ANTIPROLIFERATIVE ACTIVITY OF BIOACTIVE COMPOUNDS FROM MUSHROOMS OF INDIAN ISOLATES

KAVIYARASAN V* AND SHENBAGARAMAN R
C.A.S.in Botany, University of Madras, Guindy Campus, Chennai 600025
manikavi53@gmail.com

ABSTRACT

Biodiversity study on mushrooms of basidiomycetes was initiated and more than seven hundred species were described by Natarajan and associates during 1975-2008 from South India. But the bio-documentation of many medicinal mushrooms were initiated a decade ago by Vaidya and associates from Western India, Janardhanan and associates from Kerala and Kaviyarasan and associates from South India. *Lentinus tuberregium, Neolentinus kauffmanii* and *Agaricus heterocystis,* were studied for their medicinal properties such as antitumor, antiviral, antimicrobial and antioxidant activities. These indigenous mushrooms are effective against many cancer lines and induce apoptosis and results in tumor cell death. Antiangiogenesis effect of *Trametes hirsuta* extract was well established with fertilized hen eggs. These results, clearly established their candidature for drug formulations. Two novel anticancer compounds extracted from *Lentinus tuberregium* were filed for patent for their anticancer properties. Polysaccharides from *Tramates hirusuta,* an indigenous isolate was also very effective against many cancer lines. Currently few more edible mushrooms are being studied for their medicinal properties.

INTRODUCTION

Many clinically important drugs, such as aspirin, digitoxin, progesterone, cortison and morphine, have been derived directly or indirectly from higher plants. Less well-recognized but of great clinical importance are the widely used drugs from fungi such as the antibiotics, penicillin and griseofulvin, the ergot alkaloids and cyclosporine [1]. Mushrooms are the macrofungi with a distinctive fruiting body, which can be either hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand [2]. Mushrooms constitute at least 14,000 and perhaps as many as 22,000 known species. The number of mushroom species on the earth is estimated to be 140,000, suggesting that only 10% are known [3].

For millennia, mushrooms have been valued by mankind as an edible and medical resource. A number of bio- active molecules, including antitumor substances, have been identified in many mushroom species. During the last two decades there has been an increasing recognition of the role of the human immune system for maintaining good health. Mushrooms such *as Ganoderma lucidum* (reishi), *Lentinus edodes* (shiitake), *Inonotus obliquus* (chaga) and many others have been collected and used for hundreds of years in Korea, China, Japan, and eastern Russia. It is notable and remarkable how reliable the facts collected by traditional eastern medicine are in the study of medicinal mushrooms [4,5]. In India, the knowledge of indigenous mushroom consumption as food and medicine prevails from time immemorial. But there is no authentic record of our own. But two authentic reports on medicinal uses were recorded by Petch, [6] on the uses of *Termitomyces* mycelial mass near Thanjavur, Tamilnadu, India and Gordon Wasson of Germany. A good book on Soma drink referred in Rig Vedas stating that *Amanita muscaria* extract was the Soma drink by comparing the descriptions in the Vedas with the structural description of *Amanita* has been written. Use of compounds from *Phellinus* sp. as preservative was recorded by Sharifi *et al.* [7] and their antitumor activity was studied by Meera and Janardhanan [8]. Natarajan and associates during 1978-2008 has studied biodiversity of Agarics diversity of South India. Recently many indigenous edible and medicinal mushrooms were studied by our group for their antioxidant and antitumor activities using cell lines and presented in this paper [9-14].

CURRENT STATUS OF RESEARCH

The fruiting body and the mycelium of mushrooms contain compounds with a wide range of medicinal properties. Currently a lots of research is being carried out to prove the medicinal properties such as antitumor properties, antiviral, antibacterial

and immunomodulatory properties of the bioactive metabolites at both national and international level. Mushrooms are rich sources of β-glucan, proteoglycan, lectin, phenolic compounds, flavonoids, polysaccharides, triterpenoids, diatery fibre, lentinan, schizophyllan, lovastatin, pleuran, steroids, glycopeptides, terpenes, saponins, xanthones, coumarins, alkaloid, kinon, fenil propanoid, kalvasin, porisin, AHCC, maitake D-fraction, ribonucleases, eryngeolysin, and also have been used extensively in traditional medicine for curing various types of diseases such as antimicrobial, antiviral, anticancer, antitumor, antiinflamatory, cardiovascular diseases, immunomodulating, central activities etc. [15-17, 4]. Medicinal mushroom research has focused on discovery of compounds that can modulate positively or negatively the biologic response of immune cells. Those compounds, which appear to stimulate the human immune response, are being sought for the treatment of cancer, immunodeficiency disease or for generalized immunosuppression following drug treatment. They are also sought for combination therapy with antibiotics and as adjuncts for vaccines [18]. Wasser [4] reported that mushroom polysaccharides are regarded as biological response modifiers (BRM). This basically means that they cause no harm and place no additional stress on the body, but help the body to adapt to various environmental and biological stresses. Mushroom polysaccharides support some or all of the major systems of the body, including nervous, hormonal and immune systems as well as regulatory functions. The polysaccharides from mushrooms do not attack cancer cells directly, but produce their anti-tumour effects by activating different immune response in the host [4].

