Antihyperlipidemic Effect of *Pleurotus ostreatus* in HIV: Results of a Pilot Proof-of-Principle Clinical Trial

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Antiretroviral treatment (ART) regimens commonly contain agents which cause significant lipid elevations in many HIV patients (pts), including increases in both triglycerides and cholesterol. Standard treatments for hyperlipidemia include the HMG CoA reductase inhibitors, or "statins." Because many ART agents and statins share a common metabolic pathway that uses the CYP3A4 enzyme system, co-administration of ART with statins could increase statin plasma levels significantly. This increases the likelihood for adverse effects, including elevated liver function tests and muscle breakdown, which, if left untreated, may progress to renal failure. A safe and effective alternative to statins for treatment of hyperlipidemia in patients on ART regimens would be of value. *Pleurotus ostreatus* (Jacq.: Fr.) P. Kumm. (culinary-medicinal oyster mushroom) has been shown in animal models to decrease lipid levels – a finding that has been supported by preliminary data in a small study in humans. Our pilot study was designed 1) to determine whether there are detectable lipid-lowering effects of *P. ostreatus* specifically in patients with HIV and hyperlipidemia who are taking ART; and 2) to assess whether the concomitant administration of daily *P. ostreatus* and ART regimens in this population is safe.

Pleurotus ostreatus was cloned and maintained *in vitro* at the spawn laboratories before being expanded into edible mushrooms. Clusters of young mushrooms were harvested and flash-frozen. We designed a single-arm, open-label study of 8 weeks' duration with a target enrollment of 20 subjects. Study patients with ART-induced, elevated, non-HDL cholesterol levels (>160 mg/dL) were eligible to enroll. After screening and obtaining informed consent, pts received packets of freeze-dried *P. ostreatus* (15 gm/day) to be administered orally each day for the 8-week trial period. Pts were followed with lipid levels drawn every 2 weeks to assess efficacy. Safety assessments include self-reported incidence of muscle aches and measurement of liver and muscle enzymes.

126 pts were screened to enroll 25 subjects, of which 20 completed the study. The mean age was 46.4 years (36–60). Pts had a mean 13.2 yrs of HIV infection. Mean non-HDL chol was 199.8 mg/dL at day 0 and 198.9 mg/dL at day 56 [mean change = –0.9; 95% confidence interval (CI) = –15.5, 13.7]. HDL cholesterol levels increased from 37.0 mg/dL at day 0 to 40.2 mg/dL on day 56 (mean change = 3.1; 95% CI = 1.1, 5.1). Triglycerides dropped from 320.5 mg/dL on day 0 to 271.3 mg/dL on day 56 (mean change = –49.2; 95% CI = –105.8, 7.5). There were no adverse experiences reported other than pts' distaste for the preparation. Liver function tests and muscle enzymes were not affected by the 8 weeks of treatment.

Pleurotus ostreatus as administered in this experiment did not lower non-HDL cholesterol in HIV patients with ART-induced hypercholesterolemia. Trends towards an increased HDL and a decrease in triglycerides are encouraging, suggesting that further studies may be warranted.

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Growing Medicinal Mushrooms in Egypt as a New Strategy for Cancer Treatment

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Man has been interested in mushrooms since ancient times; the pharaohs of ancient Egypt prized mushrooms as a delicacy. According to Egyptian hieroglyphics (4,600 years ago), mushrooms were considered the plants of immortality. The delicious flavor of mushrooms intrigued the pharaohs of ancient Egypt so much that they decreed mushrooms were food for royalty and that no commoner could ever touch them. In the past twenty years researchers have extensively studied mushrooms for their immune-enhancing effect. In Japan, China, Canada, and USA, due to scientific investigation, several mushroom extracts have been approved as anticancer drugs. The incidence of cancer is gradually increasing and the spectrum of cancer prone organs is changing each year. In addition to surgery, irradiation, and chemotherapy, immunotherapy is believed to be an important cancer therapy. Beta-glucan is well known as an immunomodulator substance, which is widely distributed in nature and used as medicine and food. Beta-glucan binds to macrophages and other phagocytic white blood cells at certain receptors and activates their antitumor activity. In the last decade, there have been several studies that focused on antitumor polysaccharides from mushrooms, i.e., from beta-glucan protein complex. Antitumor polysaccharides were screened not only in the mushroom fruit body but also in liquid culture media and mycelium. Since cultivated mushrooms can grow on agricultural and industrial wastes, they constitute a source for not only degrading such wastes, but also to provide a source of food for human consumption. Mushrooms have been used medicinally for hundreds of years, mainly in eastern medicine. The Chinese used dried mushrooms as diuretics and some species have recently been getting attention as carcinostatic substances. Starting in the 1970s, Japanese researchers found that antitumor compounds in some mushroom species were polysaccharides whose basic structure is a beta-glucan. These polysaccharides were different from the usual carcinostatic drugs, and their mode of action seems to be based on the stimulation of the immune system.

