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Volatilomes of milky mushroom (*Calocybe indica* P&C) estimated through GCMS/MS

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Abstract
The volatilomes of both fresh and dried samples of milky mushroom (*Calocybe indica* P&C var. APK2) were characterized with GCMS/MS. The gas chromatogram was performed with the ethanolic extract of the samples. The results revealed the presence of increased levels of 1, 4:3, 6-Dianhydro-α-d-glucopyranose (57.77%) in the fresh and oleic acid (56.58%) in the dried fruiting bodies. The other important fatty acid components identified both in fresh and dried milky mushroom samples were octadecenoic acid and hexadecanoic acid, which are known for their specific fatty or cucumber like aroma and flavour. The aroma quality of dried samples differed from that of fresh ones with increased levels of n- hexadecenoic acid (peak area - 8.46 %) compared to 0.38% in fresh samples. In addition, α-D-Glucopyranose (18.91%) and ergosterol (5.5%) have been identified in fresh and dried samples respectively. The presence of increased levels of ergosterol indicates the availability of antioxidants and anticancer biomolecules in milky mushroom, which needs further exploration. The presence of α-D-Glucopyranose (trehalose) components reveals the chemo attractive nature of the biopolymers of milky mushroom, which can be utilized to enhance the bioavailability of pharmaceutical or nutraceutical preparations.

Keywords: GCMS/MS, Milky mushroom (*Calocybe indica* P&C var. APK2), Octa and hexadecanoic acid, Trehalose, Volatilomes

1. Introduction
Mushrooms are known to produce a wide range of volatile and flavour compounds with distinct profiles that may vary according to the species, variety and sometimes due to cultural conditions (Rapior *et al.* 1997) [21]. The flavour profile also changes when mushrooms are dried, primarily due to high level of oxidation (Morath *et al.* 2012) [17]. Solvent extraction, vacuum distillation, nitrogen flow conveyance and capillary gas chromatography are normally followed for the concentration of flavour compounds. Depending upon the extraction method, nature of sample and sample preparation procedure significant changes in flavour profiles of mushrooms have been reported (Beltran-Garcia *et al.* 1997) [2]. Analyzing the complete volatilomes of freshly harvested samples of *Calocybe indica* sporophores, (Chandravadana *et al.* 2005) [4] confirmed the presence of an eight carbon volatile compound, 1-octen-3-ol (58.3%) and n-octanol (17.9%). They also concluded that the concentration of these compounds decreased up to 10.6% and 2.4%, respectively after drying. Noticeably, benzyl alcohol and n-Hexanal present in traces in the fresh mushroom samples increased to about 10.2% and 15.3%, respectively after drying.
Linoleic and palmitic acids were the predominant fatty acid fractions reported in oyster mushroom (*Pleurotus florida*) samples by Kwon and Uhm (1984) [12]. The involvement of these compounds in fungal aroma, interactions with pests, pathogens and reproductive events have been elaborately reviewed by Combet and his co-workers. In most the fungi, it is understood that linoleic acid is oxidized to form a 10-hydroperoxide intermediate, which is then cleaved to form an eight-carbon volatile (1-octen-3-ol) and a ten-carbon oxoacid (10-ODA) by Wurzenberger and Grosch (1984c) [26]. The 10-Oxodecanoic acid (10-ODA) possesses hormone-like properties accelerating the growth of mushroom stipe and development of fungal structures. Hence, it is suggested that both 1-octen-3-ol and 10-ODA could work together to regulate the transition between vegetative and reproductive growth in fungi (Champavier *et al.* 2000) [3]. Trehalose released by the mycelium of *Laccaria bicolor* was shown to be a chemo attractant for pseudomonads (Frey-Klett *et al*. 2007) [8].

The aroma compounds of fresh and dried samples of *C. gambosa* were extracted by an aroma extract dilution analysis (AEDA) system to identify the key flavour compounds and the total volatilomes were quantified by GC flame ionization detection by Kleofas *et al.* (2015) [11]. The key odour compound detected in the fresh sample was (E)-non-2-enal, which, together with (E)-non-2-en-1-ol was found to be responsible for the characteristic flavour and cucumber-like odour. However, in the dried fruiting bodies, odour compound like 3- methylbutanoic acid were dominating and (E)-non-2-enal was not at all detected. Hence, the present study was undertaken to investigate on the volatilomes of milky mushroom, *Calocybe indica* P&C var. APK2 through GC-MS/MS flavour spectrum.

