1. INTRODUCTION

Mushrooms have been placed in a kingdom of fungi and are a "macro fungus with a distinctive spore bearing fleshy fruiting body, which can be either epigeous or hypogeous and large enough to be seen with naked eye and to be picked by hand. Mushrooms are categorized under two families of fungi which is either basidiomycetes and/or ascomycetes (Cheung, 2008). Mushrooms are consumed by humans as comestibles for their nutritional value and they are also consumed as folk medicine for their supposed medicinal value. Delicacy and palatable nature makes to be considered as vegetarian chicken. Besides their excellent flavour mushrooms have attracted much attention due to their proven healthy properties (Chiron and Michelot, 2005).

1.1. Significance of mushrooms

Drastic seasonal and climatic changes have adversely affected the agricultural pattern and crop production. The multifaceted problem on cultivable land and crop production guarantees no future security. Overall stress on land, therefore, results in nutritional security. According to the Institute of medicines, food and nutrition board, functional foods are foods or dietary components that may provide a health benefit beyond basic nutrition (Ferreira et al., 2010). Functional foods are those foods enriched/ modified and consumed as normal diet to provide health giving benefits (Patel and Goyal, 2012).

Nutraceuticals are the bioactive compound that gives functional properties to food. A nutraceutical can be defined as a substance that may be considered as food or part of food and may possibly provide medical or health benefits such as the prevention and treatment of disease. Nutraceuticals may range from isolated nutrients and dietary supplements to genetically engineered “designer” foods, herbal products and processed products such as cereals, soups and beverages (Andlauer and Feust, 2002). Intake of nutraceuticals in the daily diet improves our health. Extensive research on nutraceuticals and its production has increased with the use of biotechnological application.

The ever increasing human demand for protein rich food and the inefficiency of conventional methods have resulted in the need to explore alternatives for low cost
production of unconventional protein rich food (Mukherjee and Nandi, 2004). One of the options is edible mushrooms belonging to the basidiomycetes. To date, the mushroom production in worldwide exceeds more than 10 million metric tons/ year (Li et al., 2012a; Srivilai et al., 2013).

Mushrooms are a source of many biologically active compounds. Mushrooms manage to grow in darkness and dampness in highly competitive environments and protect themselves from hordes of attacking microbes by developing natural protective substances (Zaidman, 2005). Mushrooms can be referred as store house of biological compounds and it comprises a vast and largely untapped source of powerful new pharmaceutical products (Singh et al., 2011a).

1.2. Pleurotus mushroom

Pleurotus species are commonly called as oyster mushrooms belonging to the class basidiomycetes. The common name “oyster mushroom” comes from the white shell-like appearance of the fruiting body (Stanley et al., 2011). The word Pleurotus originates from the Greek word ‘Pleura’ i.e., formed from the lateral position of the stipe (stem like structure between mycelium and pileus relative to the position of pileus and it was originated from Florida. Species of Pleurotus generally live in nature on dead wood as saprophymes and acts as primary decomposers. However, the first attempt was made by Flack (1917) in Germany to grow oyster mushroom for human consumption. The process of cultivation on readily available substrate was taken up earlier on paddy straw in India (Bano and Srivastava, 1962) and on saw dust in Japan (Schanel et al., 1966). They are also known as wood fungus and in Northern India they are sold in the market under the local name ‘Dhingri’.

This oyster mushroom has about 40 well-recognized species, out of which 25 species are commercially cultivated in different parts of all over the world, which are as follows: P. ostreatus, P. flabellatus, P. florida, P. sajor-caju, P. sapidus, P. cystidiosus, P. eryngii, P. fossulatus, P. opuntiae, P. cornucopiae, P. yuccae, P. platypus, P. djamor, P. tuber-regium, P. australis, P. purpureo-olivaceus, P. populinus, P. levis, P. columbines and P. membranaceus (Singh et al., 2011a). Pleurotus sp. is an efficient lignin - degrading
mushroom and can grow well on different types of lignocellulosic materials. Cultivation of this mushroom is very simple and low cost production technology, which gives consistent growth with high biological efficiency. Different species of Pleurotus can grow well in variable temperature conditions; hence, they are ideally suited for cultivation throughout the year in various regions of tropical countries (Ahmed et al., 2009).