Polysaccharides are a structurally diverse class of macromolecules able to offer the highest capacity for carrying biological information due to a high potential for structural variability [4]. Whereas the nucleotides and amino acids in nucleic acids and proteins effectively, interconnect in only one way, the monosaccharide units in polysaccharides can interconnect at several points to form a wide variety of branched or linear structures [19]. This high potential for structural variability polysaccharides gives the necessary flexibility to the precise regulatory mechanisms of various cell-cell interactions in higher organisms. The polysaccharides of mushrooms occur mostly as glucans. Some of which are linked by β-(1-3), (1-6) glycosidic bonds and α-(1-3) glycosidic bonds but many are true heteroglycans.

BIOACTIVE POLYSACCHARIDES

Polysaccharides are the best known and most potent mushroom- derived substances with antitumor and immunomodulating properties [20-24]. Historically, hot-water-soluble fractions (decoctions and essences) from medicinal mushrooms, i.e., mostly polysaccharides, were used as medicine in the Far East, where knowledge and practice of mushroom use primarily originated [5]. Ikekawa *et al*. [25] published one of the first scientific reports on antitumor activities of essences obtained from fruiting bodies of mushrooms belonging to the family Polyporaceae (Aphyllophoromycetideae) and a few other families, manifested as host-mediated activity against grafted cancer – such as Sarcoma 180 – in animals [26,27]. Soon thereafter the first three major drugs were developed from medicinal mushrooms. All three were polysaccharides, specifically β-glucans: krestin from cultured mycelial biomass of *Trametes versicolor* (Turkwey Tail), lentinan from fruiting bodies of *L. edodes*, and schizophyllan from the liquid cultured broth product of *Schizophyllum commune*.

Hobbs [5] reported that *L. edodes* produces two bioactive preparations, which are efficient immune modulators, mycelium extract and lentinan. These two bioactive polymers appear to act as host defense potentiators restoring and enhancing the responsiveness of host cells to lymphocytokines, hormone and other biologically active substances. The immunopotentiation has been shown to occur by stimulating the maturation, differentiation or proliferation of cells involved in host defense mechanism. Many interesting biological activities of lentinan including increase in the activation of non- specific inflammatory response such as acute phase protein production; vascular dilation and haemorrhage-inducing factor in vivo [28], activation and generation of helper and cytotoxic T cells [15].

Chihara *et al*. [15] reported that lentinan increase host's resistance against various kinds of cancer and has the potential to restore the immune function of affected subjects. The interaction of lentinan with many kinds of immune cells was not known until recently. Ross *et al*. [29] provided an insight into receptor binding in immune cells by β-glucan from fungi and further showed that β-glucan from yeast bind to iC3b- receptors (CR3, CD11b/CD18) of phagocytic and natural killer (NK) cells. When this happens, it will stimulate phagocytosis and/or cytotoxic degranulation. Lentinan has also been shown to stimulate peripheral blood lymphocytes in vitro to increase interleukin-2-mediated LAK cell (lymphokine-activated

killer cell) and NK cell activity at levels achievable in vivo by administration of clinical doses of lentinan. This observation was made using the blood of healthy donors and cancer patients. Lentinan has also been shown to inhibit suppressor T cells activity in vivo and to increase the ratio of activated T cells and cytotoxic T cells in the spleen when administered to gastric cancer patients undergoing chemotherapy.

BIOACTIVE SMALL MOLECULES

Apart from Polysaccharides mushrooms also contains valuable bioactive small molecule that have antitumor, antimicrobial and antiviral properties. The small molecule which possess anti-microbial activity includes Applanoxidic acid A, isolated from *Ganoderma annulare* (Fr.) Gilbn., shows weak antifungal activity against *Trichophyton mentagrophytes* [30]. Steroids like 5a-ergosta- 7,22-dien-3b-ol or 5,8-epidioxy-5a,8a-ergosta-6,22- dien-3b-ol, isolated from *Ganoderma applanatum* (Pers.) Pat., proved to be weakly active against a number of gram- positive and gram-negative microorganisms. Oxalic acid is one agent responsible for the antimicrobial effect of *Lentinula edodes* (Berk.) Pegler against *S. aureus* and other bacteria [31]. Ethanolic mycelial extracts from *L. edodes* possess antiprotozoal activity against *Paramecium caudatum* [32]. The antimicrobial activity of *Podaxis pistillaris* (L.: Pers.) Morse, used in some parts of Yemen for the treatment of 'nappy rash' of babies and in South Africa against sun burn, is caused by epicorazins [33]. These substances belong to the group of epipolythiopiperazine-2,5- diones, an important class of biologically active fungal metabolites. Other antimicrobial compounds from the Aphyllophorales were summarized by Zjawiony [34].

