Bioactive Metabolites and Compound from Medicinal Mushrooms

Hiralal Sonawane, Shekhar bhosle, Gauri Bapat, Sandhya Garad, Vikram Ghole

Abstract— Mankind has used mushrooms since historic period. The records of mushrooms being used for health and medicinal purpose can be traced back in Greek/roman civilization, Chinese traditional medicine and even in the Ayurveda. Cultivation of such medicinal mushrooms for its daily use is a tradition in China, Japan and some other South Eastern Countries. The compounds, which confer the medicinal and or nutritional properties to mushrooms, are called as Bioactive compounds. The medicinal properties can be listed as Anti-tumor, anti-cancer, anti-histamine, Anti-HIV-1 etc. These bioactive compounds have been studied to a great extent during last few decades. Isolation, characterization, and identification of many such compounds have been done in case of mushrooms like, *Lentinus edodes* (Shiitake), *Ganoderma lucidum* (Reishi), *Grifiola frondosa* (Maitake), *Auricularia auricula*, *Poria coccus*, *Mycena leaiana*, *Omphalotus olearius*, *Lampteromyces japonicus* etc. These bioactive compounds ranges from compounds like Polysaccharides, Proteins, Protein – polysaccharide complex, Terpenoids, Adenine derivatives, Lectins, Ergosterol to heavy metals like Germanium etc. These have been studied for their medicinal effects against many ailments. Some medical metabolisms related to the mode of action of these bioactive compounds have also been listed in this article, which may help in understanding or studying the correct mode of action of these bioactive compounds.

Index Terms- Medicinal mushrooms, Bioactive compounds, Polysaccharide, Ergosterol, Lectins, Germanium, Anti-cancer, Anti-tumor.

1 INTRODUCTION

Fungi, has been used by mankind since historic period. The main use can be illustrated as for fermentation, to make wine. Records of some macrofungi (generally termed mushrooms) can be found in different civilizations like Chinese in the Oriental Medicine System (which is now called as Traditional Chinese Medicine) and also in "Ayurveda", one of the oldest Medicine systems in world [1]. The major civilization, which includes mushrooms in its regular diet, is the Chinese and the Japanese [2].

There are many records of different mushrooms and mushroom products used for different ailments, in man, animals and against some active diseases (Tobacco Mosaic Virus) in plants. These products are raw mushroom, dried powder, extracts of naturally growing or commercially cultivated mushroom, dried or extracted biomass of mycelium grown in a solid or submerged culture. The products are not true pharmaceuticals (real medicine) but are called as dietary supplements or nutriceuticals or also called as designer foods, nutraceuticals, mycochemicals etc. [3]

During last couple of decades a diverse and vast research has been carried out to understand the therapeutic activities exhibited by different mushrooms (and their products). The work has much accelerated due development in the culture techniques, cultivation and the biochemical assessment technique. The cultivation techniques now provide a consistent quality and quantity of mushroom and/or mycelium as per the requirement of researchers.

Many compounds have been extracted, isolated, identified and characterized from different mushrooms and their effects against different ailments have been recorded. Besides the advancement in this area their still exists a lacking in understanding the exact mechanism of action of these compounds in curing different ailments.

The ailments, which have been studied for the effects of the variety of products of mushroom can be listed as Tumor, Cancer, HIV (and its components like HIV-1 Protease enzyme etc.), different pathogenic viruses, bacteria, different physiological disorders like inflammation, hypertension, allergy, bone disorders etc and even some psychological disorders. The major compounds, which have been studied can be listed as β-D-glucan, polysaccharides, heteropolysaccharides, proteoglycans, triterpenes (terpenes), lectins, ergosterol (steroids and its derivatives), nucleosides and its derivatives.

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Name of Mushroom	Compound name	Activity	Reference
Calvatia gigantea	Calvacin	Antitumor	Wasser & Weis, 1999
Schizophyllum communae	schizophyllan	Antitumor	Wasser & Weis, 1999
Lentinus edodes	Lentinan	Antitumor, anticancer, immunostimulating, antiviral	Sasaki & Takatsuka, 1976. Chihara, 1978, 1981. Suga *et.al.*, 1984 Yoshiaki, *et.al.*,1985 Wasser & Weis, 1999
	KS-2 (glycoprotein)	Anti-tumor, interferon induction, cytocidal	
	LAP	Immunoactivation, Anti-tumor,	
	LEM, EP3	Immunoactivation, Anti-tumor,	
Ganoderma lucidum	Ganoderan A, B,C.	Hypoglycemic	Sone *et.al.*, 1985 Yoshiaki, *et.al.*,1985 Willard, 1990 Wasser & Weis, 1999
	GL-1	Antitumor, Immunostimulating	
	G-A, G-Z	Antitumor, Anti – Inflammatory, Immunostimulating	
	G-I-2a	Antitumor, Immunostimulating	
	D-6	Protein synthesis enhancer	
	FA, FI, FI-1a	Antitumor, Immunostimulating	
Trametes versicolor	PSK, Krestin	Anti-tumor, Immunostimulating, antiviral.	---
Innonotus obliqus	Befungin	Anti-tumor	Mizuno, 1996, Miles & Chang, 1997
Agaricus blazei	ATOM. AB-FP	Antitumor	Mizuno, *et.al.*, 1995c, Kawagishi, *et.al.*, 1990
Auricularia auricula	---	Antitumor	Sone *et.al.*, 1985
Petalotia	---	Antitumor	Sone *et.al.*, 1985
Volvariella volvaceae	---	Antitumor	Sone *et.al.*, 1985
Phellinus linteus	---	Antitumor	Sasaki *et.al.*, 1971, Bhonde *et.al.*, 2002
Falmmulina veluticeps	EA-6, Proflamin		Ikekawa, 1995a
Dictyophora indusiata	T-4-N, T-5-N	Weak antitumor	Hara & Ukai, 1995
Polyporus (Grifiola) umbellate, P. frondosus	---	Used in Nephritis, antitumor, Immunostimulating	---
Tremella fusiformis	---		
Cordyceps sp.	CS F30	Anticancer, Hypoglycemic	Kiho *et.al.*, 1996
	CS-81002	Immunostimulating	Gong *et.al.*, 1990
	---	Antileukemic	Chen *et.al.*, 1997

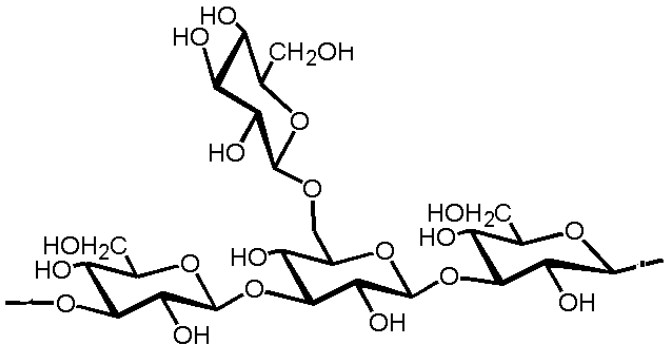
Table: 1- β – D – Glucan, Polysaccharide, Proteoglycans, Heteropolysaccharides.