1.3. Pleurotus florida

Taxonomic position (Kirk et al., 2001)

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<tr>
<th>Kingdom</th>
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<tr>
<td>Phylum</td>
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<td>Class</td>
<td>Agaricomycetes</td>
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<td>Order</td>
<td>Agaricales</td>
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<tr>
<td>Family</td>
<td>Pleurotaceae</td>
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<tr>
<td>Genus</td>
<td>Pleurotus</td>
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<tr>
<td>Species</td>
<td>P. florida</td>
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<td>Common name</td>
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Pleurotus florida mushroom was widespread in temperate, subtropical and tropical zones (Fig. 1.1). The appearance of this mushroom was similar to P. ostreatus and it was considered as subspecies of P. ostreatus. Some modern mycologists are inclined to consider it as another species with different colour and different temperature requirements. Actually, there are two groups in P. florida at the subspecies level. One group was sexually compatible with P. ostreatus and the other with P. pulmonarius. The colour of the pileus was light brown when cultivated at low temperature (10-15°C), while increasing the temperature from 20 to 25°C, it turns white pale to yellowish colour. It could be harvested in warmer temperatures as its fruiting temperature range was wider than other Pleurotus mushroom and it does not require fruiting induction like cold shock. Furthermore, P. florida shows the highest yield among the Pleurotus species (Kong, 2004).
P. florida mushroom grows well in temperature range of 20 - 32°C. It is also a thermophilic species suitable to be cultivated in warm and moist weather. From a taxonomical point of view it's name as a species doesn't exist although it is widely used across the world. It requires high relative humidity (75 - 85%) during fruit body formation. It can tolerate high CO₂ concentration during spawn run (upto 20,000 ppm or 2%) while it should be less than 600 ppm or 0.06% during cropping. Therefore sufficient ventilation should be provided during fructification. If the CO₂ concentration is high, the mushrooms will have long stipe and small pileus and it will appear like a mouth of trumpet.

White oyster mushroom, P. florida has gained popularity due to its nutritional values and ease of cultivation. This mushroom contains higher amount of protein, lipid, phosphorous, iron, thiamine and riboflavin compared to other edible mushrooms. In addition, P. florida mushroom also contains 18 essential amino acids such as isoleucine, lysine, methionine, cysteine, phenylalanine, tyrosine, tryptophan, valine, arginine, histidine, alanine, aspartic acid, glutamic acid, glycine, proline and serine (Djarijah and Djarijah, 2001).

1.4. Cultivation of P. florida mushroom

Different types of substrates like rice straw, corn cob, water hyacinth, maize straw, cotton waste and wheat bran, corn stalk and nut shells, sugarcane bagasse, beans straw and elephant grass are used for P. florida mushroom cultivation. Mondal et al. (2010) cultivated the P. florida mushroom using rice straw, banana leaves and both in different ratios (1:1, 1:3
and 3:1), the highest mycelium running rate was found in 1:1 ratio and highest yield was obtained from rice straw. Among sorghum grains, paddy grains, areca nut leaf sheath and coffee husk, sorghum grains was found to be best grain for the spawn production of P. florida (Thulasi et al., 2010).

1.5. Antioxidant and antimicrobial activities of P. florida mushroom

Antioxidants work to neutralize free radicals before they do harm to our bodies. Free radicals are atoms that cause damage to our cells. Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Mushrooms that contain antioxidants or increase antioxidant enzyme activity may be used to reduce oxidative damage in humans (Gregori et al., 2007). Various crude extracts of Pleurotus species have been shown to possess relatively strong antioxidant and antimicrobial activities. The antioxidant and antimicrobial activity of the ethanolic extract from the mycelium of P. florida, P. sajor-caju and P. aureovillosum were studied previously. Among all the species, P. florida showed a potent antioxidant activity and narrow antibacterial activity (Jagadish et al., 2008). The non enzymatic antioxidant capacity of P. florida was evaluated in two stages such as fresh and dried form of mushroom. Both fresh and dried samples of Pleurotus mushroom possess non-enzymatic activity (Selvi et al., 2007). The antioxidant activity was positively correlated with total polyphenol content. Methanol extract of P. florida fruiting bodies possessed more effective antioxidant activity than the synthetic antioxidant agent, catechin due to a higher amount of total phenols (Jose and Janardhanan, 2000).

