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Study of chemical composition in wild edible mushroom *Pleurotus cornucopiae* (Paulet) from Himachal Pradesh, India by using Fourier transforms infrared spectrometry (FTIR), Gas chromatography-mass spectrometry (GCMS) and X-ray fluorescence (XRF)

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ABSTRACT: FTIR, GC/MS and XRF study was carried out to evaluate the chemical composition of *Pleurotus cornucopiae* (Paulet), Collected from Kalpa in H.P. Thirteen peaks were recorded by FTIR spectrophotometry which corresponds to nine different functional groups. Results of GC/MS study showed the presence of 180 different compounds at different retention time. Only thirty nine compounds were found dominant. 6-Octadecenoic acid, cis-13-Octadecenoic acid and cis-Vaccenic acid occupied 52% portion of the sample. Eighteen different minerals/metals were identified by XRF analysis. Diethylpyrocarbonate (94.06%) and Potassium (4.28%) was found abundant in *Pleurotus cornucopiae* (Paulet). The results obtained during this study contribute to the knowledge of chemical composition of wild edible mushroom *viz. Pleurotus cornucopiae* (Paulet). The results of present study are assumed to be useful for pharmaceutical and food industry as many of the identified constituents of *Pleurotus cornucopiae* (Paulet) are useful in therapeutic as well as for neutraceutical purpose.

Keywords: Macro-fungi; Mushroom; Delicacy; Therapeutic; GC/MS; FTIR and XRF

INTRODUCTION

Mushrooms are macro fungus associated with mankind and provide profound biological and economical impact. These are the fruiting body of Ascomycota and Basidiomycota having short reproductive stage in their life cycle (Das, 2010). They can be epigeous or hypogeous, and seen with the naked eyes (Chang and Miles, 1992). Diversity of these macro fungus species are present in wild mostly in moisten areas. There are 140,000 species of macro fungus (mushrooms) occuring on earth, out of these only 10% species are known. It is estimated that if the undiscovered species will be described than they will be helpful in providing their benefits to mankind (Hawksworth, 2001). Many of these macro fungi have been used in medicine whereas some species are consumed by man for their nutritional value to increase the health of body (Elmastas *et al*., 2007; Ribeiro *et al*., 2007).Several workers have identified the nutritional value of various mushrooms. Nutritive value of *Pleurotus flabellatus* was determined as 0.97% ash, 1.08% crude fibre, 0.105% fat, 90.95% moisture, 0.14% non-protein nitrogen and 2.75% protein (Bano *et al*., 1963). There was a report available claiming the food value of mushrooms between meat and vegetables (Bano, 1976). Nutritional value of mushroom was estimated as high as meat, egg and milk food sources (Gruen and Wong, 1982). Mushrooms have rich nutritional value with high contents of proteins, vitamins, minerals, fibers, trace elements and low calories and cholesterol (Agahar- Murugkar and Subbulakshmi, 2005; Wani *et al*., 2010).

Different bioactive compounds like antiparasitic, antioxidant, antibacterial, antiinflammatory, antifungal, antiviral, anticancer, antitumour, cytotoxic, hypo-cholesterolemic, antidiabetic, anticoagulant and hepato-protective compounds (Wasser and Weis, 1999; Lindequist *et al*., 2005; Lakhanpal and Rana, 2005; Ajith and Janardhanan, 2007; Ferreira *et al*., 2009) are present in wild fungi. Macro fungi are useful in the bioremediation of industrial waste and the accumulation of heavy metals from the environment (Demirbas 2001; Kalac et al., 2004).

Different methods were used to analyse different constituent of edible as well as inedible macro fungi. GCMS is widely used method to identify different substances with in a test sample (Manjamalai *et al*., 2011). New drugs have been searched from mushrooms by using the GCMS techniques and polysaccharides such as xylomannans and β-glucans have been reported from edible mushroom *Flammulina velutipes* by using GC-MS and NMR techniques (Smiderle *et al*., 2006). GC-MS studies on *Pleurotus oeus* mushroom reveal the presence of fatty acids, their esters and strong antimicrobial activity. Using ion trap detector in GC-MS Riberiro *et al*. (2007; 2011) identified free amino acids composition and thirty fatty acids from twelve wild edible mushroom species.

Amino and fatty acids in wild edible mushroom species belonging to the genus *Boletus* was characterized by using HP-GC mass selective detector (Dembitsky *et al*., 2010). No such data was available on chemical composition of *Pleurotus* sp. from this region of India. So, keeping this in view, the present study has been carried out to estimate different constituent in selected wild mushroom which has edible as well as medicinal value.

MATERIAL AND METHODS

Samples were collected during September and October month during 2013 and 2014 from mix forest of *Pinus wallichiana* and *Cedrus deodara* from Kalpa area of district Kinnaur, Himachal Pradesh, India (Fig 1). This area is located at an elevation of 2600 mts amsl longitude and latitude varying from 77° 45' to 79° 00' 35" East and 31° 22' to 33° 012' 40" North.

A. Collection and identification of samples

During the survey samples were handpicked and placed in well labelled paper bags and transported to the laboratory for further study. Samples were identified morphologically and by matching with reference collection in the Department of Bio-Sciences, Himachal Pradesh University and confirmed.