The following is a report of research carried out over 2 years (in Egypt and as a Fulbright Scholar at the University of Wisconsin-La Crosse with Dr. Thomas Volk) to identify suitable conditions for growing mushrooms in Egypt and elucidating the anti-cancer effect of polysaccharides isolated from these cultivated mushrooms. A fair degree of success was obtained for growing the oyster mushrooms, *Pleurotus spp.,* in Egypt. The mode of production has been established and cultivation is presently being carried out on a small scale in mushroom growing rooms. The primary substrate used for *Pleurotus* spp. production in Egypt is chopped rice straw (3–5 cm) because of availability. Other suitable substrates include wheat straw and cottonseed hulls. The substrate is filled into galvanized metal boxes with perforated floors. The substrate is subjected to pasteurization for 1 hour in order to kill any competing microorganisms, and pH is adjusted to approximately 7.5. The dry desert climate may actually be beneficial for cultivation because of the reduced risk of airborne disease contamination. Spawn is transferred to polyethylene bags containing the sterilized rice straw substrate and incubated for 21 days at 27ºC. Humidity is also regulated to approximately 80% and the bags were carefully spaced for airflow. Humidity and temperature are controlled by using a spraying system that emits a mist of water and a cooling unit consisting of a fan and evaporative pads. In the present study mushroom fruit bodies and mycelia were grown in solid agricultural wastes and submerged culture, and the polysaccharides were extracted from fruit bodies and culture broth by hot water extraction and precipitation with 70% ethanol. The structure of polysaccharide was elucidated using IR and NMR spectra, which indicated that the polysaccharide is a highly branched glucan containing mainly 1.3 and 1.6 linkages. The polysaccharides possess anticancer activity against human cancer cell lines. The results also showed that the polysaccharides enhance the immunoresponses of the human body thereby increasing resistance to cancer disease. Mushroom polysaccharides are considered immunopotentiators or biological response modifiers.

Basidiomycetes as a Source of Food, Enzymes, Polysaccharides, Lectins, and Antioxidants

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Recently, extensive research on basidiomycetous fungi has markedly increased mainly due to their potential use in a variety of biotechnological and environmental applications, particularly for the production of enzymes, dietary supplements, physiologically active compounds and bioremediation. Two laboratories from Georgia and Israel combined efforts to evaluate the potential of Basidiomycetes strains isolated from different ecological niches and belonging to various taxonomic groups to produce lignocellulolytic enzymes, extracellular polysaccharides, lectins, and antioxidants.

Results on extensive screening prove the feasibility of mushroom submerged cultivation for the antioxidant production with high yield. When β-carotene bleaching method was used, water extracts from *Coprinus comatus*, *Agaricus nevoi*, and *Flammulina velutipes* and ethanol extracts from *Agaricus nevoi*, *Omphalotus olearius*, and *Auricularia auricula-judae* showed the highest (more than 80%) antioxidant activity with 2 mg/ml. When the DPPH radical scavenging activity of ethanol and water extracts was measured *Ganoderma lucidum* (56%–69.0%) and *Daedalea quercina* (51%–55%) were the best producers among 24 strains tested.

In the course of screening for mushroom lectins we found that all tested mushroom fruiting bodies contain hemagglutinating activity (HA) with a titer from 32 to 16,384 depending on the mushroom species. A very high HA titer was revealed in fruiting body extracts from *Agaricus pilatianus* and *Coprinus comatus* with specific HA 57490 and 60680 U mg^{-1}, respectively. It is important that all higher Basidiomycetes tested have the capability to accumulate lectin activity during their cultivation on defined liquid medium. Moreover, the accumulation of these compounds takes place in submerged and solid-state fermentation of agro-industrial wastes. The results show that the lectin HA is species- and strain-dependent and varies with culture conditions. The specificity toward a variety of sugars and the minimal inhibition concentrations have been established for different mushroom lectins.

The capability to synthesize the extracellular polysaccharide (EPS) is widespread among Basidiomycetes species, which accumulated 0.6–2.2 g/l of EPS in submerged cultivation. Glucose, maltose, and mannitol were the most appropriate carbon sources for biomass and EPS production. Organic nitrogen sources appeared to be the most suitable nitrogen sources for biomass and EPS accumulation. The cultivation process in shake flasks was successfully reproduced in a laboratory fermentor with enhanced EPS production. The highest yield of EPS (3.8–4.0 g/l) was achieved in the cultivation of *Agaricus nevoi* and *Inonotus levis*.

A common feature of white-rot Basidiomycetes is the production of cellulases, xylanases, laccases, and manganese peroxidases in submerged and solid-state fermentation of lignocellulosic substrates. Different strategies improving the enzyme production, such as the isolation of new fungal strains, optimization of growth conditions, use of inducers or cheap growth substrates, e.g., agricultural and food industry wastes, will be critically discussed.

The comparison of lignocellulolytic enzyme profiles during the life cycles of *Pleurotus ostreatus* and *Lentinus edodes* commercial strains in cultivation in plastic bags containing wheat straw or tree leaves showed that laccase and MnP activities are high during substrate colonization and decline rapidly during fruiting body development. On the contrary, low cellulase and xylanase activity was expressed during substrate colonization. When the primordia activity appeared, these enzymes sharply increased. Both cellulase and xylanase activity peaked in the mature fruit body stage. When mushrooms shifted to the vegetative growth oxidases, activity gradually increased, whereas the hydrolases activity dropped.

A Mushroom Mycelia-Soy Extract (GCP) as a Complementary Therapy for Treatment of Prostate Cancer

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Medicinal mushrooms have been used for millennia to treat cancer. A unique mushroom nutraceutical, GCP (Genistein Combined Polysaccharide, Amino Up Chemical, Sapporo, Japan) was produced by culturing soybean extract with *Ganoderma lucidum* mycelia, yielding a product rich in mushroom polysaccharides and in the highly bioavailable isoflavones genistein and daidzein.

