STUDIES ON SCREENING, ISOLATION, PURIFICATION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS PRODUCED BY SOME MUSHROOM FUNGI

SYNOPSIS

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Introduction

Mushrooms are the fruiting bodies of macro fungi. They include both edible/medicinal and poisonous species. However, originally, the word "mushroom" was used for the edible members of macro fungi and "toadstools" for poisonous ones of the "gill" macro fungi. Scientifically the term "toadstool" has no meaning at all and it has been proposed that the term is dropped altogether in order to avoid confusion and the terms edible, medicinal and poisonous mushrooms are used (Ukwuru *et al*., 2018).

Mushrooms are a nutritionally functional food and a source of physiologically beneficial medicines. Today mushrooms are becoming more and more popular among people as a continental or Chinese delicacy. As the number of diabetic patients is increasing steadily in India, mushrooms can supplement a good diet for these patients with low calories and high protein value. Due to their fast growth and simple cultivation technique without need of any chemical fertilizers or pesticides, mushroom farming is becoming a very popular cottage industry.

Cultivated mushrooms

Among 300 known genera of mushrooms and related fleshy basidiomycetes, only a few species of these fungi are cultivated commercially. The most common cultivated species are the button mushroom, *Agaricus bisporus* which was widely cultivated in Europe, the Shiitake mushroom (*Lentinus edodes)* grown for centuries in China and other oriental countries and the oyster mushroom (*Pleurotus ostreatus*) (Chakravarthy, 2011). Mushroom cultivation has been practiced for around 200 years in China but cultivation of mushrooms on commercial basis in India has started only recently.

in vitro cultivation of mushroom mycelia

The limitation of mushroom collection in the wild include their seasonal occurrence , while commercial cultivation of mushroom fruiting bodies have a different set of limitations, modern development in mushroom industry is experimenting on *in vitro* culturing of mushroom mycelia. The same compounds as in the fruiting bodies are produced in *in vitro* cultures (Muszynska *et al.,* 2017). The best way of *in vitro* culturing of mushrooms is to convert the woody substrates in to food supplement. Three fungal species from Basidiomycetes, namely *Ganoderma lucidum, Lentinus edodes* and *Pleurotus ostreatus* were grown on substrates made of wood wastes (saw dust as well as wood shavings) which could be used in this way as main ingredients for preparation of natural culture composts. This can be achieved under special conditions like controlled physical parameters like pH, temperature and moisture, as well as the optimal chemical composition of the natural compost like carbon, nitrogen and mineral salts (Petre *et al.,* 2010).

Antimicrobial activity of mushrooms

Mushrooms have increased antimicrobial activity against Gram-postive bacteria. Among all the mushrooms, *Lentinus edodes* is the most studied species and it seems to have a broad antimicrobial action against both Gram-postive and Gram-negative bacteria (Alves *et al.,* 2012). Mushrooms release various bioactive compounds such as terpenoids, flavonoids, tannins, alkaloids and polysaccharides for warding off the threat posed by microbes (Elisashvili, 2012; Vamanu *et al*., 2018).

Traditionally mushrooms have been used as potential antibacterial agents. The aqueous and organic extracts of mycelia of mushrooms have been tested as the antibacterial compounds.

The antibacterial activity in the mushroom extracts depends on the presence of phenolic compounds and various other secondary metabolites (Reid *et al.,* 2016).

Antifungal activity of mushroom extracts and isolated antifungal compounds including both high (peptides and proteins) and low (sesquiterpenes and other terpenes, steroids, organic acids, acyl cyclopentadione and quinones) molecular weight compounds. Low molecular weight terpene, grifolin has the highest antifungal activity (Rosa *et al*., 2003). The sesquiterpene rufuslactone is effective against some phytopathogenic fungi like *Alternaria* sp and *Fusarium sp.* and *Botrytis sp.* (Luo *et al*., 2005). Phenolic acids and other related compounds like p-hydroxybenzoic and cinnamic acids from *Ganoderma lucidum* are effective against *Aspergillu*s sp, *Trichoderma* sp and *Penicillium* sp (Heleno, 2013).