Some heavy metals like Germanium, Seranium (its derivatives or complexes). This article reviews such compounds, the effects exhibited by these compounds, and known modes of action while some hypothesis about the mode of action of such compounds.

1.1 β – D – Glucan, Polysaccharide, Proteoglycans, Heteropolysaccharides:

The antitumor activity of the higher Basidiomycetes was first studied in extracts of fruiting bodies of *Boletus edulis* (Bull.:) Fr.[4] In the 1960s calvacin was the most commonly cited natural antitumor product isolated from the giant puffball [*Calvatia* (=Langermannia) *gigantea* (Batsch: Pers.) Lloyd]. Numerous researchers isolated essential polysaccharide substances from several mushrooms (Basidiomycetes) [5]. The major compound was a β - D - glucan, a polysaccharide yielding D - glucose by acid hydrolysis. In addition to β - D - glucans, a number of high molecular weight antitumor components were isolated from medicinal mushrooms, including heterolglycans, chitinous substances, peptidoglycans, proteoglycans, lectins, RNA components, dietary fibers, and / or indigestible polysaccharides. As well as some lower molecular weight organic substances, such as Terpenoids, steroids, novel gamma - pyrones, and novel phenols, were isolated and identified from mushrooms [6,7,8,9,10,11,12,13,14].

Polysaccharides demonstrating a remarkable antitumor activity in vivo have been isolated from various species of mushrooms belonging to Agaricales, Auriculariales, Tremellales, Polyporales, Gasteromycetideae and Agaricomycetideae and even some members from Ascomyctes like *Cordyceps sinensis*. In Japan, Russia, China, and the United States of America, several polysaccharide carcinostatic agents have been developed and commercialized using submerged cultured mycelial biomass of *Trametes versicolor* (PSK, Krestin; Japan), Fruiting bodies of *Lentinus edodes* (Lentinan; Japan), *Ganoderma lucidum* (Japan, China, Korea),



Inonotus obliquus (Befungin; Russia), *Agaricus blazei* (USA) [9,15].

Figure 1. Structure of (1-3)-β-D Glucan with a side branch at O-6 position.

The hot water extracts obtained from fruiting bodies of seven edible wild growing higher Basidiomycetes, one out of these was *Lentinus edodes* (Berk.) Sing. The data related to component of *Lentinus edodes* water eluted fraction demonstrated a 94.8% rate of tumor inhibition [16].

A standard method of fractionation and purification of water-soluble antitumor polysaccharide from the fruit body of *Lentinus edodes* demonstrated by Cihara et al [17,18]. Antitumor properties of *Lentinus edodes*, and stated that lentinan was found to almost completely regress the solid type of tumors in synergic host tumor system A [18].

β - D - glucan from *Lentinus edodes*, Lentinan has been studied more extensively than other similar substances. Lentinan showed prominent antitumor activity not only against allogenic tumors, but also against various synergic and autochthonous tumors, and it prevents chemical and viral oncogenesis [19,20,21,22,23,24]. The molecular formula of Lentinan is (C6H10O5)n, the mean molecular weight is about one million - 5 X 105 Da, [α] D + 20° - 22° (NaOH). It is a β-D-glucan, as shown by electrophoresis and ultra-centrifugation, as well as by other chemical techniques and instrumental analysis [25]. Lentinan is not toxic to tumor cells, but inhibits tumor growth by stimulating the immune system [26].

Ganoderma lucidum, several β-D-glucans (named as Ganoderans) were isolated from the water and alkali extract of the fruiting body of this mushroom. The purified glucans were mostly water insoluble with (1-3)- D-glucose residue attached mainly with single D-glucosyl units at O-6 position (Figure 1). Few short branched (1-4)- β-D-glucan was isolated from the culture filtrate (extracellular). The glucans isolated from fruit body as well as from culture filtrate showed relatively high growth inhibition activity against 180 solid tumors in mice. Glucans when modified to D-glucan polyols by periodate oxidation and borohydrate reduction, exhibited higher antitumor activities. Thus, attachment of polyol groups to (1-3) linked backbone significantly enhances the host mediated antitumor effect. Some studies have been carried out to increase the activity or productivity of the β-D-glucan by different chemical modifications [5].

The polysaccharides act effectively with vitamin C. Since, the polysaccharides are long, chain like molecules having high molecular weight, the human body can not absorb them easily, but when these are supplemented with vitamin C the polysaccharides breaks up into oligoglucans having small molecular weight which body can easily absorb, plus these oligoglucans gets dissolved in the blood serum easily and can directly stimulate macrophages, which, otherwise comes very late in the battle [28].

The antitumor activity of the polysaccharide is also dependent on its structure, i.e. the activity is influenced greatly by the degree of branching, at least in case of Ganoderma lucidum and few other mushrooms [27]. The activity of polysaccharides decreases with the decrease in the branching. Moderately branched structures show high anti-tumor activity, higher degree of branching also in case of *Auricularia auricula*, *Pleurotus* sp. and *Volvariella volvaceae* (Fukurotake), show less activity than that of moderately branched ones. The reason for the branching affecting the antitumor activity may be partly due to the molecular rigidities of the (1-3)- β-D-glucans in triple helix form. The lower branching is also related to structure of fruit body, which may affect the intermolecular hydrogen bonding and would be characteristic of rigid, hard type of fruit body as that of *Ganoderma lucidum*.

