation was done by comparison with authentic samples.

Extraction

Samples of *P. ostreatus* mushroom were subjected to soxhlet extraction in hexane. 5 g of solid fresh material were placed inside a thimble made from thick filter paper which was loaded into the main chamber of the soxhlet

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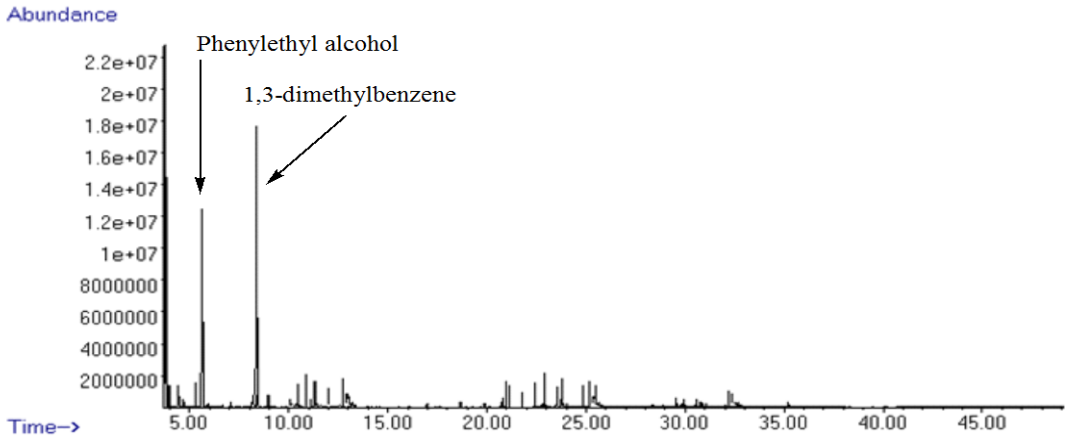

Figure 1: GC-MS profile for Oyster mushroom soxhlet hexane extract.

Table 1: GC-MS of Pleurotus ostreatus soxhlet hexane extracts

Retention time (RT)	Area %	Compound
5.2593	0.132	3-Pentanol, 3-methyl-
5.6625	21.557	Phenyl ethyl alcohol
5.8416	0.148	Heptane, 3-methyl-
6.3568	0.143	Cyclopentanol, 1-methyl-
7.0735	0.548	Butyl acetate
8.4397	32.803	Benzene, 1,3-dimethyl-
10.5003	0.161	Mesitylene
10.8586	0.864	1-Octen-3-ol
10.993	0.183	3-Octanone
11.4633	0.162	1,4-Dichlorobenzene
12.3144	0.136	2E-Tridecen-1-al
13.0312	0.127	n-Undecane
20.1758	0.106	Cedrol
21.0269	0.146	Heptadecane (C17)
22.9083	0.783	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
23.401	0.329	Methyl hexadecanoate
23.7146	0.292	Hexadecanoic acid
23.8266	0.517	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester
24.9017	0.174	gamma.-Linolenic acid, methyl ester
25.036	0.255	Methyl linoleate

extractor. The soxhlet extractor was then equipped with the condenser. The hexane solvent in the flask was heated to 55ºC to vapourise and then condensed dripping back down into the chamber housing the solid *P. ostreatus* material. The chamber containing the *P. ostreatus* was slowly filled with hexane and the oil in the *P. ostreatus* dissolved in the warm hexane. When the soxhlet chamber was almost full, the chamber was automatically emptied by a siphon side arm, with the solvent running back down the distillation. This was repeated six more times and afterwards the solvent that contained the extract was removed and subjected to GC-MS analysis.

Analysis and Identification of non polar compounds

Both qualitative and quantitative characteristics of the extract were studied using gas-chromatography (GC) and gas-chromatography/Mass Spectrometry (GC-MS) techniques[16]. The constituents of the hexane oils were identified by analysis of their mass spectra, direct comparison of their mass spectra to the Wiley NBS and NIST databases or library of mass spectra and co-injection with authentic standards on the GC.

The GC analyses were performed with a Hewlett Packard HP 5890A Gas Chromatography equipped with a flame ionization detector (at 230 °C). A fused silica capillary column (Hewlett Packard, 50 m x 0.22 mm x 0.33 mm CD) coated with methyl silicon (0.3 µm film thickness) was used with nitrogen as the carrier gas. All GC analyses were performed in the splitless mode with the injector temperature at 270 °C. The oven temperature was programmed from 60 °C isothermal for 7 min, to 120 °C at 5 °C per min, then to 180 °C at 10 °C per min and finally to 220 °C at 20 °C per min, where it was maintained for l0 min. Peak areas were calculated using a Hewlett Packard 3393 B series integrator and together with their GC retention times, compared to those of authentic samples.

RESULTS AND DISCUSSION

From the GC-MS spectrum (Figure 1), the hexane extracts from *P. ostreatus* resulted in the identification of 20 major compounds (Table 1). The identified compounds can be mainly divided into three groups according to the diverse functional groups. They are alkanes, alcohols, fatty acids and other organic compounds. Among these volatile compounds, alcohols and acid derivatives dominated over the main non- polar components. The volatile non- polar compounds for *P. ostreatus* with the highest normalized amounts were 1,3-dimethylbenzene (32.803%) and phenyl ethyl alcohol (21.557%). Other compounds like 1-octen-3-ol (0.864%) and 1,2-Benzenedicarboxylic acid (0.783%). n-undecane (0.127%), cedrol (0.106%) and heptadecane (0.146%) were obtained as minor components from the *P. ostreatus* crude extract of hexane. These aroma characteristics in *P. ostreatus* could strongly associated with these volatile compounds like phenyl ethyl alcohol, 1-octen-3-ol, 3-octanone, Tridecen-1-al and esters. The findings that

phenyl ethyl alcohol was the major constituent in the fresh *P. ostreatus* sample was consistent with that reported by Overton[17]. However, many other major constituents in this study were not reported in his analysis. Phenyl ethyl alcohol is known to provide fresh, sweet aromatic floral, strongly reminiscent of rose with a hyacinth nuance in mushrooms.

Dijkstra (2010)[18] reported 1-octen-3-ol, formed by the enzymatic breakdown of linoleic acid as the major volatile from most mushroom species. In this study, only modest amounts of 1-octen-3-ol (0.864%) were found in the "fresh' *P. ostreatus* samples which has the general mushroom-like aroma[19]. The results were in line with the recent GC-MS studies on petroleum ether Soxhlet extract of dried fruiting bodies of *Pleurotus ostreatus* by Suseem *et al*.[20]. The studies revealed the presence of volatile compounds which were identified as majorly fatty acid esters. Non polar and volatile compounds of *P. ostreatus* helps provide further explanation for its flavour and odor active properties. These results indicate that the presence of some non polar and volatile compounds present in this mushroom species give the chemistry of the aroma produced by this mushroom species.

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