Antioxidant activity of mushrooms

Antioxidant activities of mushrooms are due to the presence of various compounds like phenols, flavanoids, vitamins, peptides, polysaccharides, carotenoids and alkaloids (Ribeiro *et al*., 2006). Phenols and tocopherols present in wild and cultivated mushroom species have a major role in antioxidant activity; p-hydroxybenzoic acids, catechin, gallic acid and caffeine are the major phenolic compounds contributing to the anti oxidative property of *Pleurotus eryngii* (Oke *et al*., 2011).

Anticancer activity of mushrooms

Mushroom polysaccharides or polysaccharide-protein complexes enhance innate and cell-mediated immune responses, and they exhibit antitumor activities in animals and humans (Wasser, 2010). The chemical structures of polysaccharides in mushrooms have a role in antitumor activities. β-D- glucans isolated from fruiting bodies, cultured mycelia biomass and liquid culture media have antitumor properties. Lentinan , a β-D- glucan from *Lentinus edodes*

shows prominent antitumor activity against allogenic tumors, synergic and autochthonous tumors (Zakoany *et al.*, 1980; Suga *et al*., 1984,1985,1986,1989).

Polysaccharides

Mushroom nutriceuticals

Fungal polysaccharides derived from the mushroom genera *Ganoderma*, *Lentinus Flammulina*, *Cordyceps*, *Coriolus* and *Pleurotus* are demonstrated to have multiple bioactivities, including immunomodulating, anticancer, antimicrobial, hypocholesterolemic and hypoglycemic effects (Wang *et al*., 2017; Zhang *et al*., 2017).

Proteins

Mushrooms are rich in protein and high in essential aminoacids. Fruiting bodies and mycelia of mushrooms have a wide range of biologically active proteins. Their biological activities including antimitogenic, immunomodulating, antiviral (inhibitory activity toward VIH-1 reverse transcriptase), antiproliferative activity on different tumour cells, antifungal, antibacterial activities (Ng, 2004; Wong *et al*., 2010; Fangal *et al*., 2011).

Phenolic compounds

Mushrooms have a variety of secondary metabolites with antioxidant activities such as phenolic compounds. Phenolics and flavonoids from mushrooms can also act as free radical scavengers to terminate the radical chain reactions that occur during the oxidation of triglycerides in the food system (Barros *et al*., 2008). Antioxidant capacities can be related to the total phenolic content of the mushroom extracts.

Flavanoids

Mushrooms seem to be a potential natural source of dietary flavonoids having a great range of compounds in significant concentrations showing healthy properties (Jinting *et al*., 2017). Flavanoids have antioxidant behavior by acting at different mechanisms, like, free radical scavenging, or enzyme inhibition due to their formation of copper-flavonoid complexes.

Terpenoids

About 100 different types of triterpenoids are found in the fruiting bodies and mycelia of *Ganoderma lucidum* and *G. applanatum*. These include highly oxidized lanostatine type triterpenoids like ganoderic acids, ganoderal, lucidone, epoxyganoderia, lucidone and furanoganoderic acid and other terpenoid compounds (Wasser *et al*., 1997; Weis *et a*l., 1999). Triterpenoids like ganoderic acid from the mycelia biomass has *in vitro* inhibitory activity on the growth of hepatoma cells (Toth *et al.*, 1983; Lindequest, 1995).

Besides being functional food, mushrooms have many potential applications in various industries. Current research on mushrooms focus on the scope of using mushroom mycelia instead of the fruiting bodies as the source of compounds having industrial applications. In this context the present investigation was designed with an aim to study the bioactive compounds produced by the mycelia of edible mushrooms belonging to the genera *viz., Pleurotus, Hypsizygus, Oudemansiella* and *Schizophyllum.*

Results

Mushroom mycelial cultures

Ten species of mushrooms belonging to four genera *Hypsizygus*, *Oudemansiella*, *Schizophyllum* and *Pleurotus* were selected for the screening, isolation, purification and characterization of bioactive compounds.

