MYCORRHIZAL, ENTOMOPATHIC AND NOVEL MUSHROOMS

RP SINGH[1]* AND KK MISHRA[2]
[1]Prof. & Head Plant Pathology (Retd.), G.B. Pant University of Agriculture & Technology Pantnagar, Uttarakhand, India
[2]ICAR-VPKAS, Almora 263601, Uttarakhand, India
rp_myco@hotmail.com

ABSTRACT

Mushrooms have always been a source of good health and are being used since time immemorial as medicines. They are pro-biotic i.e. they help our body strengthen itself and fight off illness by maintaining physiological homeostasis. Mycorrhizal, entomopathic and novel mushrooms are gaining popularity since last few decades worldwide. Mycorrhizal mushrooms comprise a specific group of edible fungal species that form symbiotic associations with their host plants and includes *Tricholoma matsutake, Tuber melanosporum, Tuber magnatum, Lactarius deliciosus, Lyophyllum shimeji, Tuber borchii, Tuber formosanum, Rhizopogon rubescens,Terfezia laveryi, Boletus edulis, Cantharellus cibarius, Lactarius hatsutake, Lactarius akahatsu* and *Morchella* spp. However, currently only *Tuber melanosporum* and *Tuber uncinatum* have been cultivated commercially, although some success has been achieved with *Lactarius deliciosus, Lyophyllum shimeji, Tuber borchii, Tuber formosanum, Rhizopogon rubescens, Morchella esculenta* and *Terfezia laveryi*. Entomopathic fungi usually attach to the external body surface of insects in the form of microscopic spores. The most common and important entomopathic fungus is *Cordyceps (Ophiocordyceps)*. *Cordyceps sinensis* has long been used in folk medicine and is known to have remarkable medicinal properties. It has been determined that there is perhaps a greater biodiversity of compounds within different strains of this single species. Due to the great difference in the concentration of native compounds, a wide range of quality is found in *Cordyceps* cultivated from different strains and utilizing different culture methodology. Due to its peculiar characteristics, habitat, morphology and being a store house of medicinal properties, it is a highly prized mushroom. *Ganoderma lucidum* has gained wide popularity in recent years as a dietary supplement, not only in China and Japan, but also in North America and other parts of the world. The reason it attracts international attention as a valuable Chinese herb is due to the wide variety of its biological activities such as antitumor, immunomodulatory, cardiovascular, respiratory, anti hepatotoxic and antinociceptive effects. The diversity in the biological actions may be attributed to the fact that it is composed of different chemical entities including triterpenoids, polysaccharides, alkaloids, amino acids, peptides, inorganic elements, steroids, fatty and organic acids. *G. lucidum* products with different triterpenes and polysaccharides or combinations of these two groups are most likely to result in different pharmacological activities. However, there is lot more to be explored of this wonderful gift of nature and requires the attention of scientific community to exploit this mushroom to the benefit of mankind.

Keywords: edible ecto-mycorrhiza, entomopathic mushroom, *Cordyceps sinensis*, novel mushrooms, *Ganoderma lucidum*, medicinal uses, basic and applied research

INTRODUCTION

The world of mushrooms has always been fascinating and mystic to man owing to their sudden appearance in numbers, groups, rings, bunches and also in isolation as a single attractive and imposing structure. Man has been fascinated with this biological entity since time immemorial and references about the mushrooms are available in most ancient literatures like Vedas and Bible. Mushrooms have always been a source of good health and have been used since time immemorial as medicines. During the past 50 years, several major advancements in medicine came from lower organisms such as molds, yeasts and mushrooms (fungi). In fact, the first antibiotic was extracted from the fungi. Penicillin, tetracycline and auromycine were derived from molds and were regarded as wonder drugs for infectious and communicable diseases. Their medicinal significance has been widely appreciated throughout the world and are thus extensively used in the form of nutriceuticals, nutraceuticals and pharmaceuticals. The studies suggest that mushrooms are pro-biotic i.e. they help our body strengthen itself and fight off illness by maintaining physiological homeostasis by restoring the balance of the body. The compounds present in mushrooms are regarded as Host Defense Potentiators (HDPs) which have immune system enhancement properties and are currently extensively used for treatment of cancers and other malignancies and to increase general body health.