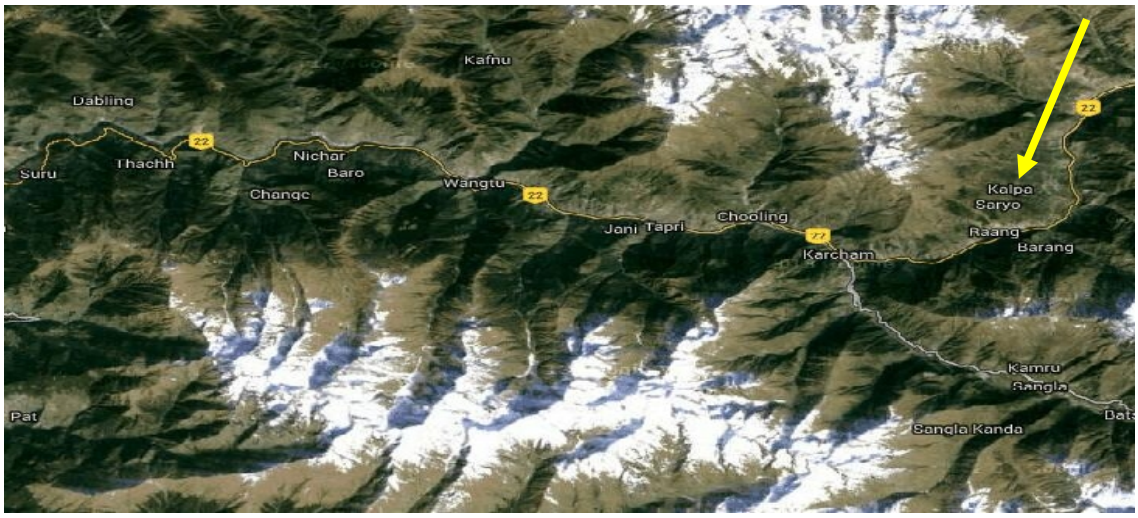

Fig 1. Map of study area in Kalpa, Kinnaur, Himachal Pradesh

Table 1: Physical characteristics of soil in Kalpa (Kinnaur)

Characters	Value
pH	6-6.30
Soil texture	Loamy and Clay
Annual rainfall	600-1200mm

Fig 2. Fruiting body of *Pleurotus cornucopiae* (Paulet)

B. Processing

Samples were washed with water to remove the debris and placed in hot air oven at 60˚C for proper drying. Dried samples were grind in liquid nitrogen into a fine powder for analysis. These samples were evaluated for different constituents of macro-fungi with the help of FTIR, GC/MS and XRF analysis. The study will be helpful in finding the useful compound which can be used in therapeutic use.

Fourier-transform infrared spectroscopy (FTIR) analysis: FTIR spectra of *Pleurotus cornucopiae* (Paulet) was recorded on Agilent Cary 630 FTIR Spectrometer. Spectra were recorded in 650-4,000 cm^{-1} range.

Gas chromatography-mass spectrometry (GC/MS) analysis: GC/MS analysis was performed by using Trace 1300 GC, Tsq 8000 triple quadrupole MS equipped with TG 5MS (30m X 0.25mm, 0.25µm) column. Helium was used as the carrier gas at a flow rate of 1ml/min and an injection volume of 1.0 µL. Injector temperature was 250˚C; ion source temperature 230˚C. The oven temperature was 50˚C isothermal for 2.0min, with an increase of 10˚C/min to 200˚C, then 5˚C/min to 280˚C, ending with 10 min isothermal at 280˚C.

X-ray fluorescence (XRF) analysis: Samples were analysed by WD-XRF (wavelength dispersive X-ray fluorescence), Model: S8 TIGER, Bruker, Germany. Homogenized fine powders of sample with particle size up to 5 microns were used for analysis.

RESULTS AND DISCUSSION

Soil of the forest is full of organic matter having 80% relative humidity and temperature ranges from 8-17˚C. Nature of the soil was acidic with pH of 6-6.30 (measured by digital pH meter) Table 1. Samples were photographed with Canon 5.1 MP camera Fig 2.

The powder of *Pleurotus cornucopiae* (Paulet) was subjected to Fourier-Transform Infrared Spectroscopy (FTIR), Gas Chromatography-Mass Spectrometry (GC/MS), and X-Ray-Fluorescence (XRF) spectroscopy analysis. The results of different analysis are discussed as:

Fourier-transform infrared spectroscopy (FTIR) Analysis: FTIR spectra of *Pleurotus cornucopiae* (Paulet) reveals 13 peaks which corresponds to nine functional groups. Peak 3260 cm^{-1} corresponds to ≡C-H stretch; 2925 cm^{-1} & 2858 cm^{-1} corresponds to C-H stretch; peak 2117 cm^{-1} corresponds to C≡C stretch; 1633 cm^{-1} corresponds to C=O stretch; 1566 cm^{-1} and 1551 cm^{-1} corresponds to N=O stretch; 1380 cm^{-1} corresponds to N=O bend; 1153 cm^{-1} and 1020 cm^{-1} corresponds to C-O stretch; 937 cm^{-1} corresponds to =C-H bend; 687 cm^{-1} and 651 cm^{-1} corresponds to C-Cl stretch (Fig 3). FTIR spectra of nine functional groups present in monosaccharide composition of four common edible mushrooms were investigated by Jin-Zhe *et al*. (2013).