The role of GCP to enhance the effect of pharmaceuticals to treat prostate cancer was examined in two sets of in vitro studies. In the first set of experiments, GCP significantly potentiated the activity of the androgen receptor antagonist bicalutamide, the anti-microtubule taxane docetaxal, and the Src kinase inhibitor pp2, resulting in growth inhibition and increased apoptosis. A schedule of docetaxel followed by GCP resulted in a highly synergistic response, while the combination appeared sub-additive if GCP was administered before docetaxel. The results suggest that GCP has significant potential in combination with the agents tested for the treatment of advanced prostate cancer.

In the second set of studies, a combination of GCP plus perifosine, an inhibitor of the protein kinase signal transduction intermediary Akt (involved in cell survival) was shown to decrease long-term cell growth and survival more effectively than either individual treatment in both androgen-dependent and –independent prostate cancer cell lines. Short-term assays showed the combination to be more effective than GCP or perifosine alone in growth arrest for a majority of cell lines, as well as increased inhibition of Akt activity and induction of the cell cycle regulator $p21^{CDKN1}$. In the androgen-dependent cell line LNCaP, the combination treatment caused nearly 100 percent inhibition of cell growth, with increased apoptosis. Pretreatment of LNCaP cells to reduce androgen receptor (AR) activity, followed by the GCP/perifosine combination, caused a further increase in apoptosis beyond that noted without knockdown of AR activity. The combination of GCP plus perifosine warrants clinical validation in prostate cancer patients. Clinical studies aimed at an evaluation of a complementary medicine approach using GCP in combination with chemotherapeutic agents seems to be warranted.

Mushroom Preparations in Combined Antitumor Treatment

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Based on our previous experience and on literature data, we determined the effects of extracts from authentic mushrooms found in the Republic of Croatia, on defined cultures of normal and tumor cells and then on experimental models of tumor growth *in vivo*. The project is supported by the Ministry of Sciences, Education, and Sport of the Republic of Croatia.

The intention of this project is:

- to isolate mushroom extract destroying tumor cells but not normal cells;
- to determine the composition of active component(s) in extracts by using modern analytical methods (NMR, spectrometry) to standardize the products,

objectively intending to obtain effective components and, further, to create the most successive extract combination with pronounced antitumor effects;
- to investigate the influence of mushroom preparations obtained on the growth of tumor transplanted in experimental mice, as the only treatment, or in combination with surgery, radiotherapy, and/or chemotherapy;
- to determine the dynamics of immunological parameters in treated tumor-bearing animals;
- to prepare the products, defined as described, for clinical evaluation.

Mushroom preparations were obtained by extraction in boiling water for 24 hours.

Until now, the effects of *Cantharellus cibarius, Ganoderma lucidum, Meripilus giganteus, Fomes fomentarius,* and *Pleurotus ostreatus* (which is the 1st contingent of cca 30 mushroom species to be tested) on tumor cells *in vitro* were investigated. We have shown that the incorporation of radioactive ^{3}H thymidine in tumor cells (mouse squamous cell carcinoma SCCVII, fibrosarcoma FsaR, and melanoma B16F1) was inhibited in the presence of particular mushroom preparation and the effect was dose-dependent.

The application of mushroom preparation to mice bearing the tumor (mentioned above) could significantly influence their survival.

Furthermore, in tumor-bearing mice, mushroom preparations used in this study increased the efficiency of chemotherapy and/or irradiation applied.

Originality of the approach is in selecting optimal extracts to prepare the products ready for clinical application.

Prebiotics from *Pleurotus ostreatus* and *P. eryngii*

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Polysaccharides isolated from mushrooms are frequently studied as imunomodulation agents. But their role in human health could be wider. Mushrooms as food were presented as a source of proteins, minerals, vitamins, and dietary fiber. Benefits of these compounds for human metabolism have been established; however, they may have other functions. Dietary fiber, in connection with other compounds, may also have a prebiotic function. We tested extracts from *Pleurotus ostreatus* and *P. eryngii* for their potential supporting function in selected probiotics.

Water and alkaline extracts of both Pleurotus strains were tested. The strains selected for tests were *Lactobacillus* ssp. (4 strains), *Bifidobacterium* ssp. (3 strains), and *Enterococcus faecium* (2 strains). The strains were chosen from our collection of probiotic strains.

Water extract was prepared by boiling smashed fruiting bodies of mushrooms in water for 6 h at a pH of 7. Alkaline extract was then prepared by extracting the solid residue from the first extraction. The extraction was performed in 1M NaOH with 0.05% $NaBH_4$ at 4°C for 4 hours. The extract was stored at 4°C and sterilized by autoclaving. The extract contained different amounts of β-glucans (9%–30% in dry matter), proteins (21%–34% in dry matter), and other compounds. Cultivation tests were performed in a medium based on MRS (oxoid) used as a standard for lactic acid bacteria cultivation. The medium was prepared without glucose and mixed with different portions of liquid extract and water (the final premix contains 50% of MRS medium). The medium was supplied with 0.05% cysteine. The growth rate was compared to the growth in medium mixed with water only. Our experiment has shown different biological activity of the tested extracts.

Strains show different growth rates not only with different extracts, but also with each other. Some *Lactobacillus* strains grew better with the water extract, some with the alkaline extract. *Lactobacillus* strain Lac A grew at the same rate as the control with water extract of *P. eryngii*, but the alkaline extract increased twice this rate. The three strains (Lac B, C, and D) used both extracts with similar fruitfulness.

Bifidobacterium strains show big differences. Strain Bifi A grew with *P. ostreatus* extracts better than with *P. eryngii* extracts. Strain Bifi B grew just with *P. eryngii* extract, but *P. ostreatus* was not able to stimulate growth. Strain Bifi C was slightly inhibited with higher concentrations of extracts.