The polysaccharides from fruiting body of P. florida mushroom was investigated for their antioxidant and antimicrobial activities. Polysaccharides showed significant antimicrobial activity against Bacillus spp. and Penicillium spp. and also it revealed potent antioxidant activity (Meera et al., 2011). On the other hand, the photo-irradiated P. florida mushroom were used for the synthesis of biofunctionalized silver nanoparticles (AgNPs)
which showed admirable antimicrobial effects against the pathogenic bacterium (Bhat et al., 2011). P. florida mushroom was proven to be a good candidate of antioxidant agent as it exhibited substantial antioxidant and radical scavenging activities which was also higher than the positive control, butylated hydroxy toluene (BHT) (Rahman et al., 2013) and the similar results were observed by Neelam and Singh (2013). Ganeshpurkar et al. (2012) investigated the antimicrobial and cytotoxicity assay of P. florida extract obtained with hydroethanol. The results exhibited an excellent antimicrobial and cytotoxic potential of hydroethanolic extract.

Mohamed Imran et al. (2011) studied the antioxidant capacity, total phenols and flavonoid for P. florida and P. ostreatus mushroom. Among the tested compounds, P. florida showed higher chelating activity against ferrous ion and also the total phenols and flavonoid contents were superior when compared to P. ostreatus. Ramkumar et al. (2010) suggested that the methanolic extract from P. florida can significantly scavenge the free radicals which were thought to be the main factor in the formation of many diseases.

1.6. Hepatoprotective activity of P. florida mushroom

Liver damage by hepatotoxic agents is of vital consequence because chronic liver injury leads to fibrosis, end stage cirrhosis and hepato-carcinoma. Hence, there is an increasing need to search of an agent which could protect the liver from such damages. There are some animal experiment–based reports that have investigated the potential of Pleurotus mushrooms to protect against liver injury. P. florida suppressed the toxin induced increased level of serum bilirubin in animals, indicating the hepatoprotective effect of this mushroom (Selvi et al., 2008; Sumy et al., 2011). The ethanolic extract of P. florida in 0.3% carboxymethyl cellulose showed hepatoprotective activity in male albino rats of which hepatic injury was induced with CCl₄ (Arunavadas and Umadevi, 2008).

P. florida showed hepatoprotective activity against paracetamol-induced liver damage in Wistar albino rats (Sumi et al., 2010). Also the P. florida protects the lipid peroxidation in hepatic tissue in hypercholesterolemic condition (Alam et al., 2009). P. florida also significantly decreased LDL/HDL (low-density lipoprotein/ high-density lipoprotein) ratio in experimentally induced hypercholesterolemic rats. The amelioration of atherogenic lipid
profile in hypercholesterolemic rats by dietary treatment with P. florida has also been reported by another study (Khan and Tania, 2012). Hypercholesterolemia-induced oxidative alteration is also recovered by the feeding of P. florida in rats (Khan et al., 2011).

Feeding on dried fruiting bodies of P. florida to hypercholesterolemic rabbits resulted in a reduction of total lipids, cholesterol and triglyceride levels of plasma and liver, whereas heart lipids were unaffected. In addition, total cholesterol and HDL / LDL cholesterol ratios increased in experimental animals providing an antiatherogenic potential (Guillamon et al., 2010). P. florida lectin protected the effect against arsenic induced oxidative stress in rat’s liver which diminished the oxidative impact by arsenic burden in the hepatocytes (Rana et al., 2012).

1.7. Anticancer and anti-inflammatory activity of P. florida mushroom

Cancer is a major health problem in developed and developing countries. After cardiovascular disease, cancer is the second leading cause of death. It is the abnormal growth of cells in our bodies that leads to death. Because of serious side effects of chemotherapy and radiation therapy, many cancer patients seek alternative complementary methods of treatment. Mushrooms have been used for treating cancer since ancient times. More than 50% of modern drugs in clinical use are of natural products. Intraperitonial administration of the methanolic extract of P. florida showed a significant tumour growth inhibition against the solid tumour induced by Ehrlich’s Ascites Carcinoma (EAC) cell lines in male Balb/c mice (Jose and Janardhanan, 2000). Selvi et al. (2011) demonstrated the anti-tumour potential of the ethanolic extract of P. florida mushroom against T24 bladder cancer cell lines using MTT and DNA fragmentation assay. The DNA fragmentation assay clearly indicates that the mushroom extract could arrest the growth of tumour cell and it could induce apoptosis in the tumour cells without the involvement of the inflammatory reactions (Selvi et al., 2011).