Antitumor polysasaccharides have also been reported from the hot water extracts of *Phellinus linteus*. The hot water extracts showed the maximum (96%) inhibition of the tumor [29].*Phellinus merillii* and *P. fastuosus* hot water, cold water and alcoholic extracts showed anticancerous activity against SiHa cell line [30].

There has been a great uncertainty about the mode of action of the bioactive compounds. β-D-glucan binds to lymphocyte surfaces or serum specific proteins, which activate macrophage, T-helper, NK, and other effector cells [31,32]. All these increase the production of antibodies as well as interleukins (IL-1, IL-2) and interferon (IFN-γ), which are released upon activation of effector cells. Thus the carcinostatic effect of lentinan results from the activation of the host's immune system [33,34]. In case of pure β-D-glucan, there is no antigen-antibody reaction nor are there any other disturbances, such as allergy, shock, Lentinan has antitumor activity significantly stronger than that of polysaccharides from other fungi [35,36,37,38,39,40,5].

α-mannan peptide (KS-2 a glycoprotein) from the hot water extracts of cultured mycelium of *Lentinus edodes*, followed by precipitation with ethanol. It contains the amino acids serine, threonine, alanine, and proline [41]. KS-2 was shown to be effective on Sarcoma 180 and Ehrlich's carcinoma, and it act via an interferon inducing activity, the mode of action of KS-2 is still unclear; the results show no KS-2 direct cytocidal effect against the tumor cells in vitro [5].

LEM (*L. edodes* Mycelial Extracts) and LAP (ethanol precipitate obtained from LEM) are glycoproteins containing glucose, galactose, xylose, arabinose, mannose, and fructose have been isolated from extracts of *L. edodes* mushroom mycelium and culture media. [42]. LEM also contains various Nucleic acid derivatives, vitamin B compounds, especially B1 (thiamine), B2 (riboflavin), and Ergosterol. LEM was prepared from an extract of the powdered mycelia of L. edodes. After incubation of mycelia on solid medium at 20 to 22°C for 80 to 120 days and before fruiting, the media was powdered and further incubated in the presence of the enzymes naturally present in the mycelia for 50 to 60 hours at 40 to 50°C when the reaction was completed, the residue was extracted with water (60°C), and the filtrate was freeze dried. The light brown powder obtained was LEM. The yield is about 6-7g/kg of medium. The precipitate obtained from the solution of LEM by adding 4 volumes of ethanol was named LAP and the yield of LAP is ≈0.3g/g of LEM. LEM and LAP have both demonstrated strong antitumor activity, both orally and by injection, in animals and humans, both working by activating the host's immune system.[6,7,43]

EP3, an immunoactive substance was obtained by fractionation of LEM. EP3 is a lignin complex composed of approximately 80% lignin, 10% carbohydrates, and 10% protein. After removal of carbohydrates, and protein, biological activity was not affected, but when lignin is removed, activity is reduced. Thus the active substance is believed to be a water soluble lignin containing numerous carboxyl groups [44].

Protein bound polysaccharides GLhw, GLhw – 01, GLhw – 02, GLhw – 03 have been isolated from the water extracts of *Ganoderma lucidum* fruit body. These compounds showed antiviral activity against Herpes Simplex Virus Type – 1 and Herpes Simplex Virus Type – 2. The GLhw – 02 exhibited the most potent antiherpetic activity among the above said compounds [45].

The polysaccharides being the main active component, the study of characteristics of these polysaccharides, the composition, the molecular weight of these polysaccharides are important in drug designing. The table 2 gives the account of characteristics of the polysaccharides from shiitake. Similar data has been developed in case of *Ganoderma lucidum*. The composition and characteristics of the polysaccharides isolated from *Ganoderma lucidum* has also been well understood.

A glucomannan – protein complex (ATOM) and a mannan – protein complex (AB-FP) have been isolated from the mycelium of *Agaricus blazei*. Both ATOM and AB-FP shows distinct antitumor activity [8,46].

A, β – glucan – protein (EA6) have been isolated from the fruiting body of *Flammulina velutipes*, which contains glucose, galactose, mannose, xylose, arabinose and 16 amino acids. This EA6 exhibited strong antitumor activity against Sarcoma 180, Lewis cancer of lung and B – 16 melanoma [47]. A glycoprotein "Proflamin" has also been studied from the cultured mycelium of Flammulina velutipes, which is effective against allogeneic and syngeneic tumors and was found effective against Sarcoma 180, B – 16 melanoma, adenocarcinoma 755 and Gardner lymphoma (dose – oral administration).

