# Chapter 1

# Introduction

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## 1 Mushroom: Source of bioactive molecules

Mushrooms are reported to be one of the important repositories of bioactive compounds. Mushroom metabolites are investigated worldwide in search of new therapeutic agent from natural source, as it has a historical use in human diet and medicine (Wassar, 2000). Recently their consumption has been increasing as natural neutraceutical, pharma food and biological response modifiers (BRM). Globally, the number of mushrooms is estimated at 140,000, of which around only 10% are known. Among the 14,000 known species, about 50% are considered as edible of which more than 2,000 are safe, and about 700 species are known to possess significant pharmacological properties (Chang, 1999a, 1999b; Wasser and Weis, 1999a, 1999b; Reshetnikov et al., 2001). Out of the 2000 safe edible mushrooms about 35 species have been cultivated commercially and 20 are cultivated on an industrial scale (Somasundaram et al., 1998; Ogundana et al., 1982; Kavishree et al., 2008). It has been reported that the gross composition of mushrooms is water (90%), and dry matter such as protein (10%–40%), fat (2%–8%), carbohydrates (3%–28%), fiber (3%–32%) and ash (8%–10%) (Ash percentage is the fraction of dry matter that remains after incineration of the organic material in a sample and is mainly composed of salts, metals and so forth) (Breene, 1990). There is a well-established consumer acceptance for cultivated mushrooms. However, a number of wild edible mushrooms have been traditionally consumed by specific groups of local people. Nevertheless, wild mushrooms are becoming more and more important in our diet for their nutritional, pharmacological characteristics as several major substances having immunomodulatory and/or antitumor activity have been isolated from these mushrooms. The compounds are mainly polysaccharides particularly β-D-glucans and polysaccharopeptides (PSP), proteins polysaccharide complex and lectins. Furthermore, other bioactive substances such as steroid, triterpenes, lipids and phenols have also been identified and characterized in mushrooms with proven medicinal properties (Sanmee et al., 2003). Thus, it is obvious that mushrooms represent a major and as yet largely unexploited source of novel potent pharmaceuticals.

## 1.1 Mushroom proteins as therapeutics

Mushrooms have been valued as highly tasty and nutritional food by many societies throughout the world from the time immemorial (Chang and Miles, 1992). Recently, apart from its dietary value, novel therapeutic proteins from mushroom have been explored using improved protein separation techniques and proteomics approach. Several research groups have isolated a variety of proteins from different mushrooms having potential applicable biological activities. These include antifungal proteins, ribosome inactivating proteins, ribonucleases, ubiquitin-like proteins and peptides, lectins, cellulases, xylanases, laccases, invertases, trehalose phosphorylases etc (Adinolfi et al., 1995; Lam and Ng 2001a; Ng et al., 2002; Ye and Ng 2002a, 2000b).

Antifungal Protein and peptides were isolated from *Tricholoma giganteum* (Guo et al., 2005), *Ganoderma lucidum* (Wang and Ng, 2006a), *Lyophyllum shimeiji* (Lam and Ng, 2001a) *Pleurotus eryngii*, (Wang and Ng, 2004a) *Pleurotus ostreatus* (Chu et al., 2005) and *Agrocybe cylindracea* (Ngai et al., 2005), *Polyporus alveolaris* (Wang et al., 2004a), *Polyporus adusta* (Ng and Wang, 2004b). Mushroom antifungal proteins exhibit a range of molecular masses of around 7-28kDa and all except alveolarin (dimeric) are monomeric in nature (Wong et al., 2011).

Ribonuclease is also an important molecule derived from different mushrooms. The mushroom RNases are known so far include those from *Volvariella volvacea* (Wang and Ng, 1999), *Pleurotus pulmonarius* (Ye and Ng, 2002a), *Pleurotus tuber-regium* (Wang and Ng, 2001a), *Lentinus edodes* (Kobayashi et al., 1992), *Pleurotus ostreatus* (Ye and Ng, 2002b), *Irpex lacteus* (Watanabe et al., 1995) *Lyophyllum shimeiji* (Zhang et al., 2010a), *Russulus virescens* (Wang and Ng, 2003), *Pleurotus djamor* (Wu et al., 2010). These RNases exhibit a range of molecular masses (MW 14-50kD), a diversity of N-terminal sequences, and different pH and temperature maxima.