MYCORRHIZAL MUSHROOMS

Edible ecto-mycorrhizal mushrooms (EEM) comprise a specific group of edible fungal species that form symbiotic associations with their host plants (Smith and Read, 1997). This includes about 200 common edible ecto-mycorrhizal mushroom species that are widely eaten in the Northern Hemisphere, although many more, particularly in Africa and South America, have to be recorded. A few species have well established worldwide markets in excess of US$ 2 billion, while many others are locally important (Hall *et al*. 2003). EEMs are not only gourmet foods, they are also a livelihood for many people who collect the mushrooms from their natural habitats or cultivate them. *Tricholoma matsutake* (matsutake) in Japan, and truffles such as *Tuber melanosporum* (Périgord black truffle) and *Tuber magnatum* (Italian white truffle) in France and Italy, are also an important part of the culture, while others have medicinal properties (Yun and Hall, 2004). Unlike saprobic edible mushrooms, with few exceptions, the market of EEMs is supplied from what can be harvested from natural forests. Unfortunately, harvests of many edible mushrooms have declined over past century, because of changes in their natural environment caused by various natural and social factors (Hall and Wang, 2002). Despite numerous scientific publications and the establishment of thousands of hectares of plantations, the downward trend in EEM production continues. Collection of ecto-mycorrhizal and other edible mushrooms in native forests for food and recreation is a popular activity in many cultures and countries. When harvest of edible mushroom is recreational, rather a source of income, generally there is little impact on the environment. However, large-scale commercial harvesting, which generates significant income, can cause serious environmental problems in forests, especially when some commercial harvesters disrespect both the mushrooms and their forest environment. As a consequence, the management of commercially harvested areas has become a major concern. For example, *Tuber melanosporum* harvests have fallen from around 2000 tons in the 1900s to sometimes less than 100 tons annually. Similarly, current matsutake production in Japan is just 5% of 1940s harvests. The decline in the availability of EEMs and increased demand has encouraged research into developing technologies for the cultivation of EEMs as well as methods for the sustainable productivity in natural forests. Currently only *Tuber melanosporum* and *Tuber uncinatum* have been cultivated commercially, although some success has been achieved with *Lactarius deliciosus*, *Lyophyllum shimeji*, *Tuber borchii*, *Tuber formosanum*, *Rhizopogon rubescens*, *Morchella esculenta* and *Terfezialaveryi*. Host plants have also been infected under sterile conditions in the laboratory or greenhouse with *Boletus edulis*, *Cantharellus cibarius*, *Lactarius hatsutake* and *Lactarius akahatsu*, although fruiting bodies have not yet been produced in the field. However, despite many years of study, attempts to cultivate species such as *Tricholoma matsutake* and *Tuber magnatum* have met with failure (Wang and Hall, 2004). The management of plantations for better EEM production is an important issue and over the years a huge knowledge has been accumulated. However, we still do not know why some truffières consistently produce large quantities of truffles, while others produce nothing. Obviously a greater understanding of this and other species of EEMs is needed. Some technologies have been developed for the cultivation of a few EEM and management of existing EEM-producing forests to maximize their production. Unfortunately, these efforts do not seem to have had an effect on diminishing production. The exploitation of new sources of EEMs, especially in developing countries, will provide income for these countries and would find ready markets in developed countries. However, over picking has to be avoided, and conservation and management are needed if EEM are not to be damaged. Mycologists facing these issues will experience considerable challenges in years to come.

ENTOMOPATHIC FUNGI

Entomopathic fungi usually attach to the external body surface of insects in the form of microscopic spores (usually asexual). Under the right conditions of temperature and humidity, these spores germinate, grow as hyphae and colonize the insect's cuticle; eventually they bore through it and reach the insects' body cavity (hemocoel). Then, the fungal cells proliferate in the host body cavity, usually as walled hyphae or in the form of wall-less protoplasts (depending on the fungus involved). After some time the insect is usually killed and new propagules are formed in or on the insect if environmental conditions are again right. The entomopathic fungi include taxa from several of the main fungal groups and do not form a monophyletic group. Many common and important entomopathic fungi are in the order Hypocreales of the Ascomycota: the asexual (anamorph) phases *Beauveria*, *Metarhizium*, *Nomuraea*, *Paecilomyces=Isaria*, *Hirsutella* and the sexual (teleomorph) state *Cordyceps (Ophiocordyceps)*.

Cordyceps sinensis: *Cordyceps* is a fungus of subphylum Ascomycotina, class Pyrenomycetes, order Clavicipitales and family Clavicipitaceae and includes more than 300 species found worldwide. A new classification of *Cordyceps* species has been suggested on the basis of chemo-taxonomy of partial nucleotide sequence of 18S rDNA obtained from four different species. *Cordyceps* species are parasitic, mainly on insects and other arthropods. Some of these are also parasitic on other fungi like the subterranean, truffle-like *Elaphomyces* and also on spiders. The mycelium invades and eventually replaces the host tissue, while the elongated fruiting body (stroma) may be cylindrical, branched, or of complex shape. The genus has a worldwide distribution and most species have been described from Asia (notably China, Japan, Korea and Thailand). *Cordyceps* species are particularly abundant and diverse in humid temperate and tropical forests. Some *Cordyceps* species are sources of important biochemical substances like cordycepin which has very high medicinal properties. The species that parasitizes the vegetable caterpillar, *Cordyceps sinensis*, is the most famous amongst all the species of *Cordyceps* and has been considered a precious ingredient of high medicinal importance.

1. Ethno-Mycological and Traditional Uses

Cordyceps sinensis, a parasitic fungus in the alpine regions has been highly valued in Traditional Medicinal System of China, Nepal, Tibet and India. In interior mountain areas, it is also locally known as 'Yarsha Gamboo', Keera ghas' and 'Keera jhar'. The Tibetan name 'Yarsha Gamboo' means 'summer-grass winter-worm'. Tibetans believe that during winter time it lives as a 'worm' and later, as metamorphosis occurs at the start of the spring season, this worm transforms into a kind of 'grass'. Two distinct phases have been recognized during the entire transformation process. Firstly, the 'grass' starts growing form the head of the larvae. The worm at this particular stage, appears to be white, is alive and can be seen moving over the ground. The blade-like part can be seen protruding out from the head of the insect like a tiny horn. This horn like structure continues to grow further. Ultimately, the worm or the insect dies and transforms into a brownish-yellow coloured 'root' like structure of the grass.