The growth rate of *Enterococcus* strains (Ent A, B) was stimulated better with alkaline extracts. However, water extracts stimulated biomass concentration to higher values than alkaline extracts. Both strains had a positive reaction to all extracts. *Enterococcus faecium* strains are known as strains with good proteolytic activity. High content of proteins in extracts is evidence for good extract utilization.

SCFA (short chain fatty acid) production is an important characteristic of probiotic strains. Acid production was established as the difference between the pH of the fermented medium control (MRS with water) and the sample (MRS with extract). Naturally, *Lactobacillus* strains have shown the highest production. *Lactobacillus* produces lactic acid as a major product of their saccharide metabolism. Highest production was registered with strains Lac B and Lac C. Strains Lac D, Lac A, Bifi A, Bifi C, Ent A, and Ent B produced comparable amounts of SCFA. Strain Bifi B produced low amounts of SCFA; moreover, the production decreased with increased concentration of extract. The result corresponds with the growth rates of this strain. The described inhibition effect supports our deduction that extracts from *Pleurotus* can be used for symbiotic construction only with carefully selected probiotic strains.

Our results demonstrate the prebiotic ability of water and alkali extracts from *Pleurotus eryngii* and *P. ostreatus*.

Antioxidative Activities of *Pholiota adiposa* Strains and Detection of MnSOD in Their Extracts

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The activity of antioxidants of DPPH and SOD, determination of SOD and pattern of SOD activity by developmental stages in the *Pholiota adiposa* mushroom extracts were investigated. Superoxide radical scavenging activity was evaluated in fruiting body extracts of 16 strains (*Ph. adiposa*) using the NBT reduction method and ESR. The antioxidative activities measured by the NBT method and ESR were from 17% (ASI 24001) to 29.4% (ASI 24018) (LSD 0.05, 6.7%) and from 40.6% (ASI 24001) to 60.1% (ASI 24002), respectively. Further assessment of antioxidant activities in fruiting body extracts using the DPPH method ranged from 3.5% to 8.8%, suggesting that the ASI 24013 (*Ph. adiposa*) extract has the highest radical scavenging activity. All strains tested showed only MnSOD activities, as no band disappeared when the gel was incubated in the presence of cyanide and hydrogen peroxide. During the fruiting process of *Ph. adiposa* species, SOD activities were fluctuated in various stages of fruiting body development. The activity of SOD was relatively high in primodia compared to the other stages, but gradually decreases with the growth of the fruiting body. The lowest level of SOD activity was observed at the mature fruiting body stage. After the fruiting stage, the level of SOD activity of the ripening stage was increased again, up to that of the young fruiting body stage.

Influence of Structural Features of Mushroom Glucans on IFN-γ Synthesis *in Vitro*

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Numerous polysaccharides (PS) or polysaccharide-protein complexes derived from Basidiomycetes's cell walls show immunomodulatory activities in animals and humans. They have the ability to enhance or suppress both innate and acquired immune responses and, as a result, act against infection and growth of tumor cells in the host. These bio-active substances bind to various host cell receptors among which Dectin-1, TLR-2, and TLR-4 are the most well-known. PS have shown a significant influence on maturation, differentiation, and proliferation of immune cells in the host.

Bioactive mushroom polysaccharides differ greatly in their chemical composition and configuration. Most mushroom PS are β(1,3-1,6) glucans, some are hetero-PS. Some are bound to protein or peptide residues and form proteoglucans. Immunomodulatory activities of these compounds depend on their chemical composition, molecular weight, highly ordered structure resp. degree of branching and tertiary structure of a triple-stranded PS helix. Structural features as β(1-3)-linkages in the backbone of the glucan and an additional β(1-6)-branch are believed to be important for receptor binding.

In this study we tried to determine which structural features of PS are of primary importance for their *in vitro* immunostimulatory properties.

Water-soluble polysaccharides prepared from *Ganoderma lucidum* spores, *Agaricus brasiliensis,* and *Phellinus linteus* fruiting bodies, were incubated in 1M NaOH and 1M H_2SO_4, respectively, and tested for stimulation of activated PBMCs and production of IFN-γ by sandwich ELISA. Changes of molecular weight (size) were observed by exlusion chromatography using Sephacryl S 200.

ELISA measurements of IFN-γ titer obtained after stimulation of PBMC's with native and 1M NaOH treated PS indicated that the primary structure of glucans is of more importance then the tertiary structure of the triple helix for their immunostimulating activity and synthesis of IFN-γ. Titers of IFN-γ measured after stimulation of cells with acid-hydrolyzed fractions confirmed that glucans of lower molecular weight are as effective as non-hydrolyzed glucans. *Phellinus linteus* glucans treated with 1M H_2SO_4 lost their characteristic suppressive effect on IFN-γ released by the stimulated cells. This indicated that the immunostimulatory activity of glucans is due to size and primary structure.

We then tested the effect of incubation with Onuzuka R-10, a 1,6 β-glucanase (Yakult Honsha Co Ltd) on the activity of the mushroom-derived polysaccharides. For *Agaricus brasiliensis* the resulting β(1-3)-glucan fragments of high molecular weight (MW>80 kDa) and the small fragments (MW<80 kDa) that were left after cellulase degradation showed strong, enhanced immunostimulatory activity compared to the native glucans. Glucans from *Ganoderma lucidum* and *Phellinus linteus* also showed an increase of their immunostimulatory activity and production of IFN-γ after exposure to cellulase. *Ph. linteus* digested glucans kept their immunosuppressive effect on PBMCs. This again shows that the immunopotentiating activity of these compounds depends on both their composition and structure.