Some ribosome inactivating polypeptides like Flammin and Velin from *Flammulina velutipes* (Ng and Wang, 2004c), Llyophyllin from *Lyophyllum shimeiji* (Lam and Ng, 2001a) were isolated.

Ubiquitin-like peptides from mushrooms *Pleurotus sajor-caju* (Ng et al., 2002), *Cantharellus cibarius* (Wang et al., 2003), *Agrocybe cylindracea* (Ngai et al., 2003) were also reported.

Lectins from *Lentinus edodes* (Ngai and Ng, 2003), *Agrocybe aegerita* (Zhao et al., 2003) are isolated having different bioactivities.

**1.2 Significance of mushroom in cancer therapy**

Cancer is ranked the most dreaded disease worldwide in terms of incidence and mortality. Despite enormous efforts made towards the development of cancer therapies over the past several decades, cancer is still a major worldwide health problem. Mortality caused by cancer is still unacceptably high. Cancer cells are highly heterogeneous and they exhibit deregulation in multiple cellular signaling pathways (Sarkar and Li, 2009). It is primarily referred to abnormal proliferation cells and their metastases to other organs or tissue. Cancers may be caused in one of three ways; incorrect diet, genetic predisposition, and via the environment (Reddy et al., 2003). There are three major modality of cancer therapies like chemotherapy, surgery, radiation therapy above that cancer vaccine and antibody therapy are now getting into mainstream treatment. Among these, chemotherapy is one of the important approaches. Existing chemotherapeutic agents or cancer drugs retard cancerous growth as they act as cell cycle inhibitors, apoptosis stimulators, signal transduction inhibitors, anti-inflammatory compounds, anti-invasive agents, anti-angiogenic compounds and differentiating agents (Amin et al., 2009). But till date there are no drugs or therapeutic strategies that prevent and inhibit the growth of cancer cells without imparting adverse effect to the normal host cell. In recent years, dietary and natural compounds with therapeutic potential and harvested from nature (natural compounds) have received increased attention (Sarkar and Li, 2009). Moreover, despite major scientific and technological progress in combinatorial chemistry, drugs derived from natural product still make an enormous contribution to drug discovery today. As mushroom is a very good source of natural bioactive compounds, there is an enormous scope to use it in cancer therapy.

Medicinal effects have been demonstrated for many traditionally used mushrooms (Ooi and Liu, 2000), including extracts of species from genera *Auricularia, Flammulina, Ganoderma, Grifola, Hericium, Lentinus* (Lentinula), *Pleurotus, Trametes* (Coriolus), *Schizophyllum*, and *Tremella* (Wasser, 2002). Over the past two to three decades, scientific and medical studies in Japan, China, Korea and more recently the United States have increasingly demonstrated the potent and unique properties of mushroom-extracted compounds for the prevention and treatment of cancer (Zaidman et al., 2005). It has been known for many years that selected mushrooms of higher Basidiomycetes origin are effective against cancer (Wasser, 2005). Lucas et al (1957) first reported the antitumor activity of the higher basidiomycetes. They used extracts of fruiting bodies of *Boletus edulis* Bull.:Fr. and other homobasidiomycetes in tests against the Sarcoma-180 tumor bearing mice model. There are approximately 650 species of higher Basidiomycetes having antitumor activity (Mizuno 1995a, 1995b; Wasser, 2002). Searching for new antitumor substances from mushrooms and studying the medicinal value of these mushrooms has become a matter of great significance.