2. The mushroom and its host

C. sinensis is an entomophagous fungus of the family Clavicipitaceae. It parasitizes a range of grass root boring caterpillars, most commonly the Thitarodes (*Hepialus armoricanus*, family Hepialidae). In all, around 40 species of *Hepialus* moth have been recognized in the Tibetan Plateau region and around 30 of these species can be infected by *C. sinensis*. The mycelium of the fungus grows in soil and colonizes the buried larvae (caterpillar) of this moth. The caterpillar becomes mummified by the growth of the mycelium and hence is given the name, "caterpillar fungus". It has been reported that *C. sinensis* has evolved and developed a special adaptation to improve chances of reproductive success. Reproduction is highly host-specific. Every single spore fragments into around 32 million propagules. These tiny propagules get attached to the larval stage of the insect. The larvae is then forced to move closer to the surface of ground (non-infected larvae will not hibernate close to the ground surface). The mycelium, which is composed of white thread like structures called hyphae, grows inside the body of the insect in the form of a cottony mesh. The hyphae fill the interior of the entire caterpillar and mummify it, leaving behind the larval exoskeleton filled with only the white mycelium of fungus. When alpine grasses start sprouting, a fruiting body develops which, surprisingly, always emerges out from the head of the caterpillar (larvae). This fruiting body is usually 5-10 cm long, brown colored and club-shaped. The propagules present on the fruiting body are dispersed by the wind and can attach to new host insects. The fruiting body resembles grass sprouting but the difference is the colour which is dark blue to black.

3. Natural Habitats

Native occurrence of this entomophagus fungus is mostly confined to the high Himalayan Mountains in Tibet, Nepal and India, at an altitude ranging from 3000 to 5000 metres. The most common occurrence of this fungus is between 3500 and 4500 metres elevation in cold and arid environment. *C. sinensis* is endemic to the Tibetan Plateau including the adjoining high altitude areas of the Central and Eastern Himalayan range (covering areas of Nepal, Bhutan and Uttarakhand, Sikkim, Himachal Pradesh and Arunachal Pradesh in India). It is found in the high altitudes of Pithoragarh, Uttarakhand (Bhatt, 2012) and other provinces at locations above 7000 feet. It is also common in the grasslands and shrub lands of the Tibetan Plateau including west Sichuan, North Yunnan and major areas of Qinghai and West Gansu. The distribution of *C. sinensis* is limited to those areas where the average annual precipitation is above the range 350-400mm. This caterpillar fungus thrives very well in sub-alpine and alpine grasslands or meadows and in open dwarf shrub lands. Extensive research has been conducted on the ecology, collection, utilization, trade route, management and significance and species diversity of *C. sinensis* in various parts of India, Tibet, Michigan, Bhutan, China and Korea.

4. Collection and trade of *C. sinensis*

Years back this fungus was collected and traded from Tibet to China in exchange for tea and other commercial goods like silk, grains, etc. The most appropriate time for harvesting of this fungus starts with the arrival of the spring season, in about starting of May. Local villagers and nomads search for the fungus in the grasslands and shrub lands. But the harvesting is slightly a difficult process as only the stroma or the grass-like part of the fungus is visible over the surface which too is quite short, not longer than 2-5cm and has to be lifted out with the help of a sharp knife. Extreme care has to be taken while pulling out the fungus from the ground surface because if the stroma breaks off from the head of the larvae, it directly affects the commercial value of the mushroom resulting in a decline in its market rate.

During the harvesting season, all other activities come to a standstill as everyone is focused on gathering more and more of the fruiting bodies. This has often led to many blooded wars in these areas. The Ministry of Population and Environment, Govt. of Nepal has banned its collection, trade and transportation. However, in Tibet it is an open trade. In some areas of Tibet, even the schools announce vacations for 15-20 day in late May so that the students can also help in the collection and harvesting of this precious mushroom. The daily collection may vary from 250g to as much as 5kg.

During recent years, caterpillar mushroom has emerged out be an important cash crop traded on a large scale and a new source of income in the rural areas in the higher altitude regions usually above 3000-5000 metres. In the river valley of Gori Ganga, India alone, the number of fungus gatherers at alpine habitats has increased about four-fold since the year 2000. During 2002, nearly 900 persons went to seven different alpine habitats in search of the fungus (average 128 persons per habitat) and collected about 200 g of the fungus per day. High price of the fungus very often make the transaction secretive in the local market while due to cross-boundary trade between countries, the rest of the trade is under the surface (Sharma, 2004). Cost of the fungus at the final destination (brokers in national and international markets) was much higher than the price paid to the field gatherers. It is believed that in the International market the fungus may fetch a price between one and two million ₹ per kg (US$ 20,000-40,000). The amount paid varies among the trade channels which start from the wild material gatherers in the field, then to the brokers and agents who collect the dried material from the various locations and sell it at a higher price. However, rapid and immediate marketing of this fungus is not required as the fungus is usually sold and consumed in a dried form. Its small size and easy storage conditions make the transportation much easier.