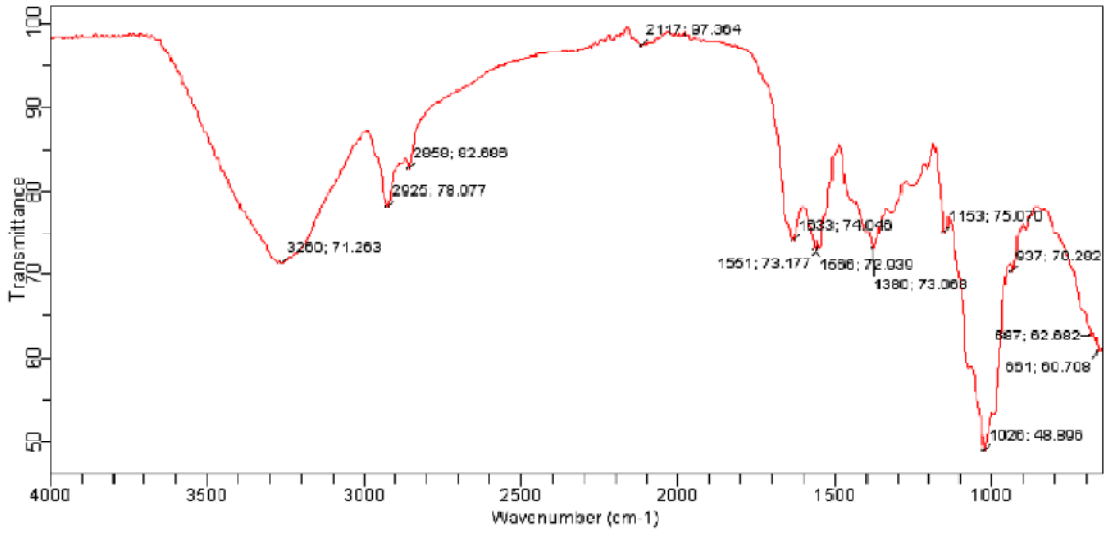

Fig 3. FTIR spectra of *Pleurotus cornucopiae* (Paulet)

Gas chromatography-mass spectrometry (GC/MS) analysis: GC/MS analysis is powerful tool for qualitative and quantitative analysis of various compounds present in natural products. GC/MS technique has been widely applied in medical, biological and food research (Ribeiro *et al*., 2007; Suseem *et* al., 2011). In present study 180 different compounds were identified by GC/MS analysis of *Pleurotus cornucopiae* sample.

Components were identified from database Library of GC/MS instrument. Identified compounds with their name, molecular formulae, retention time and peak percentage are given in Table 2 and Fig 4.

Table 2: Metabolites identified by GC/MS as component of *Pleurotus cornucopiae* (Paulet)

S. No.	Compound Name	Molecular formula	RT	%
1.	Nonane, 4-ethyl-5-methyl-	$C_{12}H_{26}$	3.05	0.06
2.	Decane, 5,6-dimethyl-	$C_{12}H_{26}$	3.05	0.06
3.	Hexane, 1-methoxy-	$C_7H_{16}O$	3.05	0.06
4.	Silane, triethylfluoro-	$C_6H_{15}FSi$	3.33	0.28
5.	Ethanone, 2-(formyloxy)-1-phenyl-	$C_9H_8O_3$	3.33	0.28
6.	Benzoyl bromide	C_7H_5BrO	3.33	0.28
7.	Propanoic acid, 2-hydroxy-, 2-methylpropyl ester	$C_7H_{14}O_3$	4.03	0.58
8.	1-Propanol, 2,2-dimethyl-, acetate	$C_7H_{14}O_2$	4.03	0.58
9.	1,3-Butanediol, (S)-	$C_4H_{10}O_2$	4.03	0.58
10	Cyclotetrasiloxane, octamethyl-	$C_8H_{24}O_4Si_4$	6.97	1.24
11.	2,6-Dihydroxyacetophenone, bis(trimethylsilyl) ether	$C_{14}H_{24}O_3Si_2$	6.97	1.24
12.	1,1,3,3,5,5,7,7-Octamethyl-7-(2-methylpropoxy) tetra siloxan-1-	$C_{12}H_{34}O_5Si_4$	6.97	1.24
13.	Cyclopentasiloxane, decamethyl-	$C_{10}H_{30}O_5Si_5$	9.44	2.17
14.	Benzoic acid, 2,6-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	9.44	2.17
15.	1,1,3,3,5,5,7,7,9,9-Decamethyl-9-(2-methylpropoxy) pentasiloxan-1-ol	$C_{14}H_{40}O_6Si_5$	9.44	2.17
16.	Cyclopropane, nonyl-	$C_{12}H_{24}$	10.06	0.21
17.	1-Undecanol	$C_{11}H_{24}O$	10.06	0.21
18.	Cyclopropane, 1-ethyl-2-heptyl-	$C_{12}H_{24}$	10.06	0.21
19.	Cyclohexasiloxane, dodecamethyl-	$C_{12}H_{36}O_6Si_6$	11.92	1.59
20.	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	$C_{14}H_{44}O_6Si_7$	11.92	1.59
21.	Silane, dimethyl(dimethyl(dimethyl(2-isopropylphenoxy) silyloxy)silyloxy)(2- isopropylphenoxy)-	$C_{24}H_{40}O_4Si_3$	11.92	1.59
22.	1-Hexadecanol	$C_{16}H_{34}O$	12.85	0.22
23.	Cetene	$C_{16}H_{32}$	12.85	0.22
24.	n-Tridecan-1-ol	$C_{13}H_{28}O$	12.85	0.22
25.	2-Allyl-3-hydroxy-2-methylsuccinic acid, 1-ethyl ester	$C_{10}H_{16}O_5$	13.86	0.75
26.	(E)-Ethyl 4,4-bis(ethylthio)-2- methylbut-2-enoate	$C_{11}H_{20}O_2S_2$	13.86	0.75
27.	2-Diisopropylsilyloxybut-3-yne	$C_{10}H_{20}OSi$	13.86	0.75
28.	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	14.16	0.68
29.	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trim ethylsiloxy) tetrasiloxane	$C_{18}H_{52}O_7Si_7$	14.16	0.68
30.	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane	$C_{19}H_{54}O_7Si_7$	14.16	0.68
31.	Cubedol	$C_{15}H_{26}O$	14.59	0.06
32.	4-epi-cubedol	$C_{15}H_{26}O$	14.59	0.06
33.	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecad iene	$C_{15}H_{26}O$	14.59	0.06
34.	Hexadecen-1-ol, trans-9-	$C_{16}H_{32}O$	15.33	0.14
35.	1-Hexadecanol	$C_{16}H_{34}O$	15.33	0.14
36.	10-Heneicosene (c,t)	$C_{21}H_{42}$	15.33	0.14
37.	5,6-Azulenedicarboxaldehyde, 1,2,3,3a,8,8a-hexahydro-2,2,8-trimethyl-, (3aà,8à,8aà)-(-)-	$C_{15}H_{20}O_2$	15.62	0.08
38.	Furan, 2-[(2-ethoxy-3,4-dimethyl-2-cyclohexen-1-ylidene)	$C_{15}H_{20}O_2$	15.62	0.08
39.	Eudesma-5,11(13)-dien-8,12-olide	$C_{15}H_{20}O_2$	15.62	0.08
40.	Urs-12-en-28-al	$C_{30}H_{48}O_8Si_8$	15.79	0.28
41.	Androst-5-en-17-one, 3,16-bis[(trimethylsilyl)oxy]-, (3á,16à)-	$C_{14}H_{42}O_5Si_6$	15.79	0.28