### 1.3 Mushroom derived compounds as anticancer and immunomodulatory agent

Mushrooms have an established history of use in nutritionally functional food as well as traditional oriental therapies. After the discovery of Penicillin in 1929, fungi were regarded as rich sources of natural antibiotics and other bioactive molecules. For hundreds of years in China, Japan, Korea and the Slave regions, mushrooms such as *Ganoderma lucidum*, *Lentinus edodes*, *Fomes fomentarius*, *Fomitopsis officinalis* and many others have been used to cure different diseases like atopic dermatitis, allergic asthma, food allergy, inflammation, atherosclerosis, autoimmune joint inflammation such as rheumatoid arthritis, thrombosis, hyperglycemia, human immunodeficiency virus (HIV) infection, listeriosis, tuberculosis, septic shock and cancer (Wassar, 2002; Lull et al., 2005). At the present time, constituent molecules of mushroom and secondary metabolites are known as bioactive compounds including polysaccharides, glycoproteins, proteoglycans, terpenoids, fatty acids, proteins, lectins, etc that possess certain medicinal properties. These compounds are found in fruiting bodies, mycelia and spores and culture broth of macrofungi (Moradali et al., 2007). In response to the increase in diseases involving immune dysfunction and cancer in recent years, medical researchers and clinicians are interested in immunotherapy as well as the discovery of novel immune-potentiators and compounds having powerful remedy potential without side effects, pathogenic resistance or affecting normal cell division. Immunomodulators or biological response modifier can be effective agents in treating and preventing diseases.

Immunomodulating activity of compounds isolated from medicinal macrofungi, related their effects to act on immune effecter cells such as haematopoietic stem cells, lymphocytes, macrophages, T cells, dendritic cells (DCs), and natural killer cells (NK) involved in the innate and adaptive immunity, resulting in the production of cytokines (Lull et al., 2005). The therapeutic effects of these compounds such as antitumor and anti-infective activity and suppression of autoimmune diseases have been associated in many cases with their immunomodulating effects. Some mushroom derived Immunomodulatory and anticancer compound are listed in table 1.1.

Table 1.1: Immunomodulatory and anticancer compounds from mushrooms

| ***Source*** | ***Compound group*** | ***Example*** | ***Activities*** | ***References*** |
|---|---|---|---|---|
| *G. frondosa*<br>*L. edodes*<br>*A. cinnamomea*<br>*P. citrinopileatus*<br>*S. sclerotiorum* | Polysacch-arides | Grifolan,<br>Lentinan,<br>Galactomannan<br>Scleroglucan | Immunomo-dulator<br>Antitumor<br>Antiangio-genic<br>Antitumor | Bhon and BeMiller 1995<br>Yap and Ng., 2003<br>Yang et al., 2009<br>Wang et al., 2005<br>Palleschi et al., 2005 |
| *Tricholoma* sp<br>*C. versicolor*<br>*T. versicolor* | Polysacch-aride-peptide | PSP<br>PSP<br>PSK | Antitumor<br>Antitumor and immunomo-dulator | Wang et al., 1995<br>Wang et al., 1995<br>Chan and Yeung, 2006<br>Price et al., 2010 |
| *G. lucidum*<br>*C. sinensis* | Steroids | Polyoxygenated derivates of ergosterol | Cytotoxic<br>Antitumor | Lin and Tome, 1991<br>Bok et al., 1999 |

Table 1.1 continued.........

| ***Source*** | ***Compoud group*** | ***Example*** | ***Activities*** | ***References*** |
|---|---|---|---|---|
| *G. lucidum* | Terpenoides | Ganolucidic acids<br><br>Lucidumols<br><br>Ganoderols<br>Applanoxidic acids | Histamine release inhibition<br>Antihypertension<br>Antiinflam-matory | Novaes et al., 2003<br>Morigiwa et al., 1986 |
| *R. delica*<br>*V. volvacea*<br>*A. aegerita* | Proteins | Lectin<br><br>Fips<br>Ganoderic acids<br>Ganoderiol<br>Ganoderenic acids<br>Lucidenic acids | Antiproliferative<br>Immunomo-dulator<br>Antitumor<br>Immunomo-dulator<br>Antitumor<br>Cytotoxic | Zhao et al., 2010a<br>She et al., 1998<br>Zhao et al., 2003<br>Ko et al., 1995<br>Chairul et al., 1991<br>Toth et al., 1983<br>Toth et al., 1983 |
| *A. blazei* | Fatty acids | Lipid | Antitumor | Nanba, 1995<br>Moradali et al., 2004 |
| *Ganoderma sp.* | Organic germanium | Bis-β-carboxyethylgermanium sesquioxide | Antitumor and Immunomo-dulating | Chiu et al., 2000 |
| *G. lucidum* | Nucleotides | Adenosine | Platelet aggregate inhibition | Shimizu et al., 1995 |