Our results suggest that the 1,6 binding of glucose monomers has probably no importance for the immunostimulatory activity and synthesis of IFN-γ by human PBMC's *in vitro*. We conclude that size, primary structure, and 1,3 bonds are the primary determinants of immunomodulatory effects of mushroom polysaccharides. The influence of the proteins or peptides present in the polysaccharide complex have not been studied; they may, however, play a more important role than the glucans.

Further elucidation of the structural aspects of mushroom polysaccharide complexes remains of the utmost importance for understanding their biological role.

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Prevention of Mammary Adenocarcinoma and Skin Papilloma Induced by 7,12-dimethyl benz(*a*)anthracene by *Ganoderma lucidum* Occurring in South India

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Natural products have been of exceptional value in drug discovery programs for cancer therapy and prevention. Mushrooms represent a major and as yet largely untapped source of potent new pharmaceutical products. *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. is a widely used herbal drug in oriental traditional medicine for the treatment of a large number of diseases. We investigated the anticarcinogenic activity of the methanolic extract of *G. lucidum*, occurring in South India, against 7,12-dimethyl benz(*a*)anthracene (DMBA)-induced mammary tumor and skin papilloma.

Female Sprague Dawley rats, 40–50 days old were used in the experiments for mammary tumor induction. DMBA (10 mg/animal) in olive oil was given once a week for 3 weeks. The mushroom extract (500 mg or 1000 mg/kg body wt.) was administered once daily for 3 weeks orally and there after 10 mg DMBA was administered once a week for 3 weeks. Female Balb/c mice (20–25 g) were used for the induction of skin papilloma. The skin papilloma was initiated by single application of 390 nmol DMBA in 200 µl acetone on the backs of mice after removing the hair by surgical clippers. One week after initiation, 200 ml freshly isolated croton oil was applied (10% in acetone), twice weekly for 8 weeks on the area where DMBA was applied, for promotion. The mushroom extract (2 mg or 10 mg in acetone/ mouse) was applied topically 40 minutes before each croton oil application.

Ganoderma lucidum extract at doses of 500 and 1000 mg/kg body wt. inhibited 51.4% to 73.8% tumor volume and 53.8% and 74.7% tumor weight of mammary adenocarcinoma, respectively. The extract also showed a profound effect on tumor development and tumor latency period. The extract significantly reduced the incidence of DMBA induced skin papilloma. A 50% reduction in tumor incidence was observed in animals treated with 10 mg extract. The tumor latency period was also markedly prolonged by treatment with the extract.

The results indicated that *G. lucidum* extract showed significant activity against DMBA induced mammary adenocarcinoma and skin papilloma in a dose-dependent manner. The mushroom extract showed profound preventive effect against both DMBA initiated mammary tumor and skin papilloma promoted by croton oil as evident from the induction of numerous tumors, tumor latency period, and tumor proliferation. The experimental results indicate that *G. lucidum* extract possessed significant protective and antipromotional activity against DMBA induced mammary and skin papilloma. The findings suggest the potential therapeutic use of *G. lucidum* in cancer chemoprevention.

Anti-Androgenic Effect of *Ganoderma lucidum*

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For thousands of years, mushrooms have been known as a source of medicine. In East Asia, the fruiting body of the fungus *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst. (Ganodermataceae) has been used for centuries in folk medicine to treat various human diseases such as cancer, hypertension, hepatitis, ne-

phritis, and so on. In this paper, we investigated the anti-androgenic effect of *G. lucidum*.

In our previous screening of 19 edible and medicinal mushrooms, the methanol extract of the fruiting body of *G. lucidum* showed the strongest 5α-reductase inhibitory activity. In addition, the treatment of *G. lucidum* significantly inhibited the growth of the ventral prostate induced by testosterone in the rat. These results led to further investigation of the ethanol extract of *G. lucidum*.

The inhibition of 5α-reductase prepared from rat liver by the ethanol extract of *G. lucidum* was concentration-dependent. As the concentrations of the ethanol extract increased, the residual enzyme activity rapidly decreased. The inhibitory concentration leading to 50% activity loss (IC_{50}) was estimated to be 93.6 μg/mL for the ethanol extract of *G. lucidum*.

The AR binding activity was also investigated. The binding active concentration leading to 50% fluorescence polarization loss (IC_{50}) was estimated to be 5.6 μg/ml for the ethanol extract of *G. lucidum*.

The LNCaP cell line becomes an attractive model for the *in vitro* studies on the biology of human prostate cancer. The effects of *G. lucidum* on androgen stimulated growth of LNCaP were examined. LNCaP cells were incubated with varying concentrations of the ethanol extracts of *G. lucidum* with or without testosterone or DHT, for 3 days. The treatment of the ethanol extracts of *G. lucidum* in the presence of testesterone resulted in a dose-dependent inhibition of LNCaP cell growth to a degree lower than that in no androgen stimulated controls at the concentrations of 100 μg/mL. The dose-dependent inhibition of the ethanol extracts of *G.lucidum* on LNCaP cell growth was also observed in the presence of DHT.

The results of the inhibitory effect on the prostate cancer cell suggested that the ethanol extracts of *G. lucidum* inhibited prostatic growth by not only the inhibition of 5α-reductase activity but also by a direct effect on the androgen receptor.