In contrast to bacterial infectious diseases, viral diseases cannot be treated by common antibiotics and specific drugs are urgently needed. Antiviral effects are described not only for whole extracts of mushrooms but also for isolated compounds. They could be caused directly by inhibition of viral enzymes, synthesis of viral nucleic acids or adsorption and uptake of viruses into mammalian cells. These direct antiviral effects are exhibited especially by smaller molecules. Indirect anti-viral effects are the result of the immunostimulating activity of polysaccharides or other complex molecules.

Several triterpenes from *Ganoderma lucidum* (M. A. Curtis: Fr.) P. Karst. [i.e. ganoderiol F , ganodermanontriol, ganoderic acid B are active as antiviral agents against human immunodeficiency virus type 1 (HIV-1). The minimum concentration of ganoderiol F and gano- dermanontriol for complete inhibition of HIV-1 induced cytopathic effect in MT-4 cells is 7.8 mg/ml. Ganoderic acid B inhibits HIV-1 protease [35]. Ganodermadiol , lucidadiol and applanoxidic acid G , isolated from *G. pfeifferi*, but also known from other *Ganoderma* species, possess *in vitro* antiviral activity against influenza virus type A. Further, ganodermadiol is active against herpes simplex virus type 1, causing lip exanthema and other symptoms [36]. *In vitro* antiviral activity against influenza viruses type A and B was demonstrated for mycelial extracts of *Kuehneromyces mutabilis* (Schaeff.: Fr.) Singer & A. H. Sm. [37], extracts and two isolated phenolic compounds from *Inonotus hispidus* (Bull.: Fr.) P. Karst [38] and ergosterol peroxide, present in several mushrooms. The antiviral activity of *Collybia maculata* (Alb.&Schwein.: Fr.) P.Kumm. (vesicular stomatitis viruses in BHK cells) is caused by purine derivatives [39]. Thus many drugs are formulated not only to treat against diseases but to stimulate the immune system to resist the pathogens using biomolecules from mushrooms.

CURRENT STATUS OF RESEARCH IN OUR LABORATORY

The major objective of research is to study the biodiversity of basidiomycetes of both Eastern Ghats (Thirumala hills, Kolli hills and Javvadi hills etc) and Western Ghats besides the plains of Tamil Nadu. Besides the biodiversity study bio-documentation of these organisms is the need of the hour. Medicinal properties of few South Indian mushroom species were charcterised by isolating few bio active molecules from indigenous mushroom species. An intracellular fibrinolytic protease from *Ganoderma lucidium* isolate VK12 (Fig 1) was isolated and purified [10], which has the potential to be used as an alternative to the commercially available Urokinases having many side effects on the patient for treatment of cardio vascular diseases. The enzyme was purified to homogeneity and molecular mass of was determined as 33.2 kDa. By enzyme kinetic studies the enzyme was characterized as metalloprotease. The purified fibrinolytic protease showed anticoagulant activity with human plasma. Moreover the purified protease protected pulmonary mice thromboemolism to the extent of 70%. The survival rate of mice treated with purified protease were 36, 72 and 81% at doses 20, 40 and 60µg/kg respectively, compared to 9% in the control [40].