The fruiting bodies of the *Cordyceps sinensis* were collected from a high altitude (3500-4500m) at different locations viz. Nahardevi, Martoli, Ralam, Laspa, Milam, Chetribugyal, Chiplakot, Malpa Top, Tola Top, Burphu and Panchachuli in district Pithoragarh, Uttarakhand, India (Singh *et al*., 2009). The ascocarps were 4.0-7.0 cm in length, mostly erect, slightly swollen at tip; emerged single, double or triple from the head of larvae. Caterpillar cadaver varied 3.0-4.0 cm in size, having eight pairs of legs with fine transverse wrinkles.

5. Composition of *Cordyceps sinensis*

The pharmacological and medicinal significance of *Cordyceps sinensis* is mainly due to its bioactive ingredients. The composition properties and structure-activity relationship of these components have been under extensive scrutiny by many researchers and have eventually been rediscovered and modified with respect to the present scenario of disease and abnormalities. It contains a wide variety of potentially important constituents, including polysaccharides, ophiocordin (an antibiotic compound), cordycepin, cordypyridones, nucleosides, bioxanthracenes, sterols, alkenoic acids and exopolymers, etc. The constituents of *C. sinensis* were thoroughly studied and a crystalline substance Cordycepic acid was isolated and identified, which was later identified as D-mannitol. Cordycepin and cordycepic acid are regarded as the most important constituents of this fungus and owe high medicinal significance.

6. Medicinal uses of *Cordyceps sinensis*

In the recent past, a variety of medicinal preparations in the form of tablets, capsules and extracts from mushrooms for the treatment of various kinds of ailments, diseases and disorders have been produced and marketed. In 1991, the value of world mushroom crop was estimated to be around 8.5 billion dollars and in the same year 1.2 billion dollars were estimated to have been generated from medicinal products from various medicinal mushrooms. Herbal medicinal preparations from different mushrooms have become a growing business in various parts of the world as for instance, Bhutan is an emerging market for *Cordyceps* and its usage has shown a tremendous increase since last few years.There are data of clinical trials that support the efficacy of *C. sinensis* as a medicinal herb, especially for disorders related to the liver, kidney and immune system. A number of studies indicate that *C. sinensis* (and also its mycelial extract) possess certain anti-cancer, anti-metastatic and immuno-stimulating properties. It is also reported to have anti-oxidant activity (Singh *et al*., 2007).

Since ages, *C. sinensis* has been regarded as panacea of life, imparting youth, vigor and longevity. Other important functions include activation of the immune responses, controlling the blood sugar levels, treatment of Hepatitis B, improvement of the respiratory functions, improvement in the functioning of the heart, maintaining the levels of cholesterol, reduction of the tumor size in cancer patients**,** protection against free radical damage**,** reduction of fatigue, combats sexual dysfunction, helping in organ transplantation, improvement in the functioning of kidney and adrenal gland etc. (Singh *et al.*, 2008a).

7. Morphological and Physiological characteristics

Isolation of the fungus was done using fresh ascocarp along with larva cadaver on Sabouraud's dextrose agar with yeast extract (SDAY) medium. The hyphe of the fungus was aerial cottony white to creamish or yellowish, septate, branched, dense and 1-3 µm wide. Colony was found initially white and later on pink red or orange and reverse cream to purplish red in colour on potato dextrose agar (PDA) medium; initially cream with lined depressions, later dark orange and from reverse dark tan in colour on sabouraud's dextrose agar with yeast extract (SDAY) medium; initially light pink which changed to purplish red and from reverse blood red colour on malt extract agar (MEA); initially creamish yellow, later light purple and dark tan colour from reverse on oat meal agar (OMA) and initially light yellow with purplish red margin, finally dark purplish red and dark tan colour in reverse on czapek dextrose agar (CDA) medium. *C. sinensis* showed the maximum and minimum growth on 12^{th} day (44.93 and 32.60mm) on SDAY and CDA media, respectively (Arora, 2008). The fungus also showed maximum (44.87mm) and minimum growth (34.27mm) at 15 ^{0}C and 25 ^{0}C, respectively and it declined below and above 15 ^{0}C. The maximum growth (44.93mm) of the fungus was reported to be at pH 6.

Addition of carbon sources significantly affects the mycelial growth of *C. sinensis* (Arora, 2008). Disaccharides (sucrose, maltose and lactose) produced a higher mycelial growth as compared to the monosaccharides (glucose and fructose). Amongst the carbon sources tested, sucrose gave a maximum dry weight of the mycelia (5.15 g/l) followed by maltose and lactose while check produced a minimum dry weight (2.83 g/l) (Table 1).

Vitamins played the significant role in the mycelial growth of *C. sinensis*. Folic acid (12.42 g/l) out yielded all the vitamins tested, while the pyridoxine produces the minimum (5.39 g/l) which was even lower than that of check. The D-biotin, thiamine and nicotinic acid were at par in terms of yield and next to folic acid. However, riboflavin was found to be at par with the check. Micro and macro elements (minerals) produced significantly higher mycelial yield as compared to that of check. Addition of micro elements was more effective in terms of mycelial dry weight per litre as compared to macro elements.Amongst the micro and macro elements the highest yield was observed in case of $ZnCl_2$ (7.87 g/l) and $CaCl_2$. $2H_2O$ (5.55 g/l), respectively (Table 2).