S. No.	Compound Name	Molecular formula	RT	%
42.	9-(Phenylthio)methyltricyclo[4.2.1.1(2,5)]deca-3,7- diene-9,10-diol	$C_{17}H_{18}O_2S$	15.79	0.28
43.	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	16.16	0.27
44.	Hexasiloxane, tetradecamethyl-	$C_{14}H_{42}O_5Si_6$	16.16	0.27
45.	Benzoic acid, 2,6-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	16.16	0.27
46.	Acetic acid, 3a,9b-dimethyl-7-oxo-2,3,3a,4,5,7,8,9,9a,9b-decahydro-1H-cyclopenta[a]naphthalen-3-yl ester	$C_{17}H_{24}O_3$	16.60	0.27
47.	6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-	$C_{15}H_{22}O_2$	16.60	0.27
48.	2(1H)-Naphthalenone,4a,5,6,7,8,8a-hexahydro-6-[1-hydroxymethyl)ethen yl]-4,8a-dimethyl-, [4ar-(4aà,6à,8aá)]-	$C_{15}H_{22}O_2$	16.60	0.27
49.	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methy l] ethyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$	16.89	0.10
50.	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	16.89	0.10
51.	2,7-Octanedione, 4,4-dimethyl 3-[2-(1-hydroxy-1-methylethyl)-3-methyl-3-butenylidene)-	$C_{18}H_{30}O_3$	16.89	0.10
52.	Acetic acid, 6-(1-hydroxymethyl-vinyl)-4,8a-dimethyl-3-oxo-1,23,5,6,7,8,8a-octahydronaphthalen-2-yl ester	$C_{17}H_{24}O_4$	17.17	0.06
53.	5,8-Dihydroxy-4a-methyl-4,4a,4b,5,6,7,8,8a,9,10-decahydro-2(3H)-phenanthrenone	$C_{15}H_{22}O_3$	17.17	0.06
54.	9,10-Secochola-5,7,10(19)-trien-24-al, 3-hydroxy-, (3á,5Z,7E)-	$C_{24}H_{36}O_2$	17.17	0.06
55.	5,8-Dihydroxy-4a-methyl-4,4a,4b,5,6,7,8,8a,9,10-de cahydro-	$C_{15}H_{22}O_3$	17.63	0.41
56.	Eudesma-5,11(13)-dien-8,12-olide	$C_{15}H_{20}O_2$	17.63	0.41
57.	5,6-Azulenedicarboxaldehyde, 1,2,3,3a,8,8a-hexahydro-2,2,8-trimethyl-, (3aà,8à,8aà)-(-)-	$C_{15}H_{20}O_2$	17.63	0.41
58.	5,8-Dihydroxy-4a-methyl-4,4a,4b,5,6,7,8,8a,9,10-decahydro-2(3H)-phenanthrenone	$C_{15}H_{22}O_3$	17.78	0.31
59.	3(4H)-Dibenzofuranone, 4a,9b-dihydro-8,9b-dimethyl-	$C_{14}H_{14}O_2$	17.78	0.31
60.	Anthracene, 1,2,3,4,5,6,7,8-octahydro-9,10-dimethyl-	$C_{16}H_{22}$	17.78	0.31
61.	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	17.89	0.23
62.	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	$C_{14}H_{44}O_6Si_7$	17.89	0.23
63.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	17.89	0.23
64.	Eudesma-5,11(13)-dien-8,12-olide	$C_{15}H_{20}O_2$	18.18	0.32
65.	5,6-Azulenedicarboxaldehyde, 1,2,3,3a,8,8a-hexahydro-2,2,8-trimethyl-, (3aà,8à,8aà)-(-)-	$C_{15}H_{20}O_2$	18.18	0.32
66.	Propanoic acid, 2-methyl-, (dodecahydro-6a-hydroxy-9a-methyl-3-methylene-2, 9-dioxoazuleno[4,5-b]furan-6-yl)methyl ester,	$C_{19}H_{26}O_6$	18.18	0.32
67.	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl] ethyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$	18.43	0.08
68.	cis-5,8,11-Eicosatrienoic acid, trimethylsilyl ester	$C_{23}H_{42}O_2Si$	18.43	0.08
69.	10,12-Tricosadiynoic acid, trimethylsilyl ester	$C_{26}H_{46}O_2Si$	18.43	0.08
70.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	18.93	0.53
71.	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	18.93	0.53
72.	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	18.93	0.53
73.	Eudesma-5,11(13)-dien-8,12-olide	$C_{15}H_{20}O_2$	19.18	1.78
74.	Furan, 2-[(2-ethoxy-3,4-dimethyl-2-cyclohexen-1-ylidene)methyl]-	$C_{15}H_{20}O_2$	19.18	1.78
75.	5,6-Azulenedicarboxaldehyde, 1,2,3,3a,8,8a-hexahydro-2,2,8-trimethyl-, (3aà,8à,8aà)-(-)-	$C_{15}H_{20}O_2$	19.18	1.78
76.	Phthalic acid, butyl tridecyl ester	$C_{25}H_{40}O_4$	19.38	1.32
77.	1,2-Benzenedicarboxylic acid, butyl decyl ester	$C_{22}H_{34}O_4$	19.38	1.32