Mycelial cultures of *Pleurotus pulmonarius, P. djamor, P. citrinopileatus, P. eryngii, P. florida P. flabellatus, P. cystidiosus, Oudemansiella radicata, Hypsizygus ulmarius* and *Schizophyllum commune* were grown in solid and liquid media and the mycelial extracts and culture filtrates were used as the materials for experiments.

Antibacterial activity

Preliminary screening was done by the analysis of antibacterial activity of culture filtrates and mycelial extracts of mushrooms, against *Escherichia coli, Proteus sp, Klebsiella sp, Pseudomonas sp,* and *Staphylococcus sp* to assess their bioactivity.

All the ten selected mushroom species significantly inhibited *Escherichia coli* and *Proteus* sp while all except *Pleurotus eryngii* inhibited *Pseudomonas* sp.

Klebsiella sp and *Staphylococcus* sp were inhibited by *Pleurotus flabellatus, Oudemansiella radicata, Hypsizygus ulmarius, Schizophyllum commune* and *Pleurotus florida. Pleurotus florida* showed the maximum inhibitory effect against these two organisms (inhibition zone diameter 26.3 mm for mycelial extract and 18.5 mm for culture filtrate).

Mycelial extracts were found to be inhibitory than culture filtrates towards the microbes tested.

Antifungal Activity

All the selected mushrooms were screened for their activity against *Aspergillus niger, A.flavus* and *Fusarium* sp. Mycelial extracts of the mushrooms were analyzed at different concentrations *viz.*, 10%, 20% and 30% aqueous extracts.

Antifungal activity of the aqueous mycelial extracts of *Pleurotus djamor, P.eryngii, P.florida P.flabellatus* and *Oudemansiella radicata* against the test fungi were found to be concentration

dependent, with the 30% aqueous mycelial extract eliciting significant loss in biomass of the mold fungi tested.

A significant observation was that mycelial extracts of *Pleurotus citrinopileatus* showed maximum inhibition against all the three mold fungi tested, markedly inhibiting *Aspergillus niger, A.flavus* and *Fusarium* sp at 20% concentration and showing the highest inhibition of *A.flavus* biomass at 10% concentration of the aqueous mycelial extract.

Antioxidant activity

All the chosen mushrooms were analyzed for their antioxidant activity by DPPH assay. The aqueous mycelial extracts were taken in different concentrations *viz.*, 5, 10 and 15% and the results were expressed in percentage. Antioxidant activity equivalent to the standard ascorbic acid (98%) was shown by *Schizophyllum commune* at 15% aqueous mycelial extract concentration. Similar significantly high antioxidant activity was shown by *Pleurotus florida* (97%), *P.flabellatus* (96.5%), *Hypsizygus ulmarius* (96.3%) and *Pleurotus pulmonarius* (93.7%) at 15% concentration of aqueous mycelial extract. At the highest concentration of aqueous mycelial extract (15%), the antioxidant activity of the mushroom fungi decreased in the order of *P.eryngii* (80.4%), *P.djamor* (70.4%) and *P.citrinopileatus* (60.5%).

Anti cell proliferation activity

Anti cell proliferation study of the mycelial extract of the selected mushroom fungi was done on Human Epidermoid Larynx Carcinoma (HEP-2) cell line. Cells were treated with different concentrations of mycelial extract for 24 hours. Of the ten screened species five mushroom fungi *viz., Pleurotus citrinopileatus, Pleurotus eryngii, Oudemansiella radicata,*

Hypsizygus ulmarius and *Schizophyllum commune* showed anti cell proliferation activity against HEP-2 cell line.