Table 1. Effect of the carbon and nitrogen sources on the mycelial growth of *C. sinensis*

C-sources	Dry wt. (g/l)	N-sources	Dry wt. (g/l)
Monosaccharides		**Inorganic**	
Glucose	3.74	Potassium nitrate	3.33
Fructose	3.18	Sodium nitrate	2.75
Disaccharides		Ammonium nitrate	2.52
Sucrose	5.15	Ammonium chloride	2.30
Maltose	4.72	Ammonium sulphate	2.16
Lactose	4.25	Urea	1.67
Check (no carbon)	2.83	**Organic**	
—	—	Beef extract	5.24
—	—	Check (no nitrogen)	1.85
CD at 5%	0.26	CD at 5%	0.31

Table 2. Effect of the vitamins and minerals on the mycelial growth of *C. sinensis*

Vitamins	Dry wt. g/l	Minerals	Dry wt. (g/l)
Folic acid	12.42	**Micro**	
D-biotin	9.40	$CuSO_4.5H_2O$	6.60
Thiamine	8.88	$FeSO_4.7H_2O$	7.13
Nicotinic acid	8.94	$MnCl_2.4H_2O$	6.20
Riboflavin	7.66	$ZnCl_2$	7.87
Pyridoxine	5.39	**Macro**	
Check (SDY)	7.42	$CaCl_2.2H_2O$	5.55
—	—	KH_2PO_4	4.50
—	—	K_2HPO_4	4.67
—	—	$MgSO_4.7H_2O$	4.20
—	—	NaCl	4.87
—	—	Check (no mineral)	3.87
CD at 5%	0.72	CD at 5%	0.13

8. Nutritional and Medicinal characteristics

The fungus was multiplied using different cereal grains viz. wheat, rice and maize grains. It was observed that the wheat grains were colonized by the fungus in minimum period of 25 days while on maize grains took 39 days maximum and on rice 31 days for colonization. Maximum (6.48 g) and minimum (2.36 g) weight loss was observed in wheat and maize grains, respectively. Whereas, the weight loss in case of rice was 4.65 g. It showed that the fungus grew well and nutritional utilization of the fungus from wheat and rice grains was higher.

Arora and Singh (2009) reported much higher amount of sugar in the mycelia in SDY broth (63.1%) as compared to that in the fruiting bodies (24.2%) while protein content was higher in fruiting bodies (28.6%) as compared to the mycelia (8.2%). The lipid content was 3.15% in fruiting bodies and 2.95% in the mycelium of *C. sinensis* (Table 3).

Table 3. Total soluble sugars, proteins and lipids in fruiting bodies and mycelium

Source	Content %		
	Total soluble sugar	**Protein**	**Lipid**
Fruiting body	24.2	28.60	3.15
Mycelium	63.10	8.2	2.95

Detection of adenosine, cordycepin and ergosterol from fruiting bodies and mycelia of *C. sinensis* by HPLC analysis are depicted in Figs. 2,3,4,5 and 6. Spectrum was taken at ë max-254 nm and the retention times were noted for the standard- adenosine and cordycepin from 50 ppm to 100 ppm. Retention time of adenosine and cordycepin were found to be 6.895 and 7.611 minute, respectively (Fig. 1). While the hot water extract of fruiting bodies were found to contain both adenosine and cordycepin (Fig. 2) but in case of *C.sinensis* mycelia, adenosine was detected while cordycepin was undetected (Fig. 3). The retention time of standard ergosterol was around 19.194 minute (Arora *et al.*, 2008) and its presence in fruiting bodies and in the mycelia of *C. sinensis* was confirmed (Figs. 4, 5 & 6) .

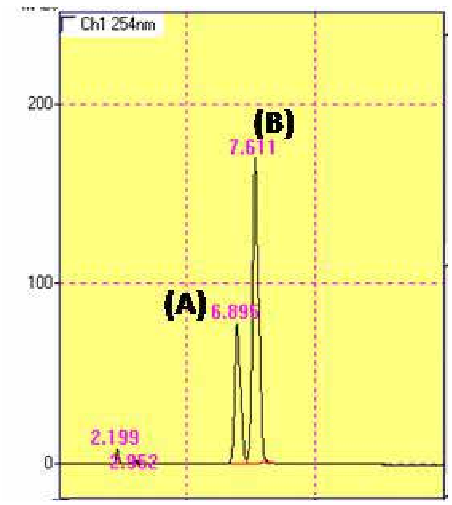

Figure 1. Chromatogram of standard adenosine (A) and cordycepin (B) at 100ppm

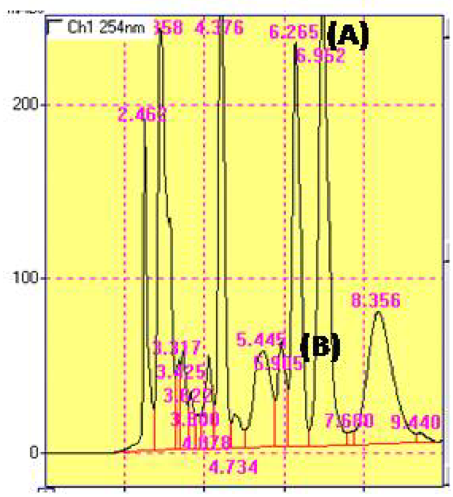

Figure 2. Chromatogram of purified extract of fruiting bodies of *C. sinensis* showing retention time were 6.895 and 7.611 min