S.No.	Compound Name	Molecular formula	RT	%
78.	Phthalic acid, butyl nonyl ester	$C_{21}H_{32}O_4$	19.38	1.32
79.	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	19.58	1.55
80.	Ethyl 13-methyl-tetradecanoate	$C_{17}H_{34}O_2$	19.58	1.55
81.	Ethyl 14-methyl-hexadecanoate	$C_{19}H_{38}O_2$	19.58	1.55
82.	Estra-1,3,5(10)-trien-17á-ol	$C_{18}H_{24}O$	19.89	0.97
83.	9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyl)oxy]-, (3á,5Z,7E)-	$C_{30}H_{52}O_3Si$	19.89	0.97
84.	1-Heptatriacotanol	$C_{37}H_{76}O$	19.89	0.97
85.	Estra-1,3,5(10)-trien-17á-ol	$C_{18}H_{24}O$	20.14	0.28
86.	7-Hydroxy-6,9a-dimethyl-3-methylene-decahydro-azuleno[4,5-b]furan-2,9-dione	$C_{15}H_{20}O_4$	20.14	0.28
87.	2-[5-(2,2-Dimethyl-6-methylene-cyclohexyl)-3-methyl-pent-2-enyl]-[1,4]benzoquinone	$C_{21}H_{28}O_2$	20.14	0.28
88.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	20.28	0.23
89.	Estra-1,3,5(10)-trien-17á-ol	$C_{18}H_{24}O$	20.28	0.23
90.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	20.28	0.23
91.	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	20.61	8.06
92.	cis-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	20.61	8.06
93.	trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	20.61	8.06
94.	Methyl stearate	$C_{19}H_{38}O_2$	20.83	2.08
95.	Heptadecanoic acid, 15-methyl-, methyl ester	$C_{19}H_{38}O_2$	20.83	2.08
96.	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	20.83	2.08
97.	6-Octadecenoic acid	$C_{18}H_{34}O_2$	21.11	52.33
98.	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	21.11	52.33
99.	cis-Vaccenic acid	$C_{18}H_{34}O_2$	21.11	52.33
100.	1-Heptatriacotanol	$C_{37}H_{76}O$	21.84	2.00
101.	Cholestan-3-ol, 2-methylene-, (3á,5à)-	$C_{28}H_{48}O$	21.84	2.00
102.	Azuleno[4,5-b]furan-2(3H)-one, decahydro-7,9-dihydroxy-6,9a-dimethyl-3-methylene -, [3aS-(3aà,6á,6aà,7à,9à,9aá,9bà)]-	$C_{15}H_{22}O_4$	21.84	2.00
103.	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	22.12	0.28
104.	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	22.12	0.28
105.	cis-Vaccenic acid	$C_{18}H_{34}O_2$	22.12	0.28
106.	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	22.41	0.33
107.	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	22.41	0.33
108.	cis-Vaccenic acid	$C_{18}H_{34}O_2$	22.41	0.33
109.	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	22.58	0.20
110.	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	22.58	0.20
111	cis-Vaccenic acid	$C_{18}H_{34}O_2$	22.58	0.20
112.	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	22.96	2.24
113.	9-Octadecenamide	$C_{18}H_{35}NO$	22.96	2.24
114.	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	22.96	2.24
115.	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	23.31	0.31
116.	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$	23.31	0.31
117.	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methy l]ethyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$	23.31	0.31
118.	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	$C_{12}H_{23}NO_2$	23.68	0.48
119.	Octanoic acid, 2-dimethylaminoethyl ester	$C_{12}H_{25}NO_2$	23.68	0.48
120.	Fumaric acid, 2-dimethylaminoethyl nonyl ester	$C_{17}H_{31}NO_4$	23.68	0.48
121.	Oleic anhydride	$C_{36}H_{66}O_3$	23.86	3.66
122.	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$	23.86	3.66
123.	i-Propyl 9-octadecenoate	$C_{21}H_{40}O_2$	23.86	3.66
124.	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$	24.04	0.41
125.	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	$C_{57}H_{104}O_6$	24.04	0.41
126.	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	24.04	0.41