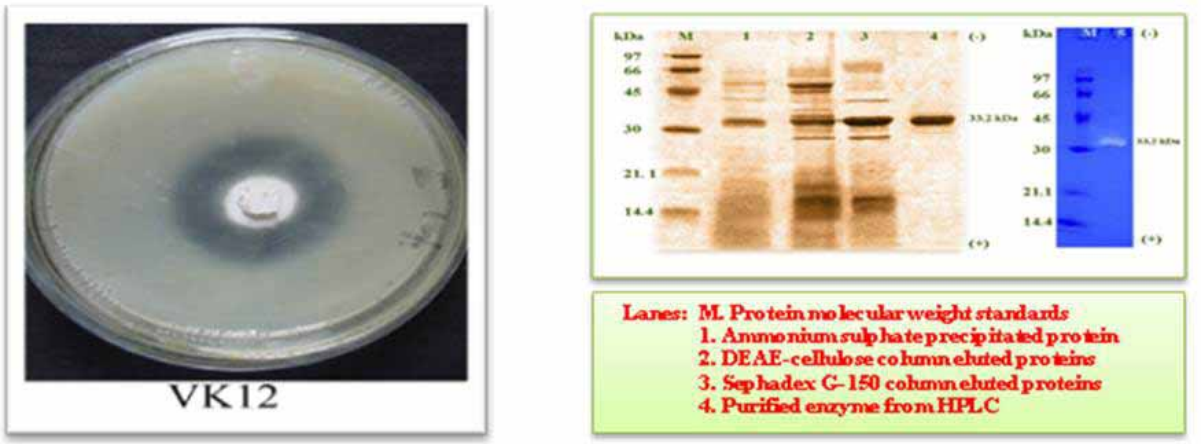

Figure 1. Extracellular fibrinolytic protease from *Ganoderma lucidum* strain VK-12 1a) Mycelium showing fibrinolytic activity on agar plate 1b) Purification of fibrinolytic protease from *Ganoderma lucidum* strain VK-12.

Figure 2. Fruiting body of Indigenous agaric *Agaricus heterocystis* Heinem and Gooss – *VKJ 17*

Though some of the agaric species are growing wildly in our tropical envirnment, no studies were carried out to cultivate them. *Agaricus heterocystis* had formed fruit bodies in the agar medium itself [41]. Cultivation of the indigenous wild edible variety *A. heterocytis strain VKJ17* (Fig. 2) was later standardized by Jagadish *et al.* [42]. In addition to the study on the nutritive value, their edibility and the medicinal properties such as antioxidant capacity and antitumor activity were evaluated [42]. Moreover the ethanolic extract of this mushroom induces apoptotic mode of cell death in HL-60 cell line. Two more terpenoid compounds were isolated namely C1-AGH and C2-AGH were shown in the Fig 3 which were active against human viruses such as HSV type1, type 2 and Influenza viruses A and B.

Lentinus tuberregium, an edible mushroom consumed by Kaani tribes of Paechi parai forest of Western Ghats (Fig. 4) are known to have antitumor, antioxidant and antimicrobial diterpene compounds. They were isolated from the *Lentinus*

Figure 3. Two antiviral compounds active against influenza virus A and B and HSV Type I and Type II viruses obtained from *Agaricus heterocystis* strain VKJ-17 3a) C1-AGH 3b) C2-AGH

Figure 4. Fruiting body of *Lentinus tuberregium* (Fr.) Fr strain VKJM 24

tuberregium VKJM 24 in another biodocumentation study [11]. Two compounds namely LT-1 and LT-2 were isolated, purified and their structure has been elucidated using various techniques and shown in the Fig. 5. They were tested against various cell lines *viz.*, SK-OV-03 (ovarian cancer), A673 (Rhabdomyosarcoma), HCT-116 (Colorectal Carcinoma) and MCF-7 (Breast Cancer) and the viability of cells was determined by the MTT assay. Of the four cancer lines tested, both LT1 and LT2 exerted maximal growth inhibition in SK-OV-03, followed by A673. On the other hand, HCT-116 showed moderate growth inhibition.

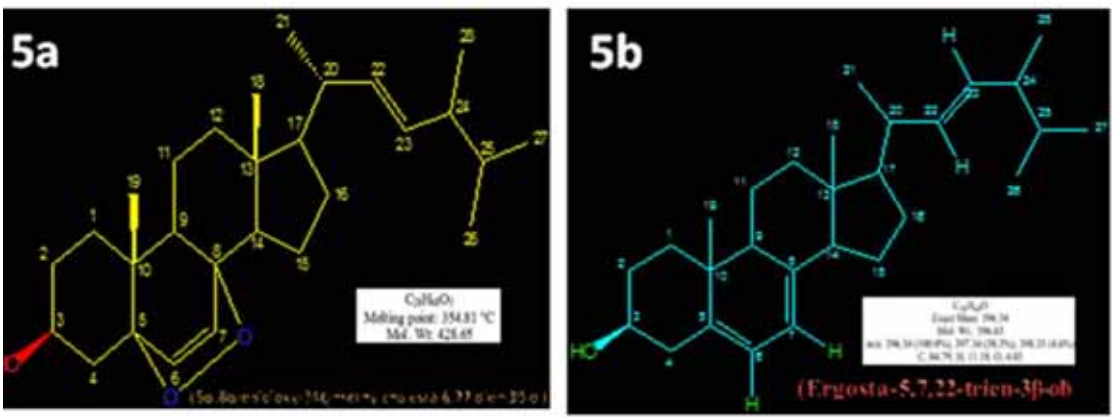

Figure 5. Two compounds namely LT-1 and LT-2 isolated from *Lentinus tuberregium* VKJM

Neolentinus kauffmanii (Fig. 6) strain *VKGJ01* was isolated during biodiversity study on Western Ghats [43] which is being consumed by the Kanni tribes of Kanyakumari forests of Western ghats as food additive and they claim many medicinal properties. Its cultivation was standardised for mass production. Both fruit body and mycelium were screened for bioactive compounds for antimicrobial and antitumor activity. A compound of steroid nature namely β-sitosterol with a molecular formula of $C_{28}H_{50}O_3$ and molecular weight 414.71 g/mol^{-1} was isolated from *N. kauffmanii* and studied for anticancer activity (Fig. 7). The study clearly showed that the compound exerted significant inhibitory effect on the HepG2 lung cancer cell lines. Both crude extract of mycelium and fruit body exhibited similar activities [14]. A Polysaccharide of glucan nature with antiangiogenic property was isolated from *Pleurotus eryngii* [12].