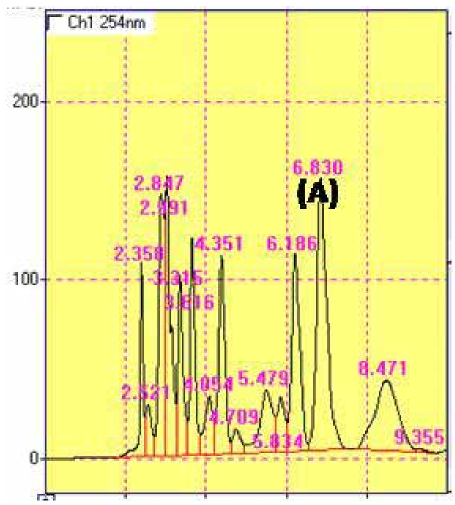

Figure 3. Chromatogram of mycelium of *C. sinensis* showing adenosine (A) adenosine (A) and cordycepin (B)

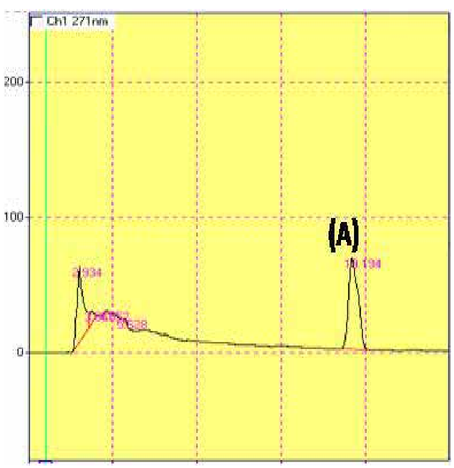

Figure 4. Chromatogram of standard-ergosterol (A) at 100ppm. Retention time was 19.194

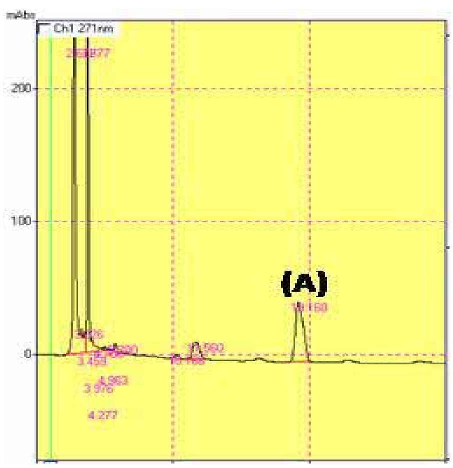

Figure 5. Chromatogram of purified extract of fruiting bodies of *C. sinensis* showing ergosterol (A)

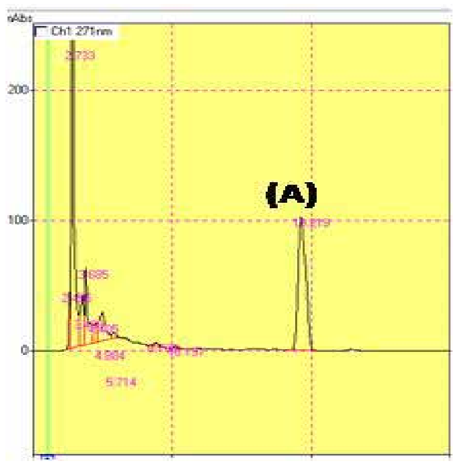

Figure 6. Chromatogram of mycelium of *C. sinensis* showing ergosterol (A)

Singh *et al*. (2007) determined anti-oxidative properties like reducing power and chelating activity on Fe^{2+} from the ethanolic extracts of *G. lucidum* and *C. sinensis* and reported that reducing power of *G. lucidum* and *C. sinensis* extracts increased with an increase in concentration and was 2.50 and 1.97 at 1.0 mg/ml, respectively (Fig .7). At the same concentration *G. lucidum* howed higher reducing power than *C. sinensis*. In addition, the reducing power of both *G. lucidum* and *C. sinensis* extracts were higher than that of L-ascorbic acid (0.71).

The *G. lucidum* and *C. sinensis* extracts showed chelating activity on Fe^{2+} in a concentration dependent manner (Fig. 8). The *G. lucidum* extract exhibited higher chelating activity on Fe^{+2} than *C. sinensis* extract. However, chelating activity of *G. lucidum* and *C. sinensis* extracts with concentration of 1.0 mg/ml (47.2 and 41.6%) was higher than that of EDTA (34.9%) and acetic acid (30.9%). The authors report that *G. lucidum* showed higher anti-oxidative properties than *C. sinensis*, probably due to presence of different compounds in the fruiting bodies.