Among the *Pleurotus* species, *Pleurotus citrinopileatus* and *Pleurotus eryngii* showed 64% and 62% inhibitory activity at 150 µg/ml and 250 µg/ml respectively. The IC_{50} value for *Pleurotus citrinopileatus* extract was found at 250 µg/ml mycelial extract concentration. *Hypsizygus ulmarius* mycelial extracts were the most effective showing an IC_{50} value at 150 µg/ml. HEP-2 cell line mortality induced by the mycelial extracts increased in the order of *Schizophyllum commune*, *Oudemansiella radicata* and *Hypsizygus ulmarius* which showed 8%-13% mortality of the cell line at 50µg/ml mycelial extract concentration and 37%-64% mortality at 250 µg/ml mycelial extract concentration.

Mycelial protein content

Total mycelial protein was analyzed using Folin-Phenol method and the results were expressed in percent dry weight. *Hypsizygus ulmarius* contained the highest mycelial protein (27.8%), followed by *Pleurotus djamor (*26. 0%*), Pleurotus florida* (23.2%), *Pleurotus flabellatus* (21.4%) and *Pleurotus citrinopileatus* (20.8%).

Mycelial total carbohydrates content

The total carbohydrate content of the mycelia of the selected mushroom species ranged between 24.3% dry weight and 45.78% dry weight. *Oudemansiella radicata* (45.78% dry weight) and *Hypsizygus ulmarius* (45.6% dry weight) had the highest mycelial carbohydrate content followed by *Pleurotus florida* (44.8% dry weight), the latter having higher carbohydrate content than all the other *Pleurotus* species.

Mycelial crude fiber content

The crude fiber content of the mycelia of the selected mushroom species ranged between 8.1% dry weight and 13.6% dry weight. *Hypsizygus ulmarius* (13.6% dry weight) and *Pleurotus citrinopileatus* (13.2% dry weight) had the highest crude fiber content followed by *Pleurotus pulmonarius* (11.4 % dry weight). *Schizophyllum commune* (8.2 % dry weight) and *Pleurotus djamor* (8.1 % dry weight) had less mycelial crude fiber content than the other mushroom species.

Mycelial lipid content

Pleurotus djamor and *Pleurotus flabellatus* contained the maximum lipid content of 2.79% and 2.42% dry weight respectively. Lipid content in the other *Pleurotus* species ranged between 1.16% and 2.24% dry weight. *Schizophyllum commune* (2.18% dry weight), *Oudemansiella radicata* (1.89 % dry weight) and *Hypsizygus ulmarius* (1.24 % dry weight) had lipid content within this range.

Quantitative ligninolytic enzyme activity

Quantitative enzyme activities of three important ligninolytic enzymes Laccase, Manganese peroxidase (MnP), and Lignin peroxidase (LiP) were studied. Laccase activities of the seven selected *Pleurotus* species ranged between 1.2 x 10^{-4} IU/ml and 5.8 x 10^{-4} IU/ml. *Hypsizygus ulmarius* showed the laccase activity within the range (2.7 x 10^{-4} IU/ml), while *Oudemansiella radicata* showed the highest 6.5 x 10^{-4} IU/ml laccase activity. *Schizophyllum commune* mycelial laccase activity was the lowest (0.6 x 10^{-4} IU/ml).

Manganese peroxidase activity of the selected mushroom fungi ranged between 1.78 x 10^{-4} IU/ml and 6.1 x 10^{-4} IU/ml. Manganese peroxidase activities of the seven *Pleurotus* species ranged between 1.78 x 10^{-4} IU/ml (*Pleurotus flabellatus*) and 4.53 x 10^{-4} IU/ml (*Pleurotus djamor*). *Hypsizygus ulmarius* mycelial MnP activity was the highest of all the selected mushroom species (6.11 x 10^{-4} IU/ml).