We isolated 13 compounds from the ethanol extracts of *G. lucidum,* as follows: ganoderic acid TR (1), ganoderic acid DM (2), ganoderic acid A (3), ganoderic acid B (4), ganoderic acid C2 (5), ganoderic acid D (6), ganoderic acid I (7), 5α-lanosta-7,9(11),24-triene-15α, 26-dihydroxy-3-one (8), ganoderol B (9), ganodermatriol (10), ganodermanontriol (11), ganoderiol A (12), and ganoderiol F (13). Compounds 1, 2, and 8 were better inhibitors of 5α-reductase, and compounds 2, 8, and 9 were strongly bound to the androgen receptor.

Prostatic enlargement is dependent on tissue androgen, namely, DHT, which is converted from testosterone by steroid 5α-reductase. In this paper, the effects of *G. lucidum* and the active compounds on prostate cancer cell and on the testosterone-induced growth of the prostate in castrated rats were tested. These results suggest that the suppression effect of prostatic growth by *G. lucidum* might come in part from its ability to inhibit 5α-reductase and bind to the androgen receptor. The clarification of the anti-androgenic effect mechanism is in progress.

Mushroom Substances as Therapeutics for Hormone-Refractory Prostate Cancer

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Prostate cancer (PCa) remains the most common non-cutaneous malignancy in the Western World. PCa tends to be a disease of older men, with more than 70% of the diagnoses amongst men over 65 years of age. Androgens play an important role in controlling the growth of the normal prostate gland, and in promoting benign prostatic hyperplasia (BPH) and PCa. Androgen action is mediated through the androgen receptor (AR). The AR is a member of the nuclear receptor superfamily of intracellular ligand-dependent transcription factors. The model of AR activation includes several sequential steps. The AR

is known to exist as a monomer in the cytoplasm of responsive cells, sequestered in a complex with heat-shock proteins (HSPs). On administration and binding of androgens, the AR changes conformation, and the HSPs are released followed by a second conformational change. The receptor dimerizes, translocates to the nucleus, binds to DNA, and activates the transcription of AR-responsive genes.

Androgen ablation therapy has been shown to produce the most beneficial responses in patients with hormone-responsive PCa. Anti-androgens compete with androgens for the AR-binding site preventing the androgen from binding to the AR, thereby inhibiting the transcriptional activation of androgen-responsive genes. Despite the initial response to anti-androgen therapy, the tumors recur in an androgen-independent form that is unresponsive to additional androgen withdrawal and chemotherapy. Potential mechanisms for the progression of prostate cancer to the hormone-refractory stage include pathways involving the AR, and pathways that bypass it.

The age-standardized incidence rate of PCa is highest in the USA, lower in European countries, and lowest in Asia, arguing for environmental and/or ethnical factors that affect prostate cancer tumorigenicity. Indeed, recent studies based on epidemiological data suggest that environmental factors, such as diet, can significantly influence the prevention or progression of PCa, hence the difference between Western and Southeastern statistics. Of special interest to us, is the growing body of research on anti-cancer properties of certain natural products isolated from mushrooms, especially in Southeastern countries.

Natural products and their derivatives have historically been invaluable as a source of therapeutic agents. The immense pool of natural products inherently contains large-scale structural diversity in comparison to synthetic compounds. For example, over the period 1981–2002, 62% of the new chemical entities in cancer therapeutics were from natural origins. Moreover, a significant number of natural product-derived drugs were ranked in the top 35 worldwide selling ethical drugs in 2000–2002.

Of about 650 mushroom species with known medicinal properties, only about 20 species are in use at present. Only from one species, *Ganoderma lucidum*, more than 130 pharmacological active triterpenoids have been isolated, arguing for the diversity and significance of mushrooms as a source for potential new pharmaceuticals. Our hypothesis is that by screening a diverse pool of natural products, using mechanism-based approaches to select the desired activity, we will be able to identify a novel chemical entity (NCE) with novel chemical structure. Isolated chemical structures are expected not to be optimized in terms of potency, selectivity, bioavailability, and their pharmakinetic and pharmadynamic properties. However, they may serve as a novel "hit" (new chemophore) that will be subjected to chemical modification and optimization.

We utilized our unique collection of medicinal and edible mushrooms to screen them for their ability to interfere with the AR function. We utilized the MMTV-luciferase reporter assay to follow the effect of fungal extracts on AR transactivation function by monitoring luciferase reporter activity. We screened a total of 201 diethyl ether, ethanol, and ethyl acetate fungal Basidiomycetes extracts for anti-androgenic activity. Out of 201 ethanol, ethyl acetate, and diethyl ether extracts screened - 11 (5.5%) exhibited anti-androgenic activity inhibiting AR-mediated reporter activity by more than 40% at a concentration of 25 μg/ml. In addition, we utilized three prostate cancer cell lines; LNCaP as an androgen-dependent cell line, PC-3, and DU 145; as androgen-independent cell lines to study the effect of selected fungal extracts on cells proliferation and viability.