Figure 6. Fruiting body of *Neolentinus kauffmanii* Smith A.H. strain VKGJ01

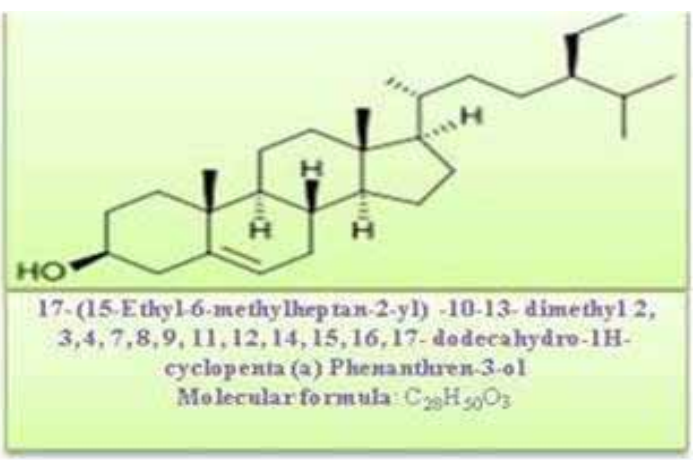

Figure 7. Structure of purified anticancer compound NK-1 active against liver cancer cell lines obtained from *Neolentinus kauffmanii* strain VKGJ01

Besides these agaric members a polypore namely *Trametes hirusuta* was isolated from suburb of Chennai (Fig 8) and its cultivation was standardized for the fruit body production. Though extensive studies on *Trametes versicolor* were carried out and the extracellular polysaccaride was shown to have antitumor activity and marketed globally as Krestin an anticancer drug [44]. Since, no such studies were carried out on indigenous *Trametes hirusuta* a study was carried out for biodocumetation of this mushroom [45]. An extracellular β-glucan was isolated from the *Trametes hirsuta strain VKESR* culture filtrate (Fig. 9) with moderate in *vitro* antioxidant and immunomodulatory potentials and showed good antiproliferative activity in colon, liver and leukemic cells lines. Further, it induced apoptosis through intrinsic mitochondrial mediated pathway in the cell lines. Moreover, it possesses good *ex vivo* antiangiogenic and anticancer potentials against DEN induced hepatocellular carcinoma in rats [45].

Figure 8. Fruiting body of *Trametes hirsuta* (Wulf.) Pil. strain VKESR

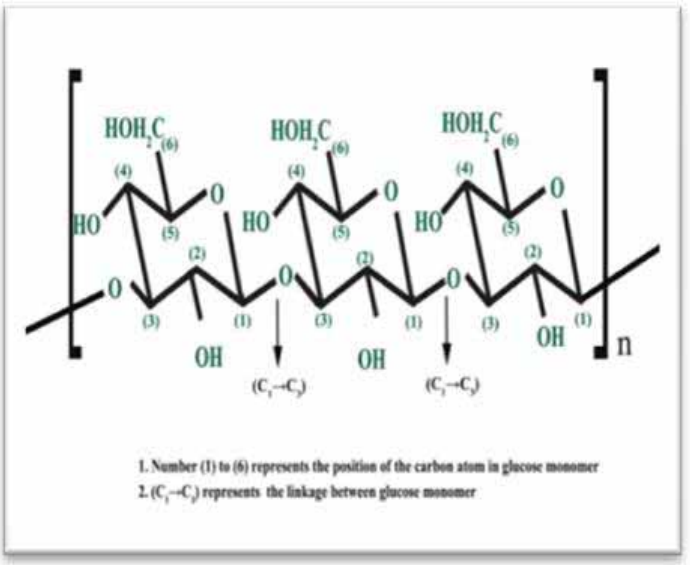

Figure 9. Structure of antitumor and immunomodulatory extracellular β– Glucan obtained from *Trametes hirsuta* (Wulf.) Pil. strain VKESR

COMMERCIAL STATUS OF BIOMOLECULES

At present, between 80 and 85% of all medicinal mushroom products are derived from the fruiting bodies, which have been either commercially farmed or collected from the wild mushrooms. Only15% of all products are based on extracts from mycelia. Examples are PSK and PSP from *T. versicolor* and Tremellastin from *Tremella mesenterica*. (Retzius): Fr. A small percentage of mushroom products are obtained from culture filtrates, e.g. Schizophyllan from *S. commune* and protein-bound polysaccharide complex from *Macrocybe lobayensis* (R. Heim) Pegler & Lodge [syn. *Tricholoma lobayense* R. Heim] [46]. After production, suitable galenic formulations like capsules, tablets or teas have to be developed, dependent on the material. Mixtures of several mushrooms or of mushroom and substrate become more and more common [47].