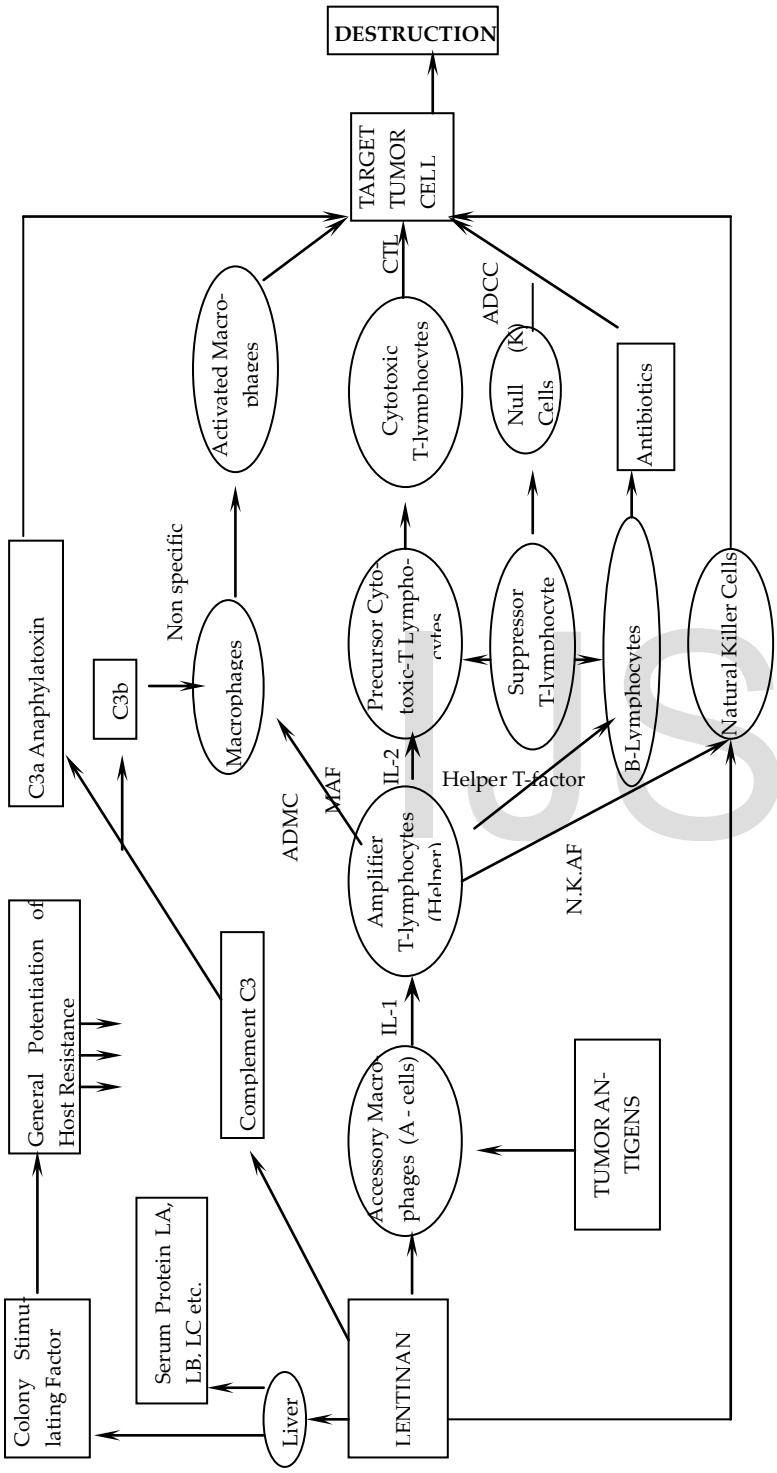
A weak antitumor (1-3) - β – D – glucans (T-4-N and T-5-N) have been isolated from *Dictyophora indusiata* [48]. The compounds were isolated with alkaline solutions and water. These β – D – glucans showed weak activity than the water extracted (1-3) – β – D – glucans isolated from the other mushrooms like *Lentinus edodes*, *Ganoderma lucidum*, *Hericium erinaceus*.

Polyporus (*Grifola*) umbellata (zhu ling) is used in nephritis. Its polysaccharides are 6-branched ß-(1-3) glucans, which promote antibody production, normalize liver function and are antineoplastic. Polyporus frondosus also called as cloud fungus. The main active ingredient of ash flower mushroom is proteoglycan, with basic structure of β-(1→6) andβ-(1→3) glucose, and small amount of xylose and mannose. The polysaccharide from this mushroom plays its anti-tumor role via enhancing cellular immunity; it has powerful inhibition of mutation caused by chemicals. It can be used in the comprehensive treatment of lassitude, leukocytopenia, reduced immunity due to chronic hepatitis and radio-chemotherapy for malignant tumors.

Name	Part from Isolated	Monosaccharide composition	Main sugar	MW ($x10^{-1}$)
Lentinan	Fruit body	Glc	Glc	500
LEM	Mycelia on bagasse medium	Xyl :Ara :Gal :Glc :Man :Fuc 39 :20.7 :15.3 :13.5 :8.7 :1.5	Xyl	- -
LAP	Mycelia on bagasse medium	Xyl :Ara :Gal :Glc :Man :Fuc :Rha 30.2 :17.4 :20.3 :19.9 :9.5 :1.6 :1.1	Xyl	- -
LAP-1	Mycelia on bagasse medium	Xyl :Ara :Gal :Glc :Man :Fuc :Rha 39 :20.7 :15.3 :13.5 :8.7 :1.5 :1.3	Xyl	- -
LAP-2	Mycelia on bagasse medium	Xyl :Ara :Gal :Glc :Man :Fuc :Rha 30.4 :14.3 :23.2 :20.3 :8.0 :2.9 :0.9	Xyl	- -
C-1-2	Mycelia on bagasse medium	Man :Xyl :Glc :Gal :Ara :Rha 35.3 :20.1 :17.3 :15.2 :10.1 :1.8	Man	>200-10
KS- 2	Culture Mycelia	Man	Man	95-60
KS-2-A	Culture Mycelia	Man :Glc :Gal :Ara :Xyl 74 :12 :12 :1 :1	Man	8
KS-2-B	Culture Mycelia	Almost Mannose	Man	75
KS-2-D	Culture Mycelia	Man :Glc :Gal :… 42 :37 :16 :…	Man	200-30
Le-2-1	Culture Mycelia	Glc:Xyl:Gal:Man:Ara:Rha 86.1:22.0:2.5:2.1:2.0:1.6	Glc	58
Le-2-2	Culture Mycelia	Glc:Xyl:Gal:Man:Ara:Rha 70.1:1.8:10.3:8.5:2.4:3.2	Glc	6

Table: 2 characteristics of the polysaccharides from shiitake

Figure 3: Possible mode of action of Lentinan as host defense potentiator [5].



It also has certain preventive role for AIDS. Oral intake of ash flower mushroom polysaccharide is both safe and effective. Tremella fuciformis, polysaccharide extracted from this mushroom is effective in treating tumor, hepatitis and mutation, hyperglycemia, promoting the production of interferon, improving the hematopoietic function of bone marrow. It is suitable for adjuvant therapy for leucocytopenia in tumor patients due to chemotherapy, radiotherapy or other causes, for chronic hepatitis and all kinds of diabetes mellitus[49].

There are very few mushrooms that have the ability of producing terpenoids in the metabolic pathway. The main mushroom, which produces this metabolite, and most worked on by many researchers, is *Ganoderma* and the terpenoids produced are named as ganoderic acids or lucidenic acids. These triterpenes occurs as fatty acids in the mushroom; are bitter in taste and show cytotoxic effects. These terpenoids are found in fruit bodies and in culture mycelium. Initially it was considred that these are found only in fruit bodies but now its known to be found in culture mycelium too (Ganoderic acid R, T, U-Z). The type of ganoderic acids varies on its location in the fruit body (stipe, hymenia etc.) and also the growing stage of the mushroom (antler stage and complete matured stage), different triterpenes occurs in different strains and varies in different stages of fruit body development [50]. The triterpenes were reported to increase after the appearance of fruiting bodies during the process of development, although these were not found in mycelia before fruit body formation. Ergosterol and fatty acids were found in both mycelia and fruiting bodies but did not increase during growth of the latter.