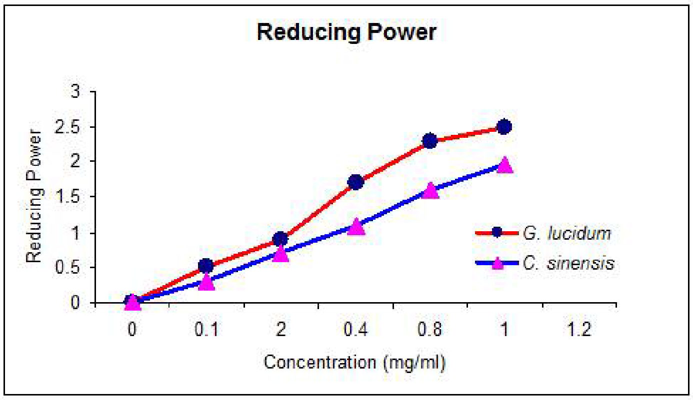

Figure 7. Reducing power of *G. lucidum* and *C. sinensis*

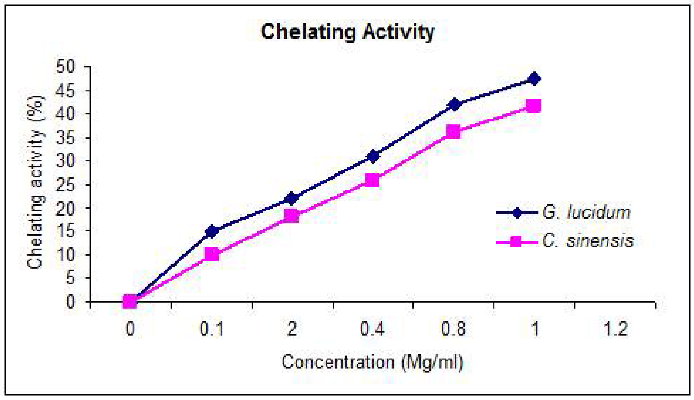

Figure 8. Chelating activity of *G. lucidum* and *C. sinensis* on Fe^{2+}

NOVEL MUSHROOMS

Edible and medicinal mushrooms can produce a variety of biologically active compounds and can be therefore described as a novel class of nutraceuticals which are widely used as dietary supplements (Jiang and Sliva, 2010). Recent epidemiological studies from Asia demonstrated that mushroom intake protects against cancer, specifically gastrointestinal (GI) cancer and breast cancer. The anticancer activities of mushrooms were mainly linked to the modulation of the immune system by branched polysaccharides (glucans), glycoproteins or peptide/protein-bound polysaccharides. Moreover, mushrooms contain minerals, vitamins (e.g., thiamin, riboflavin, ascorbic acid, and vitamin D), amino acids, and other organic compounds. Some of these natural mushroom compounds demonstrated specific activity against aberrantly activated signaling pathways in cancer cells and were able to modulate specific molecular targets in the cell function including cell proliferation, cell survival and angiogenesis. Mycophyto Complex (MC) is a dietary supplement consisting of a mixture of six varieties of mushroom mycelia, including *Agaricus blazei*, *Cordyceps sinensis*, *Coriolus versicolor*, *Ganoderma lucidum*, *Grifola frondosa* and *Polyporusum bellatus* and additional β-1,3-glucan isolated from the yeast, *Saccharomyces cerevisiae*. Interestingly, these specific mushrooms have been linked to different health promoting or disease preventing functions.

1. ***Agaricus blazei:*** *A. blazei* is traditionally believed to treat many common diseases like atherosclerosis, hepatitis, hyperlipidemia, diabetes, dermatitis and cancer (Firenzuoli *et al*., 2008). The polysaccharide phytocomplex was suggested to be responsible for the immuno-stimulant and anti-tumor properties of *A. blazei*, probably through an opsonizing biochemical pathway. In addition, recent studies demonstrated anticancer activities of *A. blazei* through the induction of apoptosis by the activation of caspase-3 in prostate cancer cells and inhibition of constitutively active NF-κB in leukemic cells. Moreover, *A. blazei* demonstrated anti-metastatic effect through the inhibition of MMP-9 in melanoma cells.

2. ***Coriolus versicolor:*** *C. versicolor* is an obligate aerobe that is commonly found year-round on logs, stumps, tree trunks, and branches. The fungus occurs throughout the wooded temperate zones of Asia, Europe and North America and may be the most common shelf fungus in the Northern Hemisphere. *C. versicolor* contains biologically active, structurally different protein-bound polysaccharide-K (PSK) and polysaccharopeptide (PSP), which were approved in Asia for immunotherapy or as biological response modifiers (BRMs) (Cui and Chisti, 2003). In addition to the immuno-modulatory activity, extracts of *C. versicolor* demonstrated direct effects on a variety of cancer cells. Therefore, *C. versicolor* induced apoptosis of breast cancer cells through p53 and Bcl-2 dependent and independent mechanisms suppressed cell proliferation and induced apoptosis of leukemia cells by mechanisms including inhibition of transcription factor NF-κB and down-regulation of expression of COX-2. Moreover, direct cytotoxic effect of PSK, through the cell cycle arrest at G0/G1 phase and induction of apoptosis associated with the caspase-3 expression, was reported in various tumor cell lines derived from leukemia, melanomas, fibrosarcomas and cervix, lung, pancreas and gastric cancers.

3. ***Ganoderma lucidum:*** *Ganoderma* is one of the members of Ganodermataceae family systematized under the order Aphyllophorales of phylum Basidiomycota. The fungus has very large climatic diversity and occurs in natural habitat of almost all around world from temperate to tropical regions. Of the 250 known species of *Ganoderma*, several species are used for medicinal purposes. Fruiting bodies of the *G. lucidum* contain polysaccharides, triterpenoids, adenosine, germanium, protein (LZ-8), amino acids etc., and found to have anti tumor and immuno-modulating effect (Singh *et al.*, 2007 and 2008b). Because of its perceived health benefits this species has gained wide popularity, not only in China and Japan but also in Korea, North America, Malaysia, India and other parts of the world. A number of products prepared from *G. lucidum* are sold throughout the world as dietary supplements. The estimated global turnover of *G. lucidum* products was approximately $2.16 billion (Lai *et al.*, 2004).