These Mushroom derived active molecules can be broadly divided in two categories such as i) macromolecules like polysaccharides, proteins and polysachharide protein complexes and ii) small molecules such as terpenoids, peptides etc (Novaes et al., 2003; Ngai et al., 2003). However majority of the studies is focused on polysaccharides and its derivatives. On the other hand, very few studies have been undertaken to understand the role of mushroom derived proteins in immunodulation and anticancer studies. Lectins and some small molecular weight proteins with RNase activity, ribosome inactivating proteins are among the reported bioactive compounds from mushrooms (Wang and Ng, 1999; Ng, 2004; Zhang et al., 2010a, 2010b; Ng and Wang, 2004a, 2004b). Considering the vast biodiversity of available mushrooms it will be of great significance to explore proteins as anti tumor and immunomodulatory response.

### 1.4 Cultivable and wild edible mushrooms as source of anticancer and immunomodulatory compound

Edible higher basidiomycetes are being evaluated for their nutritional value and as well as their pharmacological properties. Many edible mushrooms used in traditional folk medicine, including *Lentinus edodes, Grifola frondosa , Hericium erinaceus, Flammulina velutipes*, *Pleurotus ostreatus* and *Tremella mesenterica* are also a source of relatively pure bioactive compounds for medical usage, while other, non-edible, species, such as *Ganoderma lucidum*, *Schizophyllum commune* and *Trametes versicolor* are used only for their medicinal properties. Historically, most medicinal mushrooms were gathered in the wild, growing on dead or living trees; nowadays, however, most of the important species are cultivated artificially on wood derivatives by a process of low moisture fermentation, thus removing the need to harvest scarce natural sources. The cultivable mushrooms attract intense industrial interest, being a source of many pharmaceutical substances with potent and unique valuable as well as of various health enhancing substances known as dietary supplement, functional foods, nutraceuticals, mycochemicas and designer foods (Wassaer et al., 2002). Higher basidiomycetes are well recognized to posses immunomodulatory substances among various mushrooms studied. In the beginning, consumption of mushrooms developed by gathering and cultivation. Although major edible mushrooms are cultivable but still some species are collected from wild for consumption. Also, the availability of such wild edible mushroom depends on geographical distribution. Therefore, it will be of great importance to study the biological effect of the locally available mushroom species.

In Indian context, there are huge species of edible (both wild and cultivated) mushrooms; but these are not well studied for therapeutic potential for cancer treatment and also there is no tradition for the use mushroom or mushroom extracts for therapeutic purposes. At present, lot of effort is going on globally to find out alternate viable treatment for cancer therapy which has less side effects and may be used as combination therapy along with established or new chemotherapeutic drugs. Thus, Indian edible mushrooms may be suitable to explore the potential of biological response modifier in cancer therapy. At present several research groups in India are involved in mushroom research to study either the taxonomy or the structural studies of different components of mushroom derived products. But yet not much effort is exerted in therapeutic potential of mushroom derived proteins in cancer therapy. Vaidya and Lamrood (2000) studied the mushroom available from Pune region; Sarma and Vittal (2000) studied the biodiversity of fungi from mangrove forest of eastern coast. Raghukumar et al (1999) studied various marine fungi and their application. Only a few studies have been carried out with mushroom for immunomodulatory and anti-tumor properties. Bhonde et al (2000) studied anticarcinogenic activity of two Species of *Phansomba* a mushroom available in Maharasthra and Goa region. Structural aspects of soluble mushroom glucans (*P. osreatus*, *P. sajor-caju*, *A. hygrometricus*) were studied at Vidyasagar University West Bengal (Rout et al., 2004, 2005, 2006; Chakraborty et al., 2004)