Based on our screened results, in combination with the selective inhibition of prostate cancer LNCaP cells, we selected *Coprinus comatus* and *Ganoderma lucidum* for further evaluation. We demonstrated that ethanol and ethyl acetate extracts from *C. comatus* and *G. lucidum,* respectively, selectively inhibit dihydrotestosterone-induced LNCaP cell viability, suppress levels of secreted prostate-specific antigen in a dose-dependent manner, and cause a G1 phase arrest in LNCaP, but not in DU 145 and PC-3 cells. For the first time, to the best of our knowledge, we demonstrated that *C. comatus* and *G. lucidum* decreased androgen and glucocorticoide receptors transcriptional activity in breast cancer MDA-kb2 cells in a dose-dependent manner, and suppressed androgen receptor protein level in LNCaP and MDA-kb2 cells. In addition, active fractions that interfere with AR functions and consequently inhibit LNCaP cell proliferation and variability were isolated from *C. comatus* and *G. lucidum*. Our findings suggest that androgen receptor and non-androgen receptor mediated mechanisms mediate the effects of *C. comatus* and *G. lucidum.*

Effect of *Agaricus brasiliensis* Extracts on Wound Healing in Rats

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Wound healing is a fundamental biological process that allows the maintaining of tissue structural and functional integrity. Complex and powerful mechanisms are engaged in performing this important task. The wound healing process can be divided into several overlapping phases including hemostasis and inflammation, proliferation, and remodeling. The inflammatory phase is initiated by the infiltration of neutrophils, followed by monocytes and their differentiation into mature tissue macrophages. The proliferative phase of wound repair encompasses cellular proliferation, extracellular matrix production and tissue regeneration, and is characterized by fibroplasia and angiogenesis. The final phase of remodeling/resolution is the longest and results in regain of tissue homeostasis.

The effect of extracts from *Agaricus brasisliensis* S. Wasser et al. (=*A. blazei* sensu Heinem.) on the wound healing process in rats was examined by monitoring the dynamics of wound closure in rats.

Full-thickness 1 cm^2 excisional skin wounds were made on the backs of Albino Oxford (AO) rats, and *A. brasiliensis* products obtained by hot water/ethanol extraction (composed of proteoglucan complex) were administered in three different regimes of application. Proteoglucans were injected intraperitoneally (*i.p. application*) at a dose of 20 mg/kg body mass for 7 days (1) concomitantly with the wounding, (2) 3 days before wounding, and (3) 6 days before wounding. In separate experiments, a solution of proteoglucans was applied topically (*open application*) in doses of 2 mg/wound and 0.4 mg/wound for 7 days. The effect was evaluated by 7-day monitoring, when wound closure was nearly completed.

Concomitant *i.p*. and wounding as well as *i.p*. application 6 days before wounding exerted no effect on wound healing. When 3-day *i.p*. application preceded wounding, significantly faster wound closure was noted in the initial phase of the healing process. Topical application of a higher dose of *A. brasiliensis* extract resulted in significant initial reduction of wound surface, while the lower dose was without effect. Initial histologic evaluation of respective early (7-days old) granulation tissue of proteoglucan-treated wounds showed thin collagen fibers, which tend to be horizontal and parallel compared to more homogenized, short, and irregular collagen fibers in control wounds.

The data demonstrate the effect of *A. brasiliensis* extract on the early (inflammatory) phase of wound healing. As this phase of wound healing is critical for the later course/quality of wound healing, experiments are under way to supply more data on the underlying mechanisms of the observed effects.

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Agaricus brasiliensis Possesses the Ability to Stimulate the Differentiation of Naive T Cells into T-Helper Type 1

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Allergic diseases, such as asthma, allergic rhinitis, atopic dermatitis, and food allergies are steadily increasing especially in industrialized countries. Key factors driving these rising trends include an increased exposure to sensitizing allergens and a reduced stimulation of the immune system during critical periods of development. Allergic responses involving IgE-dependent mast cell degranulation and eosinophil accumulation in the sites of inflammation are considered to be related to the development and activation of Th2 cells.

The incidence of atopic dermatitis (AD) is steadily increasing in industrialized countries, and population studies suggest that 10%–20% of the population is affected by AD during childhood. A number of studies suggest a key role of Th1/Th2 balance in the generation of AD. Interest in the medicinal characters of natural products has increased due to their popular use in traditional medicine. Many available substances have been found in the foods, particularly in mushrooms. *Agaricus brasiliensis* S. Wasser et al. (ABSW) (=*A. blazei* sensu Heinem.) is well-known among these mushrooms. It was previously reported that within 12 h, there were no drastic changes in mRNA expression in IL-18 (a pro-inflammatory cytokine enhancing the Th1 immune response) during stimulation with a polysaccharide purified from ABSW. However, mRNA expression in IL-18 rapidly increased up to 5.6-fold 24 h post treatment. The levels of IL-12 p40 (a key role in Th1 differentiation) mRNA expression were different from those of IL-18, which started to show an increase only after 12 h and within 24 h post treatment. Moreover, polysaccharides from ABSW changed the percentage of splenic L3T4 (CD4)-positive cells in the T cell subsets in ABSW-treated mice. These results lead to the presumption that this mushroom possesses an ability to stimulate the differentiation of naive T cells into T-helper type1 (Th1), resulting in an antiallergic activity.

The present study examined the inhibitory effects of ABSW extract on AD model using NC/Nga mice sensitized and challenged with picryl chloride. Oral administration of ABSW extract suppressed the development of AD-like skin lesions to 5 ± 0.5 compared to 6.6 ± 0.7 in control. Moreover, ASBW treatment downregulated serum IgE levels and upregulated serum interferon (IFN)-γ levels in NC/Nga mice. When spleen cells from NC/Nga mice were directly stimulated with ABSW extract (2 μg/ml) together with T-cell mitogen, concanavalin A, IFN-γ production increased to 894.6 ± 49.8 pg/ml and interleukin (IL)-4 production decreased to 103.8 ± 1.7 pg/ml compared to 639 ± 14.7 pg/ml and 127.7 ± 4.3 pg/ml in the control, respectively. Likewise, when spleen cells were stimulated with ABSW together with both anti-CD3ε and CD28 antibody, IFN-γ production increased to 702.7 ± 41.7 pg/ml and decreased IL-4 production to 106.2 ± 1.7 pg/ml compared to 531.6 ± 37.2 pg/ml and 125.9 ± 3.2 pg/ml in the control, respectively. Further, ABSW extract activated macrophage cell lines, RAW264.7 cells on the basolateral side, co-cultured with intestinal epithelial cell line, Caco-2 cells, in apical side to produce approximately 2-fold TNF-α contents compared with the control (309.9 ± 27.4 pg/ml).