Lentinan from *L. edodes* fruit-bodies, Schizophyllan from *S. commune* mycelial broth, PSK and PSP, from mycelial cultures of *Trametes versicolor* and Grifron-D from fruit-bodies of *G. frondosa* were clinically tested commercial anticancer and immunomodulating drugs. All have been gone through Phase I, II and III clinical trials mainly in Japan and China but now in US. However, in many cases the standards of these trials may not meet current Western regulatory requirements. In many cases there have been significant improvements in quality of life and survival. Increasingly, several of these compounds are now used extensively in Japan, Korea and China, as adjuncts to standard radio and chemotherapy. While most of these clinical studies have used extracts from individual medicinal mushrooms, some recent studies from Japan have shown that mixtures of extracts from several known medicinal mushrooms, when taken as a supplement, have shown beneficial effects on the quality of life for some advanced cancer patients.

FUTURE FOCUS IN THE RELEVANT FIELD

The above studies clearly show that mushrooms, similar to plants, have a great potential for the production of useful bioactive metabolites and they are a prolific resource for drugs. The responsible bioactive compounds belong to several chemical groups, very often they are polysaccharides or triterpenes. One species can possess a variety of bioactive compounds, and therefore, has probabibility of having higher pharmacological effects. The best example is *G. lucidum*, which not only contains >120 different triterpenes but also polysaccharides, proteins and other bioactive compounds [48,49]. However, one main pre-requisition to use as drug, nutraceutical or other purpose is the continuous production of mushrooms (fruiting bodies or mycelium) in high amounts and in a standardized quality. In the opinion of Chang [46], mycelial products are the 'wave of the future' because they ensure standardized quality and year around production. A further necessity is the establishment of suitable quality parameters and of analytical methods to control these parameters. Nevertheless, the legal regulations for authorization as drug or as dietary supplements or as food should get more attention [44]. Control of possible side effects (i.e. allergies) during broad use is necessary. Finally, also the nutritional value of mushrooms should be taken into account.

Currently, biodocumentation of few more indigenous mushrooms from Indian forest ecosystem are being carried out in our group. Further, focusing on standardization of mass production of fruit bodies and mycelium for more compound production to develop novel anticancer drugs from the indigenous mushrooms in collaboration with various organizations. Thus many more miles to go before we get a fruitful drug with following vision and concepts. More studies are needed to demonstrate anti-viral, anti-tumor and anti-cholesterol process with high-quality long term double-blinded placebo-controlled studies with large trial populations to ensure safety and efficacy of medicinal mushrooms with statistical power.

ACKNOWLEDGEMENTS

I thank the former Directors of C.A.S in Botany Prof. N. Anand, and the present Director Prof. R. Rengasamy for the help and guidance.

REFERENCES

[1] A Ajith T and K Janardhanan K. (2007). Indian medicinal mushrooms as a source of antioxidant and antitumor agents. *J. Clin. Biochem Nutr*. 40(3): 157-162.

[2] Chang ST and Miles PG. (1989). Edible mushrooms and their cultivation, CRC Press.

[3] Hawksworth DL. (2001). The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological Research*. 105(12): 1422-1432.

[4] Wasser SP and Weis AL. (1999). Therapeutic effects of substances occurring in higher Basidiomycetes mushrooms: a modern perspective. *Crit Rev Immunol*. 19(1): 65-96.

[5] Hobbs C. (2000). Medicinal value of *Lentinus edodes* (Berk.) Sing. (Agaricomycetideae). A literature review. *International Journal of Medicinal Mushrooms*. 2(4): 287-302.

[6] Petch T. (1906). *The Fungi of Certain Termite Nests*.

[7] Sharif A *et al.* (2005) Evaluation of crude sesquiterpenoid extract of *Phellinus fastuosus* as a natural preservative. *Hindustan Antibiot. Bull.* 47-48(1-4): 20-23.

[8] Meera CR and Janardhanan KK. (2012). .Antitumor Activity of a Polysaccharide-Protein Complex Isolated from a Wood-Rotting Polypore Macro Fungus *Phellinus rimosus* (Berk) Pilat. *J. Environ. Pathol. Toxicol. Oncol*. 31(3): 223-232.