Lignin peroxidase activity of the selected mushroom fungi ranged between 0.67 x 10^{-4} IU/ml and 4.31 x 10^{-4} IU/ml. Lignin peroxidase activities were lower than that of laccase and manganese peroxidase activities except for *Pleurotus florida* showing the highest LiP activity (4.64 x 10^{-4} IU/ml), *Hypsizygus ulmarius* (4.31 x 10^{-4} IU/ml) and *Schizophyllum commune* (1.64 x10^{-4} IU/ml).

Total phenolic compounds content

The total phenolic compounds content of the selected mushroom fungi ranged between 32.0 and 48.2 mg/g dry weight. *Hypsizygus ulmarius* had the highest total phenolic compounds content (48.2 mg/g dry weight). *Pleurotus citrinopileatus* had higher total phenolic compounds content (45.1 mg/g dry weight) than the other *Pleurotus s*pecies. *Pleurotus pulmonarius* (32.6 mg/g dry weight) and *Pleurotus eryngii* (32.0 mg/g dry weight) had less total phenolic compounds content than the other mushroom fungi.

Condensed tannins content

The condensed tannins content in the mycelia of the selected mushroom fungi ranged between1.03 and 1.30 mg/g of dry weight of mycelia. *Pleurotus djamor* and *Hypsizygus ulmarius* had the highest condensed tannins content 1.30 and 1.18 mg/g dry weight respectively, followed by *Pleurotus citrinopileatus, P.florida, P.flabellatus, Oudemansiella radicata,*

Schizophyllum commune. *Pleurotus pulmonarius* (1.08 mg/g dry weight) and *P.cystidiosus* (1.03 mg/g dry weight) had less condensed tannins content than the other mushroom fungi.

Total flavanoids content

The total flavanoids content in the mycelia of the selected mushroom fungi ranged between 0.91% and 1.5% dry weight. Flavanoids content was the highest in the mycelia of *Hypsizygus ulmarius* (1.5 % dry weight) followed by *Schizophyllum commune* (1.4% dry weight). Except *Pleurotus pulmonarius* (1.39% dry weight) which had mycelial flavanoid content equivalent to that of *Schizophyllum commune*, all the other *Pleurotus* species and *Oudemansiella radicata* had comparatively lower mycelial flavanoid content than these three mushroom species.

Vitamins content

The wild strains among the selected mushroom fungi *viz*., *Oudemansiella radicata*, *Hypsizygus ulmarius* and *Schizophyllum commune* had significantly higher content of vitamin A, vitamin B_1, vitamin B_2 and vitamin C which ranged between 0.14-0.25 mg/g, 0.56-0.94 mg/g, 0.15-0.51 mg/g and 0.14-1.46 mg/g respectively. All the cultivated strains of *Pleurotus* species had less mycelial vitamin content with values less than the lower value of each range of content of all the four vitamins (vitamin A, B_1, B_2 and C) in the wild strains except *Pleurotus citrinopileatus* having vitamin B_2 content (0.23 mg/g) and *Pleurotus florida* having vitamin C content (0.96 mg/g) equivalent to those of *Schizophyllum commune.*

Mycelial protein profile

SDS-PAGE protein profile of the mycelial extract of *Pleurotus citrinopileatus* and *Hypsizygus ulmarius* were distributed in the molecular weight range of 10- 100 kDa. Mycelial extract of *Pleurotus citrinopileatus* showed seventeen protein types, whereas *Hypsizygus ulmarius* showed twenty protein types in SDS PAGE, observed as distinct bands in the gel.

Three number of protein bands found at 75-100 kDa in both the mushroom fungi represented cellulases and other structural proteins while three prominent bands in the 25-50 kDa region represented the ligninolytic enzymes.

Zymogram

The presence of laccase in the mycelial extract of the mushroom fungi was confirmed by the enzyme activity staining of the resolved protein fractions in the native PAGE. The native PAGE of mycelial extract of *P.citrinopileatus* showed two protein fractions,whereas *H.ulmarius* showed a single protein band, after the laccase activity staining with guiacol. The reddish brown colour developed due to activity staining showed the oxidative polymerization of guiacol in the presence of laccase.Two protein fractions in the *P.citrinopileatus* confirmed the presence of two isoenzymes of laccase in the mushroom.