S.No.	Compound Name	Molecular	RT	%
127.	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	24.41	0.23
128.	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$	24.41	0.23
129.	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-	$C_{27}H_{42}O_4$	24.41	0.23
130.	2-[5-(2,2-Dimethyl-6-methylene-cyclohexyl)-3-methyl-pent-2-enyl]-[1,4 benzoquinone	$C_{21}H_{28}O_2$	24.98	0.29
131.	3-(3,7-Dimethyl-octa-2,6-dienyl)-4-hydroxy-benzaldehyde	$C_{17}H_{22}O_2$	24.98	0.29
132.	1,4-Bis[methyl(tetramethylene)silyloxy]butane	$C_{14}H_{30}O_2Si_2$	24.98	0.29
133.	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$	25.55	3.53
134.	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$	25.55	3.53
135.	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	25.55	3.53
136.	Methyl 14-methyl-eicosanoate	$C_{22}H_{44}O_2$	25.75	0.73
137.	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans-	$C_{19}H_{36}O_3$	25.75	0.73
138.	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	$C_{19}H_{36}O_3$	25.75	0.73
139.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	26.33	0.12
140.	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	$C_{14}H_{44}O_6S_{i7}$	26.33	0.12
141.	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	$C_{12}H_{38}O_5Si_6$	26.33	0.12
142	Squalene	$C_{30}H_{50}$	26.71	0.73
143.	6,10,14,18,22-Tetracosapentaen-2-ol,	$C_{30}H_{51}BrO$	26.71	0.73
144.	3-bromo-2,6,10,15,19,23-hexamethyl-, (all-E)- Supraene	$C_{30}H_{50}$	26.71	0.73
145.	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	26.88	0.11
146.	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	$C_{12}H_{38}O_5Si_6$	26.88	0.11
147.	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4S_{i2}$	26.88	0.11
148.	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	27.06	0.07
149.	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	$C_{12}H_{38}O_5Si_6$	27.06	0.07
150.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	27.06	0.07
151.	1-Monolinoleoylglycerol trimethylsilyl etherl-	$C_{27}H_{54}O_4Si_2$	27.98	0.07
152.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethy	$C_{16}H_{50}O_7Si_8$	27.98	0.07
153.	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	$C_{12}H_{38}O_5Si_6$	27.98	0.07
154.	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	28.18	0.16
155.	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	28.18	0.16
156.	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	28.18	0.16
157.	Dehydroergosterol 3,5-dinitrobenzoate	$C_{35}H_{44}N_2O_6$	28.57	0.44
158.	9(11)-Dehydroergosteryl benzoate	$C_{35}H_{46}O_2$	28.57	0.44
159.	Anthraergostatetraenol benzoate	$C_{35}H_{46}O_2$	28.57	0.44
160.	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	28.78	0.08
161.	1-Monolinoleoylglycerol trimethylsilyl ether	$C_{27}H_{54}O_4Si_2$	28.78	0.08
162.	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	$C_{12}H_{38}O_5Si_6$	28.78	0.08
163.	Oleanolic acid	$C_{30}H_{48}O_3$	29.20	0.11
164.	3-[3-(1,5-Dimethylhexyl)-7-(2-hydroxy-1-methylethy l)-3a,6,9b-trimethyl-2,3,3a,4,5,6,7,8,9,9b-decahydro - 1H-cyclopenta[a]naphthalen-6-yl]propanoic acid, methyl ester	$C_{31}H_{54}O_3$	29.20	0.11
165.	Bornane, 2,2,5-endo,6-exo,8,9,10-heptachloro-	$C_{10}H_{11}Cl_7$	29.20	0.11
166.	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	30.10	0.15
167.	Heptasiloxane, hexadecamethyl-	$C_{16}H_{48}O_6Si_7$	30.10	0.15
168.	Tetracosamethyl-cyclododecasiloxane	$C_{24}H_{72}O_{12}Si_{12}$	30.10	0.15
169.	Anthiaergostan-5,7,9,22-tetraen-3-ol	$C_{28}H_{42}O$	31.63	0.47
170.	Dehydroergosterol 3,5-dinitrobenzoate	$C_{35}H_{44}N_2O_6$	31.63	0.47
171.	Anthraergostatetraenol	$C_{28}H_{42}O$	31.63	0.47
172.	Ergosterol	$C_{28}H_{44}O$	32.35	2.37

S.No.	Compound Name	Molecular formula	RT	%
173.	Ergosta-5,8,22-trien-3-ol, (3á,22E)-	$C_{28}H_{44}O$	32.35	2.37
174.	Silane, (ergosta-5,7,22-trien-3á-yloxy)trimethyl-	$C_{31}H_{52}OSi$	32.35	2.37
175.	5,6-Dihydroergosterol	$C_{28}H_{46}O$	32.61	0.46
176.	7,22-Ergostadienol	$C_{28}H_{46}O$	32.61	0.46
177.	Ergosta-14,22-dien-3-ol, (3á,5à,22E)-	$C_{28}H_{46}O$	32.61	0.46
178.	ç-Ergostenol	$C_{28}H_{48}O$	33.92	0.49
179.	Ergost-7-en-3-ol, (3á)-	$C_{28}H_{48}O$	33.92	0.49
180.	Campesterol	$C_{28}H_{48}O$	33.92	0.49

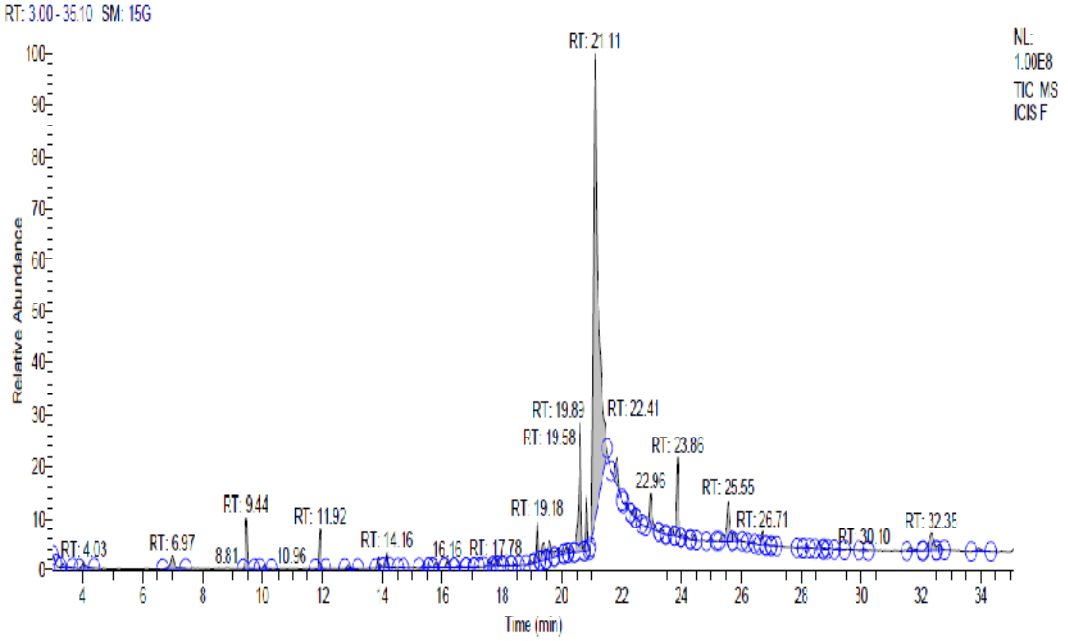

Fig 4. GC/MS Chromatogram of *Pleurotus cornucopiae* (Paulet)