Irregular shaped biofunctionalized AuNP were synthesized by photo-irradiation technique using the edible mushroom P. florida, as a reductant. It showed an effective anti cancer property against four different cancer cell lines A549 (Human lung carcinoma), K-562 (Human chronic myelogenous leukemia bone marrow), HeLa (Human cervix) and MDA-MB
(Human adenocarcinoma mammary gland) and no lethal effect were observed in Vero (African green monkey kidney normal cell) cell lines (Bhat et al., 2013).

Methanolic extracts of P. ostreatus var. florida showed significant inhibition of mutagenicity elicited through mutagens requiring activation (Lakshmi et al., 2004). Jose et al. (2004) observed that the inhibition of platelet aggregation in methanol extract of P. florida fruiting bodies which reduced ameliorated acute and chronic inflammation. A methanol extract of P. florida fruiting bodies significantly inhibited platelet aggregation. The antiplatelet-aggregating activity along with the anti-inflammatory activities, suggests its potential therapeutic use against vascular disorders (Jose et al., 2004). Ganeshpurkar et al. (2011) reported in vitro anticataract activity of P. florida on isolated goat eye lens.

Gold nanoparticles (AuNPs) were synthesized by reducing chloroauric acid with a glucan, isolated from an edible mushroom P. florida. AuNPs-glucan bioconjugates exhibited excellent heterogeneous catalytic property in the reduction of toxic pollutant 4-nitrophenol to 4-aminophenol (Sen et al., 2013).

1.8. Bioactive polysaccharides from P. florida mushroom

Different functional compounds have been found in mushrooms, including phenolic compounds, sterols, terpenes, ceramides, etc. Among them, polysaccharides can be considered as one of the main groups of compounds occurring in mushrooms that are responsible for a great range of healthy properties. Recently, several polysaccharides from mushrooms have been isolated and characterized, such as PS, PS-I and PS-II from P. florida; PS from hybrid mushroom of P. florida and Calocybe indica, PS-I, II from hybrid mushroom of P. florida and Lentinus squarrosulus; P. florida and Volvariella volvacea (Ren et al., 2013). Generally, bioactive polysaccharides from mushrooms consist of a β-linked glucose backbone displaying different patterns and degrees of branching (Villares et al., 2012). The P. florida mushroom has shown different types of glucans, which are a watersoluble α, β-polysaccharide consisting of a main chain of α-(1→3) - linked D-glucan (Santos-Neves et al., 2008) and a water-insoluble (1→3),(1→6) - linked β-D-glucan (Rout et al., 2008).
Protoplast fusion between the strains of P. florida and Lentinus edodes produced nine new hybrid strains and among them six strains, i.e., pfe 1o, pfe 1p, pfe 1q, pfe 1r, pfe 1s, and pfe 1v produced fruit bodies. Maji et al. (2012) isolated a β-D-glucan from the fruit bodies of a hybrid strain pfe 1r. Aqueous extract of the fruit bodies of another hybrid strain, pfe 1q, yielded a mannogalactan (Maity et al., 2013).

1.9. Spent mushroom substrate

The substrate remains after the harvest of one full crop of mushroom, beyond which extension of crop becomes un-renumerative is called as the ‘spent mushroom substrate’ (SMS). The uncontrolled release of industrial wastes in the open environment and lack of availability in pre-treatment facilities contributes towards the level of contaminants in the soil and water bodies. The degradation of these contaminants mainly depends upon the physical and chemical conditions of soil and the nature of microorganisms thrive in soil. In addition to being rich nutrient source for various field crops, spent mushroom substrate originated from different edible mushrooms possesses unique physiochemical and biological properties which make SMS an ideal bioremediative agent for various environmental protection activities.

1.10. Environmental contamination

With the rapid growth of industries such as metal plating facilities, mining operations, fertilizer industries, tanneries, batteries, paper industries, pesticides and heavy metal wastewaters are directly or indirectly discharged into the environment. These unacceptable events increases often and it consequently leads to encephalopathy, cognitive impairment, behavioral disturbances, kidney damage, anemia and toxicity to the reproductive system (Das, 2005). Metal processing industries are prominently located in and around Salem District; which are somehow being the sources of metal (iron) discharge that contaminates soil and water.