Detection of compounds in TLC

Aqueous mycelial extract of the two selected mushroom fungi *viz., Pleurotus citrinopileatus* and *Hypsizygus ulmarius* were analyzed for the presence of bioactive compounds using TLC and TLC spray reagents.

Compounds with conjugated double bond systems

TLC plates developed with the mycelial extract of *Pleurotus citrinopileatus* and *Hypsizygus ulmarius* were placed in the tank saturated with iodine vapour with the iodine crystals. The plates when evaluated in the visible light after the exposure to iodine vapour showed the presence of yellow zones each in the two mushroom strains which indicated the presence of compounds containing conjugated double bond systems in the two selected mushroom fungi.

Phenolic compounds

The TLC plates developed with the mycelial extract of *Pleurotus citrinopileatus* and *Hypsizygus ulmarius* showed single and two spots of brown colour respectively, when sprayed with 2% ferric chloride in ethanol. Presence of phenolics in the mycelial extract of the two selected mushroom fungi was thus confirmed.

Flavonoids

The plates analyzed for flavonoids were developed with the solvent system ethyl acetate: glacial acetic acid: water in the ratio of 90:10:10. The developed plates were sprayed with 5% ferric chloride to detect the presence of flavonoids by the grey colour developed. The appearance of single spot of grey colour in the TLC plates of *Pleurotus citrinopileatus* and *Hypsizygus ulmarius* confirmed the presence of flavanoids in the two selected mushroom fungi.

Alkaloids

Appearance of brown colour in the plates developed after spraying with 10% ethanolic sulphuric acid confirmed the presence of alkaloids. The developed TLC plates of *Pleurotus*

citrinopileatus and *Hypsizygus ulmarius* showed no brown colour spots thereby indicating the absence of alkaloids in the two mushroom fungi.

Terpenoids

Detection of terpenoids can be done with the spraying reagent vannilin phosphoric acid. The developed TLC plates of *Pleurotus citrinopileatus* and *Hypsizygus ulmarius* sprayed with the detection reagent showed single spot of blue colour, confirming the presence of terpenoids in the mushroom fungi.

Saponins

Detection of saponins was done by developing the plates with the solvent system chloroform: methanol in 30:5 ratio and sprayed with vanillin sulphuric acid. Presence of single and four spots of violet colour respectively in the developed TLC plates of *P.citrinopileatus* and *H.ulmarius* indicated the presence of saponins.

Carbohydrates

Presence of carbohydrates in the analyzed extracts was detected with the solvent system benzene: acetic acid: water in the ratio of 2:1:1. Spraying agent 10% sulphuric acid in ethanol produced black spots in the plates which confirmed the presence of carbohydrates in the samples.

Glycosides

Mycelial extact of *Pleurotus citrinopileatus* and *Hypsizygus ulmarius* developed in the TLC plates, when sprayed with the Kedde reagent showed yellowish orange colour which confirmed the presence of glycosides in the two selected mushroom fungi.

UV -Visible spectra

Mycelial extracts of *Pleurotus citrinopileatus* and *Hypsizygus ulmarius* were extracted with different solvents *viz.*, diethyl acetate, methanol, ethylacetate and water and their UV-Visible spectra were studied. *Pleurotus citrinopileatus* showed a sharp peak at 222 nm and a shoulder at 283 nm in methanol. Less defined peaks were obtained at 254 nm, 276 nm and350 nm in diethyl ether. Mycelial extracts of ethyl acetate showed less defined peaks at 342 nm and 357 nm.