Among all identified compounds, thirty nine compounds were abundant *viz.* 6-Octadecenoic acid, cis-13-Octadecenoic acid and cis-Vaccenic acid, 9-Octadecenoic acid (Z)-, methyl ester, cis-13-Octadecenoic acid, methyl ester, trans-13-Octadecenoic acid, methyl ester, Oleic anhydride, 2,3-Dihydroxypropyl elaidate, i-Propyl 9-octadecenoate, 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, 2,3-Dihydroxypropyl elaidate, 9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester, Ergosterol, Ergosta-5,8,22-trien-3-ol, (3á,22E)-, Silane, (ergosta-5,7,22-trien-3á-yloxy)trimethyl-, Cyclopentasiloxane decamethyl-, Benzoic acid, 2,6-bis[(trimethylsilyl)oxy]-trimethylsilyl ester, 1,1,3,3,5,5,7,7,9,9-Decamethyl-9-(2-methylpropoxy) pentasiloxan-1-ol, Methyl stearate, Heptadecanoic acid, 15-methyl-, methyl ester, Heptadecanoic acid, 16-methyl-, methyl ester, 1-Heptatriacotanol, Cholestan-3-ol, 2-methylene-, (3á,5à)-, Azuleno[4,5-b]furan-2(3H)-one, decahydro-7,9-dihydroxy-6,9a-dimethyl-3-methylene-, [3aS-(3aà,6á,6aà,7à,9à,9aá,9bà)]-, Eudesma-5,11(13)-dien-8,12-olide, Furan, 2-[(2-ethoxy-3,4-dimethyl-2-cyclohexen-1-ylidene) methyl]-, 5,6-Azulenedicarboxaldehyde, 1,2,3,3a,8,8a-hexahydro-2,2,8-trimethyl-(3aà,8à,8aà)-(-)-, Cyclohexasiloxane, dodecamethyl-, Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-, Silane, dimethyl (dimethyl (dimethyl(2-isopropylphenoxy)silyloxy) silyloxy) (2-isopropylphenoxy)-, Hexadecanoic acid, ethyl ester, Ethyl 13-methyl-tetradecanoate, Ethyl 14-methyl-hexadecanoate, Phthalic acid, butyl tridecyl ester, 1,2-Benzenedicarboxylic acid, butyl decyl ester, Phthalic acid, butyl nonyl ester, Cyclotetrasiloxane, octamethyl-, 2',6'-Dihydroxyacetophenone, bis(trimethylsilyl),ether and 1,1,3,3,5,5,7,7-Octamethyl-7-(2-methylpropoxy)tetra siloxan-1-ol. Earlier, thirteen compounds were identified by GC/MS study in *Pleurotus ostreatus* from India (Priya *et al*., 2012). Twenty one different volatile constituents were reported in two species of *Agaricus* by the similar analysis (Lacheva, 2014).

X-ray fluorescence (XRF) analysis: XRF analysis of *Pleurotus cornucopiae* (Paulet) shows the presence of Diethylpyrocarbonate ($C_6H_{10}O_6$) and seventeen different minerals/metals i.e. K, P, S, Si, Al. Mg, Fe, Ca, Na, Re, Cl, Rb, Ti, Cu, Mg, Se and Zn (Table 3).

Table 3: XRF analysis showing proportion of different components present in *Pleurotus cornucopiae* (Paulet).

C6H10O5	K	P	S	Si	Al	Mg	Fe
24.1KCps	248.6 KCps	19.5 KCps	24.6 KCps	7.3 KCps	1.8 KCps	1.8 KCps	23.7 KCps
94.06%	4.28%	0.55%	0.36	0.32%	0.11%	0.11%	0.07%

Ca	Na	Re	Cl	Rb	Ti	Cu	Mn	Se
	0.2 KCps	10.2 KCps	0.9 KCps	41.3KCps	0.6 KCps	5.7 KCps	0.8 KCps	3.5 KCps
0.03%	0.03%	0.02%	0.01%	0.0085%	0.0067%	0.0033%	0.0033	0.0029%

Zn	Sum
10.2 KCps	
0.0002%	100.00%

The concentration was determined on dry weight basis of sample. Majority of proportion was Diethylpyrocarbonate (94.06%) and Potassium (4.28%) whereas other minerals/metals were present in a very less quantity. Earlier, accumulation of nine heavy metals from substrate of edible mushroom species *viz. Lycoperdon perlatum* and *Pleurotus ostreatus* were identified from Romania (Stihi *et al*., 2011). Eight minerals and their proportion were recognised in five *Pleurotus* sp. (Bano *et al.*, 1981; Bisaria *et al.*, 1987).

CONCLUSION

High level of chemical components were found in wild edible as well as medicinal mushroom *Pleurotus cornucopiae* (Paulet). Identified compounds contributes beneficial role to human being and have been considered as new source for different supplement and curative for many ailments with low risk for resistance development by pathogenic microorganisms due to natural origin. According to these results, *Pleurotus cornucopiae* (Paulet) could be suggested as a new potential source of natural therapeutic use in many remedies.

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REFERENCES

Agrahar-Murugkar, D. and Subbulakshmi, G. (2005). Nutritional value of edible wild mushrooms collected from the Khasi hills Meghalaya. *Food Chemistry*. **89:** 599-603.