The oxygen is said to be the functional group and the hydrophobic moieties play an important role in generating the bitterness Figure 3a (the group R_1 R_2 R_3 R_4 plays important role in the identification or classifying the triterpenes). Triterpenes were found more in the outer section than in the inner section of fruiting bodies. This infers that aging of a fruiting body increases its triterpenes because the outer section is older than the inner section [51].

About 100 and more triterpenoids have been identified form the fruit bodies of *Ganoderma lucidum* and *Ganoderma applanatum*. These include highly oxidized lanostane type triterpenoids such as Ganoderic acids Alpha, A, B, C_1, C_2, D_1, D_2, E_1, E_2, F, G, H, I, J, K_1 K_2, L, Ma, Mb, Mc, Md, Me, Mf, Mg, Mi, Mj, Mk, Mn, N, O, P, Q, R, S, T, U, V, W, X, Y, Z. Ganoderenic acids A, B, C, D, E, F, G, H, I; **Ganolucidic acids** A, B, C, D, and E; **Lucidenic acids** such as A, B, C, D_1, D_2, E_1, E_2, F, G, H, I, J, K, L, M; **Ganodermanomtriol**. The structures of terpenoids from different species of *Ganoderma* are of lanostane type and are grouped on the basis of carbon number and the state of oxidation. Ganoderol A and B, [5,52,53,54]

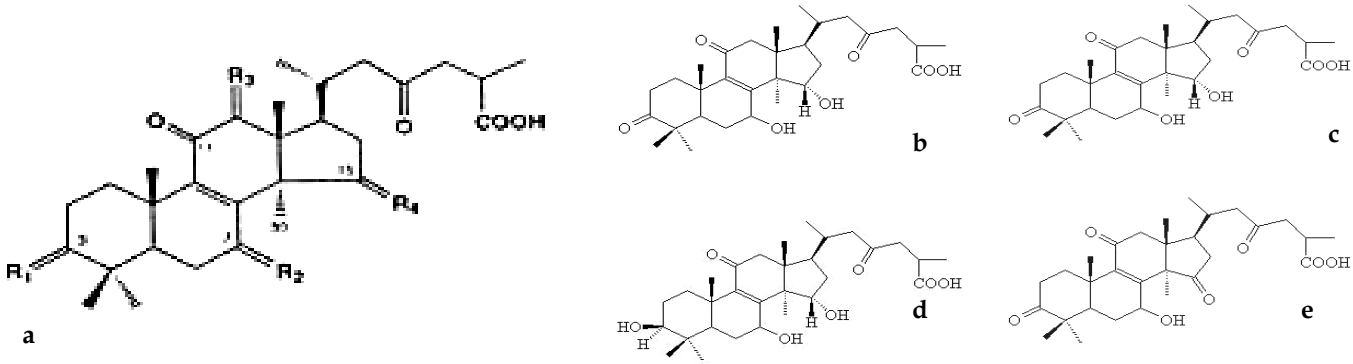


Figure 3a: Sesquitriterpene from *Ganoderma lucidum*, and **b-e.** Ganoderic acids A, B, C, D [51]

The terpenoids have cytotoxic effects and are candidates for anti-tumor agents. Ganoderic acids isolated from submerged cultured mycelial biomass have been reported to inhibit Hepatoma cells in vitro. Triterpenes isolated from the spores of *Ganoderma lucidum* shows cyto-toxicity against Meth-A and LLC tumor cells [55].

The triterpenes also show hepato (liver) – protective activity. The CCl4 (carbon tetrachloride) induced liver damage in mice showed lowered SGOT and SGPT values and prominent effects to the hepatocytes around central veins. Extract of G. lucidum administered with glutathione against liver damage induced by CCl4 proved to be beneficial against hepatic necrosis and hepatitis. It has also been discovered that the extract of G. lucidum could probably supplement the rate of toxin transformation and subsequent bile excretion, thereby acting as a liver detoxicant and protectant [56].

Ganoderic acids A, B, C and D showed inhibition of histamine release from the rat mast cells, induced by Concanavalin A and compound 48/80 proving the anti-inflammatory or anti allergic activity of the ganoderic acids. These triterpenes were isolated from the methanolic extract of *G. lucidum*. Ganoderic acids C and D showed the distinctive inhibitory activity on histamine release [54].

Other than the cytotoxic effect the triterpenes also have a harmonizing effect on the body, on the immune system and on circulatory system. These triterpenes have an adaptogenic effects, it helps the body to adapt to wide range of environmental, biological and sociological stress [50].

The triterpenes also show anti-hypertensive (high blood pressure) action and the mode of action is through inhibition of ACE (Angiotensin Converting Enzyme). The triterpenes (compounds 1-10) isolated from the fruit body of *G. lucidum* showed inhibitory activity on Angiotensin Converting Enzyme. The ganoderic acid F exhibited the highest inhibitory effect on the enzyme (IC50= 4.7 X 10-6 M) while the other IC50 values of the other triterpenes were of the order of 10-5 M [14].

The Angiotensin Converting enzyme cleaves the decapeptide angiotensin I (biologically inactive) to form active angiotensin II. Angiotensin II causes contraction of vascular smooth muscle and thus raises blood pressure and stimulates aldosterone release from the adrenal glands. Angiotensin is finally broken down by angiotensinases. Elevations in angiotensin converting enzyme are seen in sarcoidosis, histoplasmosis, alcoholic cirrhosis, diabetes, hyperthyroidism, amyloidosis, idiopathic pulmonary fibrosis, pulmonary embolism, scleroderma, silicosis, tuberculosis, leprosy etc. The normal values are 18 to 67 U/ml over 20 years of age (people under 20 have higher levels).