   Uttarakhand a hill state of India is gifted with a rich medicinal mushroom flora that includes *Ganoderma* (Singh *et al.*, 2007). The fungus occurs in natural habitat of almost all parts of the zone A and B of the state spread over 100-1500m in height. *Ganoderma* is composed of a vast number of bioactive compounds of pharmacological activities appear to be triterpenes, polysaccharides and adenosines. *G. lucidum* isolated from different altitudes in Uttarakhand, India were designated as GL-1, GL-2, GL-3, GL-4, GL-5, GL-6, GL-7, GL-8, GL-9 and GL-10.

Table 4. Yield performance of isolates over different substrate

Sl. No.	Isolate	Av. yield 'g'/500g dry substrate								
		WS	WS+ RB 5%	BE%	SD	SD+ RB 5%	BE%	CP	CP+ RB 5%	BE%
1	GL-1	103.00	111.00	22.20	64.0	81.10	16.2	27.50	38.50	7.70
2	GL-2	93.00	93.00	18.60	56.5	71.00	14.2	24.50	31.25	6.25
3	GL-3	75.25	85.60	17.12	43.0	52.75	10.5	24.00	32.33	6.46
4	GL-4	71.50	79.25	15.85	33.2	54.50	10.9	20.50	35.00	7.00
5	GL-5	67.50	75.60	15.12	37.1	57.66	11.5	18.25	21.50	4.30
6	GL-6	55.10	74.10	14.82	33.3	51.20	10.2	15.75	21.50	4.30
7	GL-7	60.31	71.25	14.25	31.0	47.00	9.40	14.20	25.25	5.05
8	GL-8	68.80	71.00	14.20	40.0	46.20	9.20	14.50	21.00	4.20
9	GL-9	61.20	69.50	13.90	32.2	45.10	9.02	15.00	19.10	3.82
10	GL-10	39.50	42.25	8.45	27.0	31.50	6.30	19.00	29.33	5.86

RB = Rice bran; WS = Wheat straw; SD= sawdust; CP= Coir pith

CD at 5% ; Isolate a= 2.40; Substrate b= 2.82 and Interaction a x b=5.12

The results revealed that all the substrate, either alone or with 5% rice bran resulted in the development of fruit bodies of all the isolates of *G. lucidum* and all the substrate varied significantly from each other in terms of yield (Table 4). Wheat straw supplemented with 5% rice bran out yielded all other substrate. However, the lowest biological efficiency was obtained on coir pith alone or with rice bran. Among the isolates, the isolates GL-1 GL-2 and GL-3 gave significantly higher yields 111.00g, 93.00g, 85.60g/500g dry WS substrate, respectively though, the yield varied significantly from each other (Singh *et al*., 2009). Mishra and Singh (2008) reported wheat straw as a better substrate as compared to sawdust which is widely used for cultivation of *G. lucidum*.

Mishra and Singh (2010) reported that total soluble and non reducing sugar content varied from 14.58–14.88 and 14.55-14.80%, respectively among the different wild isolates of the fungus. However, protein content varied from 21.0-27.0% in wild and their cultivated isolates. The wild and their cultivated isolates showed presence of ganoderic acids-A, C & H. These ganoderic acids were higher in wild isolates as compared to their cultivated isolates (Singh *et al*., 2012). Wild isolates of *G. lucidum* showed higher content of glucosaminoglycan (Singh *et al*., 2008b), however, the genetic makeup of the wild and cultivated isolates was the same.

4. ***Grifola frondosa:*** *G. frondosa* is a popular culinary mushroom originally recognized in Japan and Korea. The anticancer activities of *G. frondosa* were originally described more than 30 years ago and are associated with the presence of 1,3-ß glucan named grifolan LE (Kodama *et al.*, 2002). 1,3-branched-1,6-ß-glucan isolated from *G. frondosa*, was associated with the stimulation of natural killer (NK) cells and activation of macrophages and differentiation of T-cells. The direct anticancer effect was demonstrated on the gastric cancer cells by the induction of apoptosis via caspase-3-dependent and independent pathways.

CONCLUSION

Nature is the source of all the raw materials that we need. About 2–3 decades ago, most of the drugs were of herbal origin. A variety of reasons under pin why people like to use natural medicines as it is evident that patients are getting even more distressed after using chemically synthesized drugs, rather than natural means like medicinal mushrooms that can conquer life claiming diseases, leaving no side effects on human health. To maintain proper growth, the pharmaceutical industries need innovation and access to high output rate on low-cost materials with reasonable safety. The combination of modern chemistry with bio-based starting materials, like, bio-metabolites, offers the scope for revolutionizing mushroom based pharmaceutical industries. In the near future, bio-metabolites (cordycepin, polysaccharides, ganoderic acids etc.) extracted from medicinal mushrooms will have a role that compares with that of oil and gas crackers today. Therefore, bio-metabolites of medicinal mushrooms will be the key future driving force in the realm of green pharmacology and pharmacognosy.

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