[9] Jagadish KL *et al.* (2009). Comparitive study on the antioxidant, anticancer and antimicrobial property of *Agaricus bisporus* (J E Lange) Imbach before and after boiling. *African Journal of Biotechnology*. 8(4). 654-661.

[10] Kumaran S *et al.* (2011). Studies on screening, isolation and purification of a fibrinolytic protease from an isolate (VK12) of *Ganoderma lucidum* and evaluation of its antithrombotic activity. *Med. Mycol. J*. 52(2): 153-162.

[11] Manjunathan J *et al.* (2011). Taxonomic Studies on *Lentinus tuberregium* (GQ292711) Tamil Nadu, India. *Research J. Pharm. and Tech*. 4(2)

[12] Shenbhagaraman R *et al*. (2012). Optimization of extracellular glucan production from *Pleurotus eryngii* and its impact on angiogenesis. *Intl. J. Biol. Macromol*. 50(4): 957-964.

[13] Johnsy G and Kaviyarasan V. (2012). Nutritive Value of Edible Wild Mushrooms Collected from the Western Ghats of Kanyakumari District. *World journal of Dairy and Food Sciences*. 11(1).

[14] Johnsy G. (2012). Studies on nutritional and medicinal value of an indigenous isolate of *Neolentinus kauffmanii* Smith A.H. (VKGJ-01). Ph.D., Thesis submitted to University of Madras, Chennai, India.

[15] Chihara G. (1992). Immunopharmacology of Lentinan, a polysaccharide isolated from *Lentinus edodes*: its applications as a host defence potentiator. *Intl. J. Oriental Medicine* 17: 57-77.

[16] Wang H *et al*. (2000). A new lectin with highly potent antihepatoma and antisarcoma activities from the oyster mushroom *Pleurotus ostreatus*. *Biochem. Biophys. Res. Commun.* 275(3): 810-816.

[17] Bobek P *et al*. (2001). Effect of pleuran (beta-glucan from *Pleurotus ostreatus*) in diet or drinking fluid on colitis in rats. *Nahrung* 45(5): 360-363.

[18] Jang M *et al*. (1997) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* 275(5297): 218-220.

[19] Sharon N and Lis H. (1993). Carbohydrates in cell recognition. *Sci Am*. 268(1): 82-89.

[20] Mizuno T *et al*. (1996) .Antitumor activity and chemical modification of polysaccharides from niohshimeji mushroom, *Tricholma giganteum*. *Biosci Biotechnol Biochem*. 60(1): 30-33.

[21] Lorenzen K and Anke T. (1998). Basidiomycetes as a source for new bioactive natural products. *Current Organic Chemistry*. 2(4): 329-364.

[22] Borchers AT *et al*. (1999). Mushrooms, tumors, and immunity. *Proc Soc Exp Biol Med*. 221(4): 281-293.

[23] Mizuno M *et al*. (1999). Anti-tumor polysaccharide from the mycelium of liquid-cultured *Agaricus blazei* mill. *Biochemistry and Molecular Biology International*. 47(4): 707-714.

[24] Reshetnikov SV *et al*. (2001). Higher basidiomycota as a source of antitumor and immunostimulating polysaccharides (Review). *Intl. J. Med. Mushr*. 3(4): 361-394.

[25] Ikekawa T *et al*. (1969). Antitumor Activity of Aqueous Extracts of Edible Mushrooms. *Cancer Research* 29(3): 734-735.

[26] Ikekawa T *et al*. (1982). Studies on antitumor polysaccharides of *Flammulina velutipes* (Curt. ex Fr.) Sing.II. The structure of EA3 and further purification of EA5. *J. Pharmacobiodyn* 5(8): 576-581.

[27] Ikekawa T *et al*. (1992). Antitumor activity of *Hypsizigus marmoreus*. I. Antitumor activity of extracts and polysaccharides. *Chem. Pharm. Bull. (Tokyo)*. 40(7): 1954-1957.

[28] Maeda YY *et al*. (1991) Genetic control of the expression of two biological activities of an antitumor polysaccharide, lentinan. *Intl. J. Immunopharmacology* 13(7): 977-986.

[29] Ross GD *et al*. (1999) .Therapeutic intervention with complement and beta-glucan in cancer. *Immunopharmacology* 42(1-3): 61-74.

[30] Smania EFA *et al*. (2003) .Antifungal activity of sterols and triterpenes isolated from *Ganoderma annulare*. *Fitoterapia*. 74(4): 375-377.

[31] Bender S *et al*. (2003). A Case for Caution in Assessing the Antibiotic Activity of Extracts of Culinary-Medicinal Shiitake Mushroom *Lentinus edodes* (Berk.) Singer] (Agaricomycetideae). 5(1): 6.