2. Materials and Methods

Samples of fresh milky mushroom sporophores, *C. indica* P&C var. APK2 grown on paddy straw substrate were collected from the Mushroom Research and Training Centre, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India. The fresh experimental samples included mixed preparation of both pileus and stipe, collected at harvest stage from five randomly selected mushrooms. For making dried mushroom samples, freshly cut sporophores were oven dried at 55º C for 6 h, until the moisture content of the sample reached 12 per cent. The dried samples were macerated with liquid nitrogen to obtain powdered form for the extraction of flavour compounds.

2.1 Extraction of volatilomes

Volatile compounds from the samples were extracted following the method suggested by Srinivasan and Kumaravel (2015) [23]. A sample size of 30 g of fresh or dried and powdered milky mushroom tissue was soaked in 30 ml of ethanol overnight and then filtered through a rough filter paper. The filtrate was then concentrated to one ml by flushing nitrogen gas into the solution. The concentrate along with 2g sodium sulfate (used to remove the sediments and traces of water in the filtrate) was once again filtered through Whatman No.41 filter paper. The final sample was subjected to volatilomes analysis.

2.2 GC-MS/MS analysis

GCMS/MS analysis of the samples through electron ionization (GC-MS/EI) mode was performed at Food Safety and Quality Testing Laboratory, Indian Institute of Crop Processing Technology, Thanjavur, Tamil Nadu. The GC-MS/MS (Scion 436-GC Bruker model) coupled with a triple-quadruple mass spectrophotometer with fused silica capillary column BR-5MS (5% diphenyl / 95% dimethyl polysiloxane); length-30m; Internal diameter-0.25 mm; thickness- 0.25µm was used during the experimentation. Helium gas (99.99%) was used as the carrier gas at a constant flow rate of one ml per min and an injection volume of 2 µl was employed (split ratio of 10:1). The column oven temperature program is given below: 110 °C hold for 2 min; up to 200 °C @ 10 °C per min-no hold; up to 280 °C @ 5 °C per 10 min hold. The injector temperature was maintained at 280 °C and the total GC running time was 38.50 min. This last increase was to clean the column from any residues. The mass spectrometer was operated in the positive electron ionization (EI) mode with ionization energy of 70eV. The solvent delay was 0-3.5 min. A scan interval of 0.5 sec. and fragments from m/z 50 to 500 Da was programmed. The inlet temperature was set at 290 °C, source temperature at 250 °C and total running time was 37.50 min. The relative percentage of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was MS Work station 8. The NIST Version 2.0 library database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for identifying the chemical components. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

3. Results and Discussions

The major volatilomes identified through GC-MS/MS in fresh and matured fruiting bodies at harvest stage of milky mushroom was 1,4:3,6-Dianhydro-α-d-glucopyranose with the highest peak area of 57.77% followed by α,β-Gluco-octonic acid lactone recording a peak area of 22.16% (Table 1 and Fig.2). European Food Safety Authority (2010) determined that the 1,4:3,6-Dianhydro-α-d-glucopyranose is one of the smoke flavouring primary product, which was obtained from beech wood sawdust (*Fagus grandifolia*) used for preservation purposes and they demonstrated that the smoke flavour is produced by controlled thermal degradation of wood in a limited supply of oxygen (pyrolysis). It has also been sufficiently demonstrated that, it does not cause risk to human health. The most abundant compound that was found to be present in dry milky mushroom was oleic acid, which showed the highest peak area of 56.58% followed by 9-Octadecenoic acid, methyl ester, (E)- with the peak area of 16.94 % (Table 2 and Fig 2). In addition to these compounds (both in fresh and dried milky mushroom samples) different types of terpenes, alcohols, fatty acids and sterols. The most abundant compound was present in dry milky mushroom was oleic acid with the highest peak area of 56.58% followed by 9-Octadecenoic acid, methyl ester, (E)- with the peak area of 16.94 % (Table 2 and Fig. 2). Pedneault *et al.* (2006) [19] reported that oleic acid is a major component available in some mushrooms that belong to the genus *Boletus*. This bioactive compound is known to strongly inhibit the activity of human telomerase in a cell-free enzyme assay (Masako *et al.* 2002) [14]. Further, Won *et al.* (2007) [25] proved that, it is an efficient inhibitor of glucosyl transferase.