With this view, the focus of the present study was concentrated to explore bioactive proteins with respect to immunomodulatory and antitumor potential in five different species of locally available mushrooms. Three types of cultivated edible mushrooms i) *Pleurotus ostreatus* (Oyster mushroom), ii) *Volvariella volvacia* (Straw mushroom), iii) *Calocybe indica* (Milky mushroom) and two wild edible mushroom iv) *Astraeus hygrometricus* (Kurkuri mushroom) and v) *Termitomyces clypeatus* (Termite mushroom) were undertaken in this study. Oyster mushroom and paddy mushroom are well cultivated in India and the anticancer properties of these mushroom derived glucans were reported (Mahajan et al., 2000; Rout et al., 2004; Sarangi et al., 2006; Shah et al., 2007). There is not much work on anticancer potential of *A. hygrometricus* except some recent reports on the immunomodulatory and anticancer potential of this mushroom derived glucans (Mallick et al., 2009, 2010a, 2010b, Chakraborty et al., 2004). But more detail investigation of these mushroom derived molecules especially protein having anticancer properties are needed. The mechanism action of also these mushroom derived proteins need to be investigated to evaluate their anticancer efficacy.

### ***1.4.1 Pleurotus ostreatus*** (Oyster mushroom)

The cultivated mushroom varieties are mainly *Agaricus bisporus, Lentinus edodes* and the oyster mushroom, *Pleurotus ostreatus* (Suguimoto et al., 2001). Cultivation of these mushrooms represents a major industry in the countries of south East Asia (Ragunathana and Swaminathan, 2003). *Pleurotus* species with a 24.2% of world production (Stamets, 2000.) after *Agaricus* spp. become very popular edible mushrooms in different parts of the world. It is gaining popularity due to low cost technology and easy availability of various substrates for its cultivation. In India, prevalence of varied, agro-climatic conditions and availability of vast quantities of lignocellulosic raw materials have stimulated the cultivation of *Pleurotus* spp (Ragunathana and Swaminathan, 2003).

The medicinally beneficial effects of *P. ostreatus*, such as their anticancer activities, immunomodulatory effects, and antiviral, antibiotic anti-inflammatory and cholesterol lowering activities are known worldwide (Sarangi et al., 2006). The bioactive compounds derived from *P. osrteatus* are mainly polysaccharides, lectins and proteins. Wang and Ng (2000) isolated a novel ubiquitin-like protein from *Pleurotus ostreatus* having anti-HIV, translation inhibitory and ribonuclease activities. *Pleurotus ostreatus* mycelia derived proteoglycans has antitumor and immunomodulating effects (Sarangi et al., 2006).

### ***1.4.2 Volvariella volvacea*** (Straw mushroom)

*Volvariella volvacea* or straw mushroom is grown on an industrial scale in many tropical and subtropical regions and ranks fifth among cultivated mushrooms in terms of annual world-wide production (Wang et al., 2008). Among different species of *Volvariella spp.*, *V. volvacea, V. bombycina* and *V. diplasia* have been cultivated in India (Ghosh et al., 2008). The local people consume these as delicious food materials. Nutritive values of the fruit body *V. volvacea* have been analyzed and were reported to possess (92.41%) moisture, (2.19%) carbohydrate, (2.94%) protein, (0.40%) crude fat, (1.15%) crude fiber, (0.99%) ash, and (0.80%) nitrogen on a dry weight basis (Kurtzman, 2005). Apart from its culinary properties of a unique aroma and texture (Ahlawat et al., 2008; Mau et al., 1997), it is reported to also have pharmaceutical potential since it produce antitumor polysaccharides, immunosuppressive proteins and immunomodulatory lectins (Hsu et al., 1997; Kishida et al., 1992; She et al., 1998). Due to its cellulolytic characteristics, *V. volvacea* had been cultivated on an array of agro-industrial residues (Chang, 1993), but low biological efficiency (i.e. conversion of growth substrate into mushroom fruiting bodies) has limited its production on lignocellulosic wastes (Ding and Jhon, 2006). *V. volvacea* contains a fungal immunomodulatory protein (FIP- Vvo) that induces Th1-specific cytokine (IL-2, IFN-γ, LT) and Th 2-specific cytokine (IL-4) (Hsu et al., 1997; She et al., 1998)