[32] Badalyan S. (2004). Antiprotozoal activity and mitogenic effect of mycelium of culinary-medicinal shiitake mushroom *Lentinus edodes* (Berk.) Singer (Agaricomycetideae). 6(2): 8.

[33] Lindequist U. (1998). *Schizophyllum* In: Schneider G, Hänsel R, Blaschek W, editors. HAGERs Handbuch der Pharmazeutischen Praxis. Berlin, Heidelberg, New York: Springer-Verlag;. pp. 528–34.

[34] Zjawiony JK. (2004). Biologically active compounds from aphyllophorales (polypore) fungi. *J. Natural Products*. 67(2): 300-310.

[35] El-Mekkawy S *et al*. (1998). Anti-HIV-1 and anti-HIV-1-protease substances from *Ganoderma lucidum. Phytochemistry* 49(6): 1651-1657.

[36] Mothana RAA *et al*. (2003). Antiviral lanostanoid triterpenes from the fungus *Ganoderma pfeifferi. Fitoterapia* 74(1–2): 177-180.

[37] Mentel R *et al*. (1994). *In vitro* antiviral effect of extracts of *Kuehneromyces mutabilis* on influenza virus. *Pharmazie*. 49(11): 859-860.

[38] Awadh Ali *et al*. (2003). Antiviral activity of *Inonotus hispidus*. *Fitoterapia*. 74(5): 483-485.

[39] Leonhardt K *et al*. (1987). 6-Methylpurine, 6-methyl-9-beta-D-ribofuranosylpurine, and 6-hydroxymethyl-9-beta-D-ribofuranosylpurine as antiviral metabolites of *Collybia maculata* (Basidiomycetes). *Zeitschrift fur Naturforschung. C. J. Biosciences* 42(4): 420-424.

[40] Kumaran S. (2008). Studies on production, purification and characterisation of fibrinolytic and antithrombotic protease from a South Indian isolate of *Ganoderma lucidum* (FR.) Karst. Ph.D., Thesis submitted to University of Madras, Chennai, India.

[41] Natarajan K *et al.* (2002). Basidiocarp production in *Agaricus heterocystis* Heinem. and Gooss. in nutrient agar media. *Current Science*. 82: 9-10

[42] Jagadish L. (2010). Cultivation and medicinal properties of Indigenous Agaric. *Agaricus heterocystis* Heinem and Gooss – *VKJ 17*. Ph.D., Thesis submitted to University of Madras, Chennai, India.

[43] Davidson Sargunam S *et al.* (2012). Mushrooms in the food culture of the Kaani tribe of Kanyakumari district. *Indian Knowledge of traditional knowledge* 11(1): 150-153.

[44] Wasser SP. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology* 60(3): 258-274.

[45] Shenbhagaraman R. (2012). Therapeutic potential of extracellular â- glucan from *Trametes hirsuta* (Wulf.)Pil. strain VKESR against malignant neoplasm. Ph.D., Thesis submitted to University of Madras, Chennai, India.

[46] Chang ST. (2001). A 40-Year Journey Through Bioconversion of Lignocellulosic Wastes to Mushrooms and Dietary Supplements. 3(2-3): 1.

[47] Wasser SP *et al.* (2000). Dietary Supplements from Medicinal Mushrooms: *Diversity of Types and Variety of Regulations* 2(1): 19.

[48] Kim HS *et al.* (1999). *In vitro* chemopreventive effects of plant polysaccharides (*Aloe barbadensis Miller*, *Lentinus edodes*, *Ganoderma lucidum* and *Coriolus versicolor*). *Carcinogenesis* 20(8): 1637-1640.

[49] Gao Y *et al.* (2004). Immunomodulating activities of *Ganoderma*, a mushroom with medicinal properties. *Food Reviews International* 20(2): 123-161.

[50] Hobbs C. (1995). Medicinal Mushrooms: An Exploration of Tradition, Healing & Culture, Botanica Press.

[51] Ikekawa T. (2001). Beneficial Effects of Edible and Medicinal Mushrooms on Health Care. 3(4): 8.

[52] Kumar M. (2009). Taxanomic studies on Agaric diversity of Kolli hills, Eastern ghats, India. Ph.D., Thesis submitted to University of Madras, Chennai, India.

[53] Manjunathan J. (2011). Isolation, Purification, Characterization and Evaluation of Antimicrobial and Anticancer Compounds from Indigenous Isolate of *Lentinus tuberregium* (Fr.) Fr. Ph.D., Thesis submitted to University of Madras, Chennai, India.

[54] Sekar Kumaran *et al.* (2011). Screening of Fibrinolytic protease from south Indian isolates of *Ganoderma lucidum*. *International J. Pharma. and Bioscience.* 2(1): 419-431.