Table 1: Volatile compounds identified in the freshly harvested milky mushroom

No.	RT	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area %
1.	6.55	1,4:3,6-Dianhydro-α-d-glucopyranose	$C_6H_8O_4$	144	57.77
2.	10.25	α-D-Glucopyranose, 4-O-β-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	342	18.91
3.	12.33	α,β-Gluco-octonic acid lactone	$C_8H_{14}O_8$	238	22.16
4.	13.11	Undecanoic acid, 11-mercapto-	$C_{11}H_{22}O_2S$	218	0.04
5.	13.92	Pyrano[4,3-b]benzopyran-1,9-dione, 5a-methoxy-9a-methyl-3-(1-propenyl)perhydro-	$C_{17}H_2O_5$	308	0.04
6	14.29	10-Undecynoic acid, trimethyl ester	$C_{14}H_{26}O_2$	254	0.20
7.	15.53	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.38
8.	23.79	Glycerol 1-palmitate	$C_{19}H_{38}O_4$	330	0.36
9.	26.83	d-Galactose, 1,2:3,4-di-O-isopropylidene-, 6-decanoate	$C_{22}H_{38}O_7$	414	0.13

Table 2: Volatile compounds identified in the dried milky mushroom in powder form

No.	RT	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area %
1.	6.08	3-Hydroxymethyl-2-trimethyl pentane	$C_9H_{22}O_2Si$	190	0.54
2.	9.39	Allyl (2tetrahydrofurylmethoxy) dimethyl	$C_{10}H_{20}O_2$	200	0.78
3.	11.72	β-D-Glucopyranose, 4-O-β-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	342	0.22
4.	14.30	10-Undecynoic acid, trimethyl ester	$C_{14}H_{26}O_2$	254	0.15
5.	15.10	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.55
6.	15.68	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	8.46
7.	17.50	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	1.23
8.	18.28	Oleic Acid	$C_{18}H_{34}O_2$	282	56.58
9.	18.47	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296	16.94
10.	22.68	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	310	1.08
11.	23.54	Glycerol 1-palmitate	$C_{19}H_{38}O_4$	330	1.20
12.	24.64	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3β,5Z,7E)-	$C_{27}H_{44}O_3$	416	1.06
13.	26.15	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	356	2.99
14.	29.80	9(11)-Dehydroergosteryl benzoate	$C_{35}H_{46}O_2$	498	1.33
15.	33.51	9(11)-Dehydroergosterol tosylate	$C_{35}H_{48}O_3S$	548	0.68
16.	34.38	Ergosterol	$C_{28}H_{44}O$	396	5.51
17.	34.76	Ergosta-14,22-dien-3-ol, (3β,5α,22E)-	$C_{28}H_{46}O$	398	0.71

The other important compounds found both in dry and fresh mushrooms were Ergosterol (5.51%) and sugar related compounds like α-D-Glucopyranose (18.91%), respectively. Ergosterol is the principal sterol of the cell membrane of fungi by Czub and Baginski (2006) [5] is known to activate expression of a number of defense genes and increase the resistance of plants against the pathogens by Lochman and Mikes (2006) [13]. The ergosterol is also an important dietary source of vitamin D. Novaes *et al.* (2011) [18] reviewed that the ergosterol or provitamin D2 is the precursor of ergocalciferol, an important substrate in vitamin D biosynthesis frequently found in the lipid fraction of *Agaricales* extracts. The pharmacological effects of ergosterol rich mushrooms have been reported in several clinical studies with promising results in the treatment of breast cancer mainly mediated through the improvements in immunological and hematological parameters, ultimately enhancing the quality of life in cancer prone patients. Afieroho and Ugoeze (2014) [1] performed GCMS and reported about the presence of α-ergosterol in *Lentinus tuber regium* with a peak area percentage of 2.16. This steroid component predominantly possessed anti-cancer, antioxidant, hypoglycemic, hypocholesterolemic and thyroid inhibiting properties. The results of present study obtained through GCMS analysis obviously indicated increased levels of ergosterol (peak area - 5.51%) in milky mushroom samples. This may be a good indication relating vitamin D synthesis by the milky mushroom fungus, *Calocybe indica* (P&C) var. APK2. Jedinak and Sliva, (2008) [9] suggested that *P.ostreatus,* known to contain ergosterol could significantly inhibit the proliferation of human breast and colon cancer cells by the means of cell cycle arrest.