Ganoderic acids B & C from the fruit body of *G. lucidum* to be an active inhibitor of cholesterol biosynthesis. The oxygen position present in the structure is the key to the cholesterol biosynthesis inhibition. Similarly two more triterpenes are recorded to be isolated from the mycelium of *G. lucidum* namely Ganoderic acid Mf and ganodermic acid T – O exhibiting cholesterol biosynthesis inhibition [50, 57]

Ganoderic acid alpha is proved to be Anti-HIV – 1 (The type species of lentivirus and widely recognised as the aetiologic agent of acquired immunodeficiency syndrome (AIDS). It is characterised by its cytopathic effect and affinity for the t4-lymphocyte) and Anti-HIV – 1 protease. Ganoderiol F and Ganodermanotriol is anit-HIV-1 at a dose of 7.8µg/ml. Ganoderic acid B, ganoderiol B, ganoderic acid C1, 3β-5α dihydroxy - 6β – methoxy ergosta – 7, 22-diene, ganoderic acid α, ganoderic acid H and ganoderiol A are moderate active inhibitors against HIV-1 Protease [58]. Lucidenic acid O and lectone, have been isolated from the fruit body of G. lucidum which prevented activities of calf DNA polymerase alpha and rat DNA polymerase beta, besides it has also shown to prevent the HIV – 1 reverse transcriptase [59].

HIV – 1: The type species of lentivirus and widely recognised as the aetiologic agent of Acquired Immuno Deficiency Syndrome (AIDS). HIV – 1 Protease: the proteins that comprise the human immunodeficiency virus (HIV) are produced in the form of long "polyproteins." These polyproteins must be cleaved to yield the active protein components of the mature virus. The HIV-1 protease is a novel aspartic protease that functions to cleave the nascent polyproteins during viral replication. When viewed as a space-filling model, the novel structural features of this enzyme are not readily apparent.

The triterpenes isolated from water extracts of *G. lucidum* were screened for their antioxidative effect against pyrogallol induced erthrocyte membrane oxidation and Fe (II) – ascorbic acid induced lipid peroxidation. All the tested samples showed antioxidant activities in a dose dependent manner [60].

Other than Ganoderma, another polyporous fungus

Cryptoporous volvatus, shows bitter taste and are named as cryptoporic acids A-G. These are determined as sesquiterpenoid ethers. The dimeric cryptoporic acid C, E, F and G showa inhibitory activity on the release of oxygen from guinea-pig peritoneal macrophage [61].

Omphalotus olearius (DC.: Fr) Fay. and *Lampteromyces japonicus* (Kawamura) Sing. produce the cytotoxic tricyclic sesquiterpene, namely illudin S. This illudin S shows anticancer properties and inhibits cancer cell growth. It is understood that illudin S undergoes activation by glutiathione, and the activated form then covalently binds with DNA. This stops the progress of DNA replication and leads to cell death [5]. A similar compound to illudins has also been isolated from Mycena leaiana, which also shows cytotoxic effect. Illudin A and B were isolated from *Omphalotus olearius* and illudin S and M from *Lampteromyces japonicus*.

Antihistamine: Drugs that combat the histamine released during an allergic reaction by blocking the action of the histamine on the tissue. Antihistamines do not stop the formation of histamine nor do they stop the conflict between the IgE and antigen. Therefore, antihistamines do not stop the allergic reaction but protect tissues from some of its effects. Antihistamines frequently cause mouth dryness and sleepiness. Newer non-sedating antihistamines are generally thought to be somewhat less effective. Antihistamine side effects that very occasionally occur include urine retention in males and fast heart rate.

Poria cocos triterpenoids are not immunologically active unless treated chemically, but if chemically treated has sedative effects [62].

Lectins:

Name of Mushroom	Compound name	Activity	Reference
Grifiola frondosa	GFL	Cytotoxic	Kawagishi, 1995
Pleurotus ostreatus	POL	Anti hemagglutinating activity	Hexiang et.al., 2000
Tricholoma mongolicum	TML	Antitumor	Wang H.X.,1996
Agaricus bisporus	ABL (?)	Antitumor	Wang et.al., 1996 & 1998
Volvariella volvaceae	VVL (?)	Antitumor	Wang et.al., 1996 & 1998
Ganoderma lucidum	GLL-M	Antitumor, mitogenic	Kawagishi, et.al., 1997
Lentinus edodes	---	Mitogenic	Jeune, et.al., 1990

Lectins are protein or glycoproteins substances of 60,000 – 100,000 molecular weight. Usually of plant origin and of non immunoglobulin nature. Lectins are capable of specific recognition of and reversible binding to carbohydrate moieties of complex glyco conjugates without altering the covalent structure of any of the recognized glycosyl ligands. This group includes monovalent lectins (i.e. bacterial and plant toxins). These lectins binds to sugar moieties in cell walls or membranes and thereby change the physiology of the membrane to cause agglutination, mitosis or other biochemical alterations in the cell.

It has been known since 1880's that extract of certain plants could agglutinate red blood cells. Many members of lectinic protein family agglutinate red blood cells. Although the term "Lectins" was originally coined to define agglutinins, which could discriminate among types of red blood cells, today the term is more general and includes sugar binding proteins from many sources regardless of their ability to agglutinate cells.

Lectins have been found in plants, viruses, microorganisms, animals and fungi, but still their function in nature is unknown. Lectins with a certain carbohydrate are very specific. Plants, animals, fungi and other life forms have cell membranes that contain carbohydrates, which are half embedded in the membrane while the half is projected out from the membrane. The interaction is a specific as in the case of enzyme – substrate in the form of Lock and Key model: Lectin is protein containing molecule as a key which fits in a certain type or a specific type of carbohydrates locks, and as in the case of antigen – antibody interaction. Lectins may bind with free sugars or with sugar residue of polysaccharide, glycoproteins or glycolipids, which can be free or bound (as in cell membrane). The term lectin refers to the specificity of the reaction. If a lectin with the molecular structure concurrent to that of carbohydrate and comes in contact with such carbohydrate, it gets integrated/coupled. As this happens, it is hypothesized that the membrane gets disrupted and or damaged thus initiating cell death and thus the process considered in the case of lectins having cytotoxity or anti-cancer effect. Due the specificity of lectins, each lectin has affinity towards a particular carbohydrate structure; even oligosaccharides with identical sugar compostions can be distinguished or separated on the basis of the specificity.

The different ways of binding of lectin with carbohydrates:

Some lectins will bind only to structures with mannose or glucose, while other may recognize only galactose residue.

Some lectins prefer (require) a particular sugar be at the terminal non-reducing position of the oligosaccharide, while some bind in between the chain of the oligosaccharide.