Mycelial extract of *Hypsizygus ulmarius* in methanol showed a sharp peak at 219 nm and shoulders at 261 nm and 357 nm. Peak at 200 nm and a less defined peak at 266 nm were observed with water. Less defined peaks were obtained at 256 nm, 270 nm and 345 nm for extract with ethyl acetate and two less defined peaks at 253 nm, 274 nm with an inflection at 337 nm was observed with diethyl ether extract. The absorbance peak at 222 nm and 260 nm indicated the presence of protein and nucleic acid respectively in the mushroom samples. Peaks at visible range indicated the presence of brown coloured phenolic compounds.

FTIR spectra

FTIR spectra of *Pleurotus citrinopileatus* and *Hypsizygus ulmarius* were recorded. Aqueous extract of *P. citrinopileatus* showed a sharp peak at 3397.53 cm^{-1} indicated O-H stretching and alcohol groups. Another sharp peak at 2923.66 cm^{-1} is due to N-H stretching indicated the presence of amines. A broad peak at 2126.98 cm^{-1} indicates the presence of C= C stretching and alkynes group. A sharp peak at 1644 cm^{-1} indicates C= C stretching and alkenes. A medium peak at 1383.68 cm^{-1} showed the O-H bending. A strong peak at 1325.61cm^{-1} showed

C-N stretching in aromatic amines. Peak at 1075 cm $^{-1}$ indicated the C- C stretching in macro molecules

Aqueous extract of *Hypsizygus ulmarius* showed an absorption peak at 3395.85cm^{-1} indicated O-H stretching and peak at 2923.85 cm $^{-1}$ indicated the N- H stretching. The peak at 2154.87 cm^{-1} indicates N=N=N stretching. The peak at 1645.76 is C=N stretching. C-H bending is indicated by the presence of a peak at 1385.71cm$^{-1.}$ A peak at 1044.25 cm^{-1} indicates the CO-O-CO stretching. C- Br stretching is indicated by the peak at 575.02 cm^{-1}. Peak at 1044 cm $^{-1}$ indicated the C- C stretching. Results of FT-IR spectra of the two mushrooms have confirmed the presence of various functional groups in proteins, phenolic compounds, polysaccharides,terpenoids and sterol thereby showing the presence of various bioactive compounds.

GC – MS spectra of mycelial extracts of selected mushroom fungi

Mycelial extract of the mushroom fungi *Pleurotus citrinopileatus* and *Hypsizygus ulmarius* were analyzed in GC –MS. The compounds present in the extract were identified by comparison of mass spectra and retention time, area, height of the peak and the ratio of area to the height of the samples data complemented with NIST GC-MS library.

Twenty six compounds were identified from the spectra of P. *citrinopileatus.* The compounds identified as major component were 2,6-Diflurobenzoic acid, ethylene oxide, gamma tocopherol, The compounds identified as major components were 2, 4 ,dihydroxy benzoic acid (phthalic acid) a phenolic acid, fat soluble vitamin E, mono and unsaturated fatty acids, , triglycerides, unsaturated carboxylic acid esters, aldehydes, tannin, flavonoid, 9 - Octadecenoic acid and methyl esters of fatty acids.

Twenty compounds were identified from the spectra of *H.ulmarius*. The major compounds detected were 2, 4 ,dihydroxy benzoic acid(phthalic acid), a phenolic acid, saturated primary alcohol, mono and poly unsaturated fattyacids, Z-10-Methyl -11-tetradecen-1-ol propionate – tannin, phenolic acids, triglycerides, saturated carboxylic acids, , 9 - Octadecenoic acid and methyl esters of fatty acid.

GC-MS spectra of the mycelial extracts of *Pleurotus citrinopileatus* and *Hypsizygus ulmarius* validated the results obtained in the quantification experiments, UV-Visible spectra and FTIR spectra and confirmed the presence of phenolic compounds, terpenoids, flavonoids, tannins, sterol, proteins and polysaccharides in both the mushrooms. Results of the present investigation indicate the scope of using mushroom mycelia as a source for extraction of bioactive compounds, the quantity of which directly correlated with their antimicrobial, anti oxidant and anti cell proliferation activities.