Raina *et al.* (2014) [20] quantified the ergosterol content in four different mushrooms using HPLC and indicated that *Calocybe indica* contained 243 µg/g while, other commonly cultivated mushrooms like *Pleurotus florida* and *Volvariella volvacea* contained 113µg and159 µg/g of samples, respectively. They further reported that the well known medicinal mushroom, *Ganoderma lucidum* contained comparatively increased levels of ergosterol (403 µg/g). Working with *Agaricus, Boletus, Amanita, Canthatrellus* and *Coprinus*, Kalac (2016) [10] reported the presence of mannitol and trehalose (α,α, trehalose formed by two molecules of α-D-glucopyranose bound by 1-1 glycosidic bond) in those mushrooms. These water soluble sugars partly contributed to the taste of such mushrooms. He also concluded that these sugars could participate to supply a considerable proportion of C for the growth and firmness of fruiting bodies. Trehalose was found to have growth-promoting effects on the mycorrhization helper bacteria (MHB), *Pseudomonas monteilii,* when inoculated with the ECM fungus *Pisolithus albus* (Duponnois and Kisa, 2006) [6]. The chromatogram of the ethanolic extract of fresh and dry fruting bodies of milky mushroom is given in Fig. 1 and 2. The results indicated that among the compounds identified in *Calocybe indica* var. APK2, the notable ones were polyunsaturated fatty acids like Octadecenoic acid and Hexadecanoic acid (present in both dry and fresh samples), which are known for their fatty or cucumber like flavour. The aroma quality of dried fruiting bodies differed from that of fresh samples by the presence of n- hexadecenoic acid (peak area- 8.46 % compared to 0.38% in fresh samples). Moliszewska (2014) [16] reported that the most characteristic flavour compound is defined mainly by C8 volatiles, which are known to exhibit fruit-like, cucumber, potato, garlic, cheese-garlic, and even flour-like smell in mushrooms.