### *1.4.3 Calocybe indica* (Milky mushroom)

*Calocybe indica,* is an edible mushoom and can be cultivated throughout the year in the entire plains of India. It is commonly known as Dudh Chattu (milky mushroom) and a new addition to the world of edible mushrooms from India (Purkayastha and Chandra, 1976). Dudh Chattu is a robust, fleshy, milky white, umbrella like mushroom. It can grow at hot humid climate, 25 ºC -35 ºC. *C. indica* reported to contain different types of vitamins, minerals,6 volatile flavored compounds,7,8 and can be cultivated using various substrates. A protein fraction (Purkayastha and Nayak, 1981; Maiti et al., 2008) and 6,9 di-ethyl ether extract of *C. indica* showed antimicrobial, antiproliferative, and immunostimulatory activities (Mandal et al., 2010).

### *1.4.4 Astraeus hygrometricus* (Kurkuri mushroom)

*Astraeus hygrometricus* is a non cultivated wild edible mushroom, which grows on nutrient poor soil in association with mycorrhiza primarily during rainy season. *Astraeus* is one of the most common gasteromycete genera in temperate and tropical ecosystems. Morgan (1889) was the first to recognize *Astraeus* as a distinct genus with *Astraeus* *hygrometricus* (Pers.) Morgan as the type and only species, whereas, Phosri et al (2007) described four species of this genus on the basis of its rDNA region. Although this species of mushroom is wide spread however very few studies with respect to biological activity are conducted. Earlier studies have reported a lectin with no agglutination potential from this species. Chakraborty et al (2004) reported a polysaccharide from this species with splenocyte proliferating potential *in vivo*. It has been found a heteroglucan, designated as AE2 obtained from the fruit body to have immunomodulatory potential. It activated macrophages, NK cells as well as stimulated proliferation of splenocytes and thymocytes in murine model. Further AE2 augumented the immune system of tumor bearing host and that resulted in tumor regression. Further AE2 had no cytotoxicity to cells in vitro or to the host in vivo (Mallick et al., 2009, 2010a, 2000b). Owing to the local availability of this mushroom and its potential of bioactive component make it a suitable candidate for investigation for bioactive components.

***1.4.5 Termitomyces clypeatus*** (Termite mushroom)

*Termitomyces clypeatus* have been identified as wild edible mushrooms with high nutritive value. It grows abundantly in the termites guts of the laterite forest soil in Southwest Bengal, India during September–October every year (Mondal et al., 2006). It contains 31% protein, 32% carbohydrate and 10–14% ascorbic acid (Ogundana and Fagade, 1982). Local people consume them as delicious food material. Several enzymes of high therapeutic values have been reported from *Termitomyces clypeatus* (Rouland et al., 1992). It is known as a potential producer of different enzymes in culture media (Khowala and Sengupta, 1992; Khowala, et al., 1992). The fungus *Termitomyces clypeatus* has been found to be a potential producer of a broad spectrum of extracellular glucosidases (cellulase, sucrase, cellobiase etc.), capable of hydrolysing the polysaccharides, e.g., hemicellulose, cellulose, and starch. Different enzymes such as endo 1,4-β-D-Xylanase, 1,4-β-D-Xylosidase, a-L-arabinofuranosidase, acetyl esterase, a-amylase, and amyloglucosidase were also purified from the fungus. All these enzymes have various industrial applications (Ghorai et al., 2009).

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