ANTIPROLIFERATIVE ACTIVITY OF BIOACTIVE COMPOUNDS FROM MUSHROOMS OF INDIAN ISOLATES

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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 Trametes 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, dietary 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, antiinflammatory, 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 immunosupression 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 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 lymphokynes, hormone and other biologically active substances. The immunopotentiation has been shown to occur by stimulating the maturation, differentation 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 lentian. 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 Applanoxic 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 ganodermanontriol for complete inhibition of HIV-1 induced cytopathic effect in MT-4 cells is 7.8 mg/ml. Ganodermic acid B inhibits HIV-1 protease [35]. Ganodermadiol , lucidadiol and applanoxic 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* (Scheaff.: 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 biodocumentation 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 proteose 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].
Though some of the agaric species are growing wildly in our tropical environment, 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 type 1, 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*
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.

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 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].
Besides these agaric members a polypore namely Trametes hirsuta 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 polysaccharide 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 hirsuta a study was carried out for biodocumentation 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].

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. Only 15% 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 Macrocystis 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 probability 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.

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REFERENCES


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