Ajith, T.A. and Janardhanan, K.K. (2007). Indian medicinal mushrooms as a source of antioxidant and antitumor agents. *Journal of Clinical Biochemistry and Nutrition*. **40:** 157-162.

Bano, Z., Srinivasan, K.S. and Srivastava, H.C. (1963). Amino acid composition of the protein from a mushroom (*Pleurotus flabellatus*). *Applied Microbiology*. **11**: 184-187.

Bano, Z. (1976). Nutritive value of Indian mushrooms and medicinal practices. *Economic Botany*. **31:** 367-371.

Bano, Z., Bhagya, S. and Srinivasan, K.S. (1981). Essential amino acid composition and proximate analysis of Mushroom, *Pleurotus florida. Mushrooms News Letter for Tropics*. **1**: 6-10.

Bisaria, R., Madan, M. and Bisaria, V.S. (1987). Biological efficiency and nutritive value of *P. sajor-caju* cultivated on different agro-wastes. *Biological Wastes*. **19:** 239-255.

Chang, S. T. and Miles, P. G. (1992). Mushroom biology-a new discipline. *Mycologist*. **6:** 64-65.

Dambitsky, V. M., Terentev, A. O. and Levitskyc, D. O. (2010). Amino and fatty acids of wild edible mushrooms of the genus *Boletus*. *Records of Natural Products*. **4**(4): 218-223.

Das, K. (2010). Diversity and conservation of wild mushrooms in Sikkim with special reference to Barsey Rhododendron Sanctuary. NeBIO. **1**(2): 1-13.

Demirbas, A. (2001). Concentrations of 21 metals in 18 species of mushrooms growing in the east Black Sea region. *Food Chemistry*. **75**: 453-457.

Elmastas, M., Isildak, O., Turkekul, I. and Temur, N. (2007). Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *Journal of Food Composition and Analysis*. **20:** 337-345.

Ferreira, I.C.F.R., Barros, L. and Abreu, R.M.V. (2009). Antioxidants in wild mushrooms. *Current Medicinal Chemistry*. **16:** 1543-1560.

Gruen, V.E.C. and Wong, H.X. (1982). Immunomodulatory and antitumour activities of a polysaccharide-peptide complex from a mycelial culture of *Trichoderma* sp. *Sciences*. **57**: 269-281.

Hawksworth, D.L. (2001). Mushrooms: the extent of the unexplored potential. *International Journal of Medicinal Mushroom*. **3**: 333-337.

Jin-Zhe, H., Qiao-Mei, R., Dan-Dan, D. and Pei-Long, S. (2012). Chemical characteristics and antioxidant properties of crude water soluble polysaccharides from four edible mushrooms. *Molecules***. 17**: 4373-4387.

Kalac, P., Svoboda, L. and Havlickova, B. (2004). Contents of detrimental metal mercury, cadmium and lead in wild growing mushroom. A Review: *Energy, Education Science and Technology*. **13**(1): 31-38.

Lacheva, M. (2014). GC-MS studies of the chemical composition of two inedible *Agaricus* species (Section *Xantho dermatei*) in Bulgaria. *Journal of Biodiversity and Environment Science*. **4**(4): 287-295.

Lakhanpal, T.N. and Rana, M. (2005). Medicinal and nutraceutical genetic resources of mushrooms. *Plant Genetic Resources*: *Characterization and Utilization*. **3**: 288-303.

Lindeqiust, I.L., Niedermayer, T.H. and Julich W.D. The pharmacological potential of mushroom. Evidence Based Complement. *Alternative Medicine*. **2**: 285–299.

Manjamalai, A., Yasaswini, N., Harida, A. and Berlin Grace, V. M. (2011). Antifungal, anti-inflammatory and GC-MS analysis of methanolic extract of *Plectranthus amboinicus* leaf. *International Journal of Current Pharmaceutical Research*. **3**: 2.

Priya, V., Jananie, R. K. and Vijayalakshmi, K. (2012). GC/MS determination of bioactive components of *Pleurotus ostreatus*. *International Research Journal of Pharmacy*. **3**(3): 150-151.

Ribeiro, B., Valentao. P., Baptista, P., Seabra, R.M. and Andrade, P.B. (2007). Phenolic compounds, organic acids profiles and antioxidative properties of beefsteak fungus (*Fistulina hepatica*). *Food and Chemical Toxicology*. **45**: 1805-1813.

Ribeiro, B., Guedes de Pinho, P., Andrade, P. B., Oliveira, C., Ferreira, A. C. S., Baptista, P., and Velentao, P. (2011). Do bioactive carotenoids contributes to the color of edible mushrooms?. *Open Chemical and Biomedical Methodology Journal*. **4:** 14-18.

Smiderle, F. R., Carbonero, E. R., Mellinger, C. G., Sassald, G. L., Gorin, P. A. J. and Iacomini, M. (2006). Structural characterization of a polysaccharide and a b-glucan isolated from the edible mushroom *Flammulina velutipes*. *Phytochemistry*. **67**: 2189-2196.

Stihi, C., Radulescu, C., Busuioc, G., Popescu, I. V., Gheboianu, A. and Ene, A. (2011). Studies on accumulation of heavy metals from substrate to edible mushrooms. *Roman Journal of Physics*. **56**(1): 257-264.

Suseem, S. R., Marry Sarai, A., Neelakanda, R., P. and Marslin, G. (2011). Evaluation of the analgesic activity of ethyl acetate, methanol and aqueous extracts of *Pleurotus eous* mushroom. *Asian Pacific Journal of Tropical Medicine*. **4**, 117-120.

Wani, B.A., Bodha, R.H. and Wani, A.H. (2010). Nutritional and medicinal importance of mushrooms. *Journal of Medicinal Plants Research*. **4**(24): 2598-2604.

Wasser, S.P. and Weis, A.L. (1999). Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives. *International Journal of Medicinal Mushrooms*. **1**: 31–62.