Some lectins require a specific anomeric structure with a specific sequence of sugars for binding whereas some cannot discriminate between a & b anomers.

The affinity between a lectin and its receptor may vary to a great deal due to small change in the carbohydrate structure. Generally a lectin has at least two sugar binding sites (sugar – binding proteins with a single binding site will never agglutinate structures that contain sugar residue and so are not classified as lectin). Lectins may be soluble or membrane bound, they may be glycoproteins. Sugar specific enzymes, transport proteins and toxins having multiple sugar binding sites may qualify as lectins.

Families of lectins classified under concept on protein (gene) families are: Galectin, Ca (calcium) dependent (C-type), Selectins (C-type), Collectins (C-type), Lectins in invertebrates, Annexins, Legume lectin family and Ricin. The types other that the last are said to be of animal origin but there some galactins reports from fungi.

An N-acetylgalactosamine specific lectin was isolated from the fruiting body of Grifiola frondosa (GFL)), which agglutinated all types of erthrocytes equally [63]. GFl is cytotoxic against HeLa cells. The inhibition of hemagglutinating activity of this POL was studied using different agents like melibiose, N-acetyl-D-galactosamine, Raffinose, inulin and D-galactose, out of which N-acetyl-D-galactosamine was the most potent one. The temperature effect test of this POL was carried on the background of the lectin TML isolated from the mushroom Tricholoma mongolicum [64]. The antitumor activity of *Agaricus bisporus* lectin, *Tricholoma mongolicum* lectin, *Volvariella volvacea* lectin has been previously demonstrated by Wang et.al., [64,65].

Ergosterol

Sterols are widely distributed in nature in different animals and plants and some microorganisms like fungi. Sterols are structurally related to some of the sex harmones in animals and to bile acids, e.g. cholesterol in animals is a sterol, which naturally gets converted and becomes bile acids and steroid hormones by some natural metabolic pathways. Ergosterol is a steroidal compound found in fungal membranes and not found in any other organism. It is the precursors of hormones and Vitamin D2 (also called as pro Vitamin D2). This Ergosterol gets converted in vitamin D2 when exposed to ultra violate irradiation (Figure 5). As ergosterol is found only fungi it has become an accepted measure of fungal biomass [66].

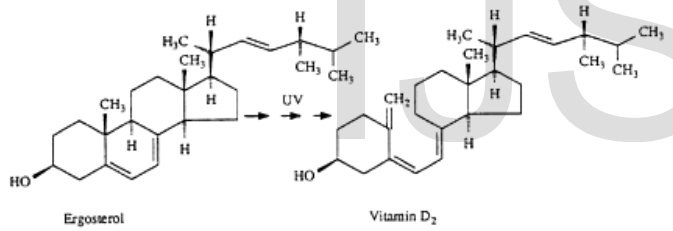


Fig.5: Chemical structure of Ergosterol and Vitamin D2 (conversion on UV irradiation)

Adolf revealed that ergosterol when irradiated with UV assumes exactly the same properties as that of Vitamin D (which was later confirmed). It was also stated that 5mg of irradiated ergosterol has the same action in the respect as one liter of good cod liver oil. It was also discovered that it had anti rachitic activity. Rickets is a disease in which there is a failure of proper development (mineralization) of growing bone. This disease is majority found in children (age group 6-24 months) and occurs as a deficiency of vitamin D. in rickets cases children may have deformities in bones or if not may show weak bones and muscles, thus risk of bone fracture is more in this children. There are some symptoms of rickets like thickening of wrists and bones.

The activity of ergosterol can be listed as ergosterol peroxide from *Polyporus* sp. strongly inhibits (100% inhibition) the bladder tumor promoters and that to in a dose dependent manner. Ergosterol and its peroxide (ergosterol peroxide) inhibit carcinogenesis in the skin of mouse. Thus it not only inhibits the promotion of the carcinogenesis in bladder but also inhibits effects of other carcinogenesis promoter. Only ergosterol has the ability to inhibit two or more tumor promoters having different mechanism [67].

Ergosterol derivatives like ergosta – 7, 22 – dien – 3 beta-yl linoleate and 5 alpha, 8 alpha – epidioxyergosta – 6, 22dien – 3 beta – yl linoleate from Ganoderma sp., exhibit potent inhibition of KB cells and human PLC/PRF5 cells in vitro [68]. The ergo peroxide and two more ergo derivatives from the mushroom *Cordyceps sinensis* show antitumor activity. Ergo peroxide was found to be greater inhibitor to the proliferation of K562, Jurkat, WM – 1341, HL – 60 and RPMI – 8226 tumor cell lines [69]. The ergo peroxide from Meripilus giganteus was identified as immunosuppressive component [70].

Ergosterol is detected from different mushrooms like *Ganoderma lucidum*, *G. amboinenese*, *Lentinus edodes* [71], *Cordyceps* sp. *Polyporous* sp. *Meripilus giganteus*. A spectrophotometric method for detection and estimation of ergosterol is given by Arthington-Skaggs [72].

Germanium

Reports of germanium in medicinal plants like ginseng and mushroom from polyporaceae are quite old. It is recorded and regularly used against many ailments in oriental drugs in Traditional Chinese Medicine.

Nutritionally useful germanium is organic germanium i.e. Germanium sesquioxide (Ge – 132). A Japanese scientist Kazuhiko Asia first synthesized organic germanium in 1967. The inorganic germanium (germanium dioxide) is commercially used in industries like semi-conductor, electronics industries. These industrial forms of germanium are potentially toxic. The pure organic germanium (Ge – 132) is used as nutritional supplements only. While the germanium dioxide shows renal toxicity at the dose such as 300gm.

Medicinal properties of organic germanium (Ge – 132).

Immune system enhancement:

Germanium (Ge – 132) can lower the energy levels of the tissue /organ which is increased due to uncontrolled growth of cancerous cells. This effect is de-nitrogenation effect. It is also related to induction of interferon. A significant level of interferon (IFN) activity was detected in the sera of mice at 20hr and reached a maximum of 320U/ml at 24hrs (Dose: Ge – 132 orally administered at rate of 300mg/kg) [73]; but this IFN activity was lost after heat or acid treatment, which suggests that the induced IFN is of gamma – nature (the molecular weight of the IFN was estimated to be 50,000 Daltons by gel filtration). The NK activity of spleen was increased 24hrs after the oral administration and the cytotoxic macrophages were induced in the peritoneal cavity by 48hrs. Further study shows that both increase in NK activity and activation of macrophages in mice after oral administration of Ge – 132 are mediated by the induced IFN.