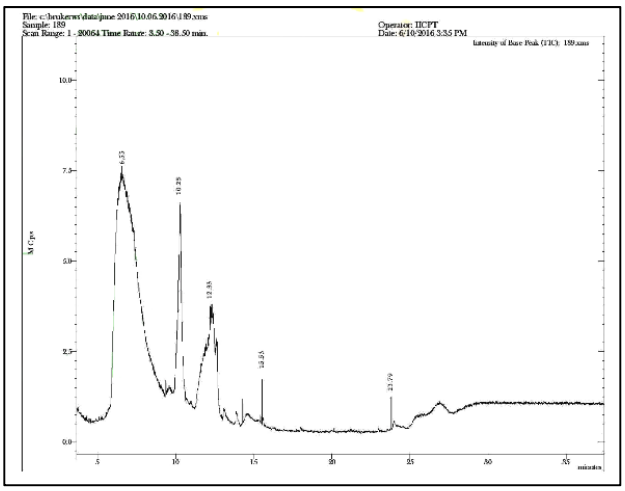

Fig 1: GCMS-MS Chromotogram for fresh milky mushroom

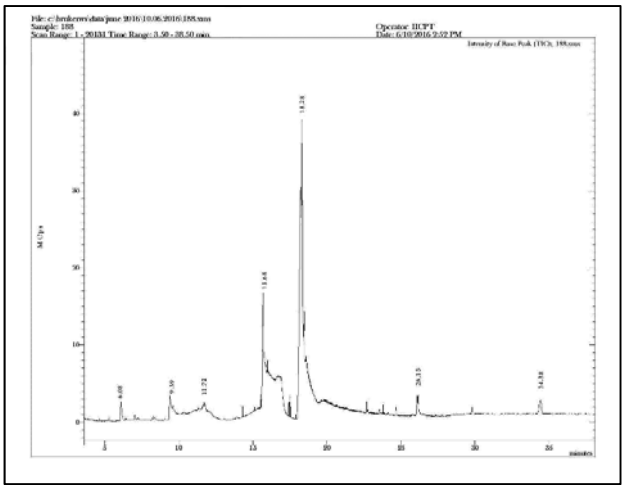

Fig 2: GCMS-MS Chromotogram for dry milky mushroom

Mau and Beelman, (1996) [15] have identified that 10-oxo-trans-8 decenoic acid (ODA) is a major mushroom aroma component, which is a product formed coincidentally with 1-octen-3ol through two enzyme catalyzed reaction and also he concluded that the ODA was found to stimulate mycelial growth, post harvest development and stipe elongation in *Agaricus bisporus* under *in vitro* condition at a concentration of 900 ppm. Also, it might be involved in the initiation of fruiting bodies, which could be proved by means of ODA supplementation (in the form of mushroom powder) to compost at spawning. It can be considered as a mushroom growth hormone. Venkateshwarlu *et al.,* (1999) [24] identified the volatile flavour compounds from three different types of mushrooms by simultaneous distillation and extraction and concluded that, 1-octen-3-ol was the major constituent, and its relative percentage was found to be the highest in *Pleurotus florida* (68%) as compared to 56.7% in *Agaricus bisporus* and 48.7% in *Calocybe indica* correlating with the strong mushroom flavour of *P. florida*. The aliphatic aldehydes*, i.e*. pentanal, hexanal, octanal and 2-octenal, were recorded mainly in *P. florida* (5.93%) and *C. indica* (5.85%) compared to 0.84% in *A. bisporus.*

Chandravadhana *et al.* (2005) [4] reported that the volatile flavour composition of dry milky mushrooms (*Calocybe indica*) could be analysed by capillary GC. They have identified 20 different compounds both in fresh and dry mushroom samples. They further reported that the 1-octen-3-ol,n-octanol and 3-octanone were present in lesser quantities as compared to compounds like n-hexanal, 2,4-decadienol, 2,4-nonadienol, 2-octen-1-ol,1-hexanol, decanol and t-linalool oxide in dried mushroom sample. The reason for reduced concentrations of alcohols and aldehydes in the dried samples might be due to mild air-drying process at 40–45 °C. Kleofas *et al.,* (2015) [11] suggested that the typical flour- and cucumber-like odour of fresh fruiting bodies of *C. gambosa* has been attributed mainly to the key compound (E)-non-2-enal, which was analyzed by GC-MS/MS-O. They further indicated that the volatilome of fresh fruiting bodies of *C. gambosa* contained seven typical C8-compounds, which were absent in the extracts of dried sporocarp samples.

4. Conclusion

From this study, it is concluded that different volatilome profiles have been exhibited by dry and fresh milky mushroom (*Calocybe indica* P&C var.APK2) samples. GCMS analyses indicated the presence of 17 different compounds in dry and 9 different compounds in fresh mushroom samples. The results also indicated that milky mushroom samples are rich in polyunsaturated and essential fatty acids. The presence of specific compounds like Octacecenoic and Hexadecenoic acid in both fresh and dry mushroom samples could be responsible for the cucumber like extra mushroom flavour of the samples. The increased level of polyunsaturated fatty acids like n-Hexadecenoic acid and Octadecenoic acid is more related to the growth and development of mushroom fungi (Mau and Beelman, 1996) [15]. The presence of increased levels of ergosterol (5.5%) and α-D-Glucopyranose (trehalose) (18.91%) in milky mushroom could be useful in anti-cancer therapy, which needs further exploration. The presence of trehalose component reveals the chemo attractive nature of the biopolymers of milky mushroom, which can be utilized to enhance the bioavailability of pharmaceutical or nutraceutical preparations.

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References

1. Afieroho OE, Ugoeze KC. Gas chromatography-mass spectroscopic (GC-MS) analysis of n-Hexane extract of *Lentinus tuber-regium* (Fr) Fr (Polyporaceae) syn *Pleurotus tuber regium* Fr sclerotia. Tropical Journal of Pharmaceutical Research, 2014; 13(11):1911-1915.
2. Beltran-Garcia MJ, Estarron-Espinosa M, Ogura T. Volatile Compounds Secreted by the Oyster Mushroom (*Pleurotus ostreatus*) and Their Antibacterial Activities. J Agric Food Chem. 1997; 45:4049-4052.
3. Champavier Y, Pommier MT, Arpin N, Voiland A, Pellon G. 10-Oxo-*trans*-8-decenoic acid (ODA): production, biological activities, and comparison with other hormone-like substances in Agaricus bisporus. Enzyme Microb Technol. 2000; 26:243-25.
4. Chandravadana MV, Vekateshwarlu G, Babu CSB, Roy TK, Shivashankara KS, Pandey M T *et al*. Volatile flavor components of dry milky mushrooms (*Calocybe indica*). Flavour Fragr J. 2005; 20:715-717.
5. Czub J, Baginski MJ. Comparative molecular dynamics study of lipids membranes containing cholesterol and ergosterol.Biophys J. 2006; 90:2368-82.
6. Duponnois R, Kisa M. The possible roles of trehalose in the mycorrhiza helper effect. Can. J. Bot. 2006; 84:1005-1008.
7. EFSA. Guidance from the Scientific Opinion on safety of smoke flavour Primary Product –TRADISMOKE™ A MAX1. EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) 2010; 2:3. Published on 8.