1.11. Biosorption

Biosorption is the binding of metal contaminants onto its cellular structure from aqueous solutions by certain types of inactive and dead microbial biomass (Hubbe et al., 2011). The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. Biosorption mechanisms may be either
metabolism dependent or non-metabolism dependent. Microbial cells carry biosorption by secretion of extracellular accumulation, precipitation, cell surface sorption and intracellular accumulation (Ahalya et al., 2003).

1.12. Significance of the study

The questions of whether medicines discovered today are safer, more efficacious and more affordable than generic medicines (whose patents have expired) or medicines that are centuries old could be answered “no” for most of the modern medicines. If so, then it is logical to revisit and revive these age-old medicines for the welfare of mankind. Mushroom is one such medicine. Its history goes back over 5000 years, to the heyday of Ayurveda (which means the science of long life).

Medication on the basis of allopathic treatment is shooting up more and more particularly in the developed nations. Not only the cost but also the side effects of the drugs are projected exorbitantly high as the science and technology instrumentation getting more and more sophisticated. Moreover, the allopathic treatment is considered to be a kind of arresting technique rather than curing one. In view of that, even the developed nations are slowly trickling towards alternative medicine. Further alternative medicine in the countries like China and India successfully demonstrates the curing of the major complaints like arthritis, leucoderma, cancer, etc. Nevertheless, the cost involved in following the alternative medicine is comparatively much cheaper and even the common man is able to sustain the expenditure involved.

With this aim of alternating the common man’s problem in this country and finding out a gate way to the developing nations in projecting a cheap, edible drug, which conform, to the economic revenue of this country. The developments of the newly born chemical and pharmaceutical industry have brought about great social enthusiasm. The on-going discovery of more and more powerful new medicines, though not less toxic, seems to promise a bright future in which there is a specific pharmacological product to treat every disease.

Many physico-chemical methods like coagulation, flocculation, ion exchange, membrane separation and oxidation are available for the treatment of heavy metals. Major
drawbacks of these methods are high sludge production, handling and disposal of the solid waste, high cost and technical constraints. This necessitates are cost effective and environmentally sound techniques for treatment of waters containing metals. Adsorption is now recognized as an effective and economic method for heavy metal water treatment. Spent Mushroom Substrate (SMS) is used as a biosorbent for the removal of environmental contaminants in ground water.

In the present study, an attempt was made to evaluate the in vitro antioxidant activity, anticancer effects of the compounds isolated from methanolic extract of P. florida mushroom and also the study was focused on the adsorption of ferrous iron (Fe$^{2+}$) using P. florida SMS. The literature survey revealed that there are no scientific reports carried out regarding antioxidant and anticancer properties of the compounds isolated from methanolic extract of horse gram supplemented P. florida mushroom and adsorption of Fe$^{2+}$ using SMS. To the best of our knowledge this is the first report of isolation and purification of bioactive compounds 4-hydroxy-3-methoxycinnamic acid and cholest-5-en-3-ol from methanolic extract of P. florida mushroom and also this is the novel report for the adsorption of Fe$^{2+}$ using SMS of P. florida. However, no systematic work has been under taken on analyzing the antioxidant and anticancer activity of these compounds from P. florida mushroom, horse gram as a source of supplement and adsorption of Fe$^{2+}$ using SMS of P. florida. Hence the present study was formulated with following objectives.
OBJECTIVES OF THE STUDY

To cultivate the P. florida mushroom using different nutrient supplement.

To analyze the phytochemicals present in P. florida mushroom, in order to understand the chemical nature of biological active components.

To evaluate the antioxidants and antimicrobial properties of P. florida mushroom extracts.

To isolate and purify the active compounds from methanolic extract of P. florida using column and preparative thin layer chromatography.

To characterize the purified bioactive compounds from methanolic extract of P. florida using $^1$H - NMR and GC/MS.

To determine the antioxidant activity for the purified active fractions.

To analyze the anticancer activity for the purified active fractions against human lung cancer cell line by in vitro methods.

To remove the environmental contaminant (Fe$^{2+}$) from metal contaminated water using Spent Mushroom Substrate of P. florida.

The vast literature available pertaining to the study was collected and scrutinized. A very brief review of the same is presented in the next chapter.