Pain neutralization:

Organic germanium is said to control the electron movement between nerve cells, therefore exerting pain-killing effect. Even the organic germanium alleviates angina when therapeutic doses are consumed (Angina: - Chest pain that occurs secondary to the inadequate delivery of oxygen to the heart muscle).

Organic germanium temporarily alleviates Epilepsy (chronic nervous affection, characterized by sudden interruption of consciousness). According to Komuro, derivatives of Ge – 132 (carboxyethyl germanium sesquioxide) are expected to have analgesic effects due to their inhibition of the degradation of endogenous opioid peptides [74].

Detoxification effect:

Organic germanium is said to have ability to wash out different pollutants, contaminants and the debris, remained after anti-tumor or anti-microbial activity of WBC/immune system, which is released in the blood serum. This removal of toxic compounds from blood and other organs is possible for organic germanium because of its ability to free flow along with the blood.

Oxygen carrying capacity:

Organic germanium increases the oxygen carrying capacity of blood; it helps the contraction or expansion of blood vessels and thereby regulates blood circulation. Germanium improves the health of arteries and also lowers blood pressure in some afflicted with Hypertension. Germanium (at a dose of 100-300mg per day) lowers total serum cholesterol levels and enhances the body's utilization of oxygen. Germanium attaches itself to oxygen to improve cellular oxygenation.

Free radical protection/scavenging:

Germanium is reported to have high ability of shielding irradiation. It can reduce the destruction of tissue due to over irradiation in case of radiotherapy. Radiotherapy destroys the abnormal tissue as well as the normal surrounding tissue, which is the major side effect of the radiotherapy used in the cancer treatment. The germanium gets adhered to the cells shielding the beam of rays from damaging. Since radiation damage is oxidative and Ge – 132 is anti-oxidant this is another evidence for the protection from radiotherapy. Goodman enlists the therapeutic effects of Germanium, some of these are immuno-enhancement, oxygen enrichment, free radical scavenging, analgesic and heavy metal detoxification [75]. The Ge – 132 raises the level of reduced glutathione, which is another free radical scavenger thus providing more protection against free radical [76].

Protection and increase of bone density:

Some reports validate that Ge – 132 may be beneficial in protecting the bone density which is also supported by a of twelve month clinical study on elderly people taking Ge – 132 had a significant decrease in parathyroid hormone levels at the end of the 12 months with increase in bone mass while the bone mass continued to decrease in control (without germanium dosage).[77]

Other effects of organic Germanium:

Control of inflammation; control of allergic reaction; control of LDL cholesterol; immune enhancement; oxygen enrichment, free radical scavenging activity, analgesic, heavy metal detoxification, used against cancer, arthritis, senile osteoporosis, anti-viral. Germanium also induces interferon, macrophages, T- suppressor cells, and increases of natural killer cell activity [75].

Organic germanium is absorbed by mushrooms like *Ganoderma* and Shiitake (*Lentinus edodes*) in wild and also in cultivated mushroom if substrate is supplemented with germanium and reports says that the germanium content can be increased significantly in cultivated mushrooms. The germanium in mushrooms can be estimated using spectrophotometric quantitative estimation method.

Nucleotides and its derivatives

Like other mushrooms, REISHI contains adenosine, 5'-GMP, 5'-XMP,RNA, etc. as basic components, all of them being related to taste (deliciousness) (Mizuno. It has been found recently that nucleotides, such as adenosine and guanosine in a water/alcohol extract of REISHI were possessed of a platelet aggregation inhibition action (anti-thrombotic activity). RNA exhibits interferon induction activity as well antiviral, while the Uridine/Uracil shows neuro muscular restorative effect [50]. Some compounds have been isolated from mushrooms as platelet aggregation inhibitor. We have also isolated and identified adenosine and guanosine as potent inhibitors from *Ganoderma lucidum*. The water-soluble fraction of *Ganoderma lucidum* was found to suppress platelet aggregation. It was further shown by gel chromatography, high performance liquid chromatography and nuclear magnetic resonance spectroscopy that the inhibitory substance present in this fraction was adenosine. The platelet aggregation inhibition is also exhibited by nucleosides from *Lentinus edodes*, few species of *Auricularia* and *Cordyceps* [78,79].

4 Conclusion

Mushrooms have been valued through out the world both as food and medicine. Europeans have always appreciated their gastronomic value. In Japan, pushcart vendors sell medicinal mushrooms on the streets, which are regularly used in diet to maintain health and promote longevity. Some Japanese people travel hundreds of miles to collect wild mushrooms, such as Reishi (*Ganoderma*), those grows only on old plum trees and are renowned for their ability to help fight cancer and degenerative diseases. For over 3,000 years, the Chinese have used and revered many fungi for their health – especially as tonics for the immune system [80].

Today an array of products from medicinal mushrooms is available in the global markets. The market value of mushroom dietary supplement products worldwide, which was approximately US $ 3.3 billion in 1990 had reached US $ 14 billion in 2000, while the consumption increasing by 20 - 40% annually [81]. The nature of the products available today in market are raw mushrooms, dried powder, extracts of naturally growing or commercially cultivated mushrooms, dried or extracted biomass of mycelium grown in a solid or submerged culture. The products are not true pharmaceuticals (real medicine) but are called as dietary supplements or nutriceuticals or also called as designer foods, nutraceuticals, mycochemicals etc. [82]. Mushroom based dietary supplements have advantage over other herbal drug as the mushrooms used in preparation of these products are commercially grown under controlled conditions, having genetic uniformity and majority times the strains are of biochemical consistency.

regulations regarding the dietary supplements from medicinal mushrooms and there is a serious need for improved quality and legal control, which is essential for both to increase and

maintain consumer confidence and to meet current and future standards, set by regulatory authorities. To prepare a product and to standardize it on the basis of one or two of its main active constituents, in order to assure that there is a consistent chemical composition between subsequent batches and prescribed levels of chemicals in each batch.

But with the recent development in pharmaceutical and biotechnology industry newer true pharmaceuticals (real medicine) will be available in market for all the above discussed ailments.

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