www.efsa.europa.eu/sites/default/files/scientific_output/files/main.../1394.pdf.
8. Frey-Klett P, Garbaye J, Tarkka M. Themycorrhizahelperbacteria revisited. New Phyt. 2007; 176:22-36.
9. Jedinak A, Sliva D. *Pleurotus ostreatus* inhibits proliferation of human breast and colon cancer cells through p53-dependent as well as p53-independent pathway. International journal of oncology. 2008; 33(6):1307.
10. Kalač P. Edible mushrooms: chemical composition and nutritional value. Academic Press, 2016, 236.
11. Kleofas V, Popa F, Niedenthal E, Rühl M, Kost G, Zorn H. Analysis of the volatilome of Calocybe gambosa. Mycological Progress. 2015; 14(10):1-11.
12. Kwon VJ, Uhm TB. A studies on the lipid components in oyster mushroom (*Pleurotus florida*). Journal of the Korean Society of Food Science and Nutrition. 1984; 13(2):175-180
13. Lochman J, Mikes V. Ergosterol treatment leads to the expression of specific set of defence- related genes in tobacco. Plant Mol Biol. 2006; 62:43-51.
14. Masako ODA, Takamasa UENO, Kasai N, Takahashi H, Yoshida H, Sugawara F *et al*. Inhibition of telomerase by linear-chain fatty acids: a structural analysis. Biochemical Journal. 2002; 367(2):329-334.
15. Mau JL, Beelman RB. Role of 10-oxo-trans-8-decenoic acid in the cultivated mushroom, Agaricus bisporus. In Mushroom biology and mushroom products: proceedings of the 2nd International Conference. University Park. Pennsylvania, 1996.
16. Moliszewska E. Mushroom flavour. Folia Biologica et Oecologica. 2014; 10(1):80-88.
17. Morath SU, Hung R, Bennett JW. Fungal volatile organic compounds: A review with emphasis on their biotechnological potential. Fungal Biol Rev. 2012; 26:73-83.
18. Novaes MRCG, Valadares F, Reis MC, Gonc alves DR, Menezes MC. The effects of dietary supplementation with Agaricales mushrooms and other medicinal fungi on breast cancer: Evidence-based medicine. Clinics. 2011; 66(12):2133-2139.
19. Pedneault K, Angers P, Gosselin A, Tweddell RJ. Fatty acid composition of lipids from mushrooms belonging to the family Boletaceae. Mycological research. 2006; 110(10):1179-1183.
20. Raina S, Sodhi HS, Sethi S. Identification of Ergosterol in Mushrooms. Proceedings of the 8thInternational Conference on Mushroom Biology and Mushroom Products (ICMBMP8), New Delhi, India. 2014, 247-251.
21. Rapior S, Marion C, Pelissier Y, Bessiere JM. Volatile composition of fourteen species of fresh wild mushrooms (Boletales). J Essent Oil Res. 1997; 9:231.
22. Sharma SK, Lall AM, Sharma M, Reishi M. Response of organic supplementation on yield and nutritional parameters of *Calocybe Indica.* Vegetos- An Inter J Plant Res. 2013; 26:36-39.
23. Srinivasan K, Kumaravel S. Unraveling the Potential Phytochemical Compounds of Gymnema Sylvestre through GC-MS Study. International Journal of Pharmacy and Pharmaceutical Sciences. 2015; 8(1):450-453.
24. Venkateshwarlu G, Chandravadana MV, Tewari RP. Volatile flavour components of some edible mushrooms (Basidiomycetes). Flavour and Fragrance Journal. 1999; 14(3):191-194.
25. Won SR, Hong MJ, Kim YM, Li CY, Kim JW, Rhee HI. Oleic acid: an efficient inhibitor of glucosyltransferase. FEBS letters, 2007; 581(25):4999-5002.
26. Wurzenberger M, Grosch W. Stereochemistry of the cleavage of the 10 hydroperoxide isomer of linoleic acid to 1-octen-3-ol by a hydroperoxide lyase from mushrooms Psaliotta bispora. Biochim Biophys Acta. 1984; 795:163-165.