These results suggested that oral administration of ABSW extract upregulated IFN-γ production which differentiated naive T cells into Th1-type cells via activation of macrophages through intestinal epithelial cells, resulting into downregulated serum IgE levels through regulation of Th1/Th2 balance.

Bioprospecting for Naturally Occurring Antimicrobial Agents in Mushrooms

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The curative properties of mushrooms are known in India since time immemorial. Mushrooms are emerging as a source of novel antimicrobial natural products. *Ganoderma lucidum, Lentinus edodes*, *Trametes versicolor, Schizophyllum commune, Pleurotus ostreatus, Agaricus bisporus, Auricularia auricula,* and *Grifola frondosa* are known to possess antimicrobial activities. So far, the antimicrobial potential of extracts of several types of medicinal mushrooms belonging to Basidiomycetes have not yet been screened for their antimicrobial potential and thus deserves investigation in this field. The antimicrobial agents from naturally occurring mushrooms would be very useful against opportunistic infections the world, e.g., AIDS and cancer.

Mushroom products and derivatives obtained through biotechnological processes from fruiting bodies, mycelia, and culture media have shown activity against *Mycobacterium tuberculosis* bacilli resistant to antituberculosis drugs, *Bacillus subtilis, Staphylococcus aureus, Micrococcus lenteus, Candida albicans,* and *Saccharomyces cerevisiae.* Drug resistance of microbes is also increasing very fast, and, therefore, there is a pressing need to search for potential antimicrobial agents of natural origin, e.g., plants and mushrooms.

Antimicrobial drugs have long been used for prophylactic and therapeutic purposes. Unfortunately, the recent increase in the occurrences of drug-resistant bacterial strains is creating serious treatment problems. Recently, there is growing scientific evidence indicating that natural products from biota contain biocompounds and micronutrients, which boost the body's immune system. Consequently, the antimicrobial activities of various antitumor polysaccharides from medicinal mushrooms are being re-evaluated in terms of their clinical efficacy. Such compounds would be expected to function by mobilizing the body's humoral immunity to ward off microbial infections resistant to current antibiotics. Sulfated Schizophyllan polysaccharide is reported to displayed strong activity against *Staphylococcus* sp. infection.

Researchers have shown that a water extract of *Lentinus edodes* demonstrated growth-enhancing effects on colon-inhabitating beneficial lactic acid bacteria, *Lactobacillus brevis* and *Bifidobacteria breve*. The affective factor in the extract is considered to be the disaccharide sugar, trehalose. The authors suggest that the *L. edodes* extracts can improve the beneficial intestinal flora of the gut and reduce the harmful effects of certain bacterial enzymes such as β-glucosidase, β-glucuronidase, and tryptophanase as well as reducing colon cancer formation.

In countries like China, Japan, and Western countries medicinal mushrooms are used as various therapeutical agents. However, in India work on antimicrobial agents from mushrooms is in its infancy. Amravati, in general, and Melghat forest, in particular, are blessed with mushroom biota. The main mushroom species include: edible and medicinal species of *Ganoderma lucidum, G. applanatum, Schizophyllum commune, Agaricus bisporus, Lycoperdon pusilum, Boletus edulis, Cantharellus cibarius, Calvatia gigantea, Pleurotus sajor-caju, P. ostreatus, Termitomyces heimii, T. microcarpa, Trametes versicolor, Volvariella volvaceae, Morchella conica, Geaster* sp., *Amanita* sp., etc. These species are known to possess antimicrobial activities. The research work on antimicrobial activity of mushrooms is in progress.

Prevention Effect of Bone Loss in Calcium Deficient Ovariectomized Rats by the Ethanol Extract of *Ganoderma lucidum*

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Menopause brings about changes in the health of postmenopausal women that have a major impact on their lives. After menopause, osteoporosis associated with estrogen deficiency is the most common cause of age-related bone loss. Hormone replacement therapy (HRT) can resolve most postmenopausal problems, and the almost universal loss of skeletal mass in postmenopausal women can be prevented by estrogen replacement. However, compliance with HRT is poor because of the risks of breast and endometrial cancers associated with the long-term use of HRT.

Thus, a safer estrogenic-ingredient originating from edible and medicinal natural products is needed. Estrogens play an important role in bone maintenance, in the central nervous system, and in the cardiovascular system. Estrogens carry out their action by binding to a high-affinity nuclear receptor, the estrogen receptor (ER). Bound ER undergoes conformational change, interacts with chromatin, and modulates the transcription of target genes in estrogen-responsive tissues. Many naturally occurring compounds such as flavonoids, coumestan derivatives, and lignans are nonsteroidal agents that have demonstrated estrogenic activity.

The fruiting body of *Ganoderma lucidum* (Reishi or Ling-Zhi) is a well-known Chinese crude drug that has been used clinically in China, Japan, and Korea for a long time as a tonic and sedative, as well as for the treatment of hypertension and chronic hepatitis. This led us to further investigate the medicinal properties of *G. lucidum*, especially its estrogenic activity.

The present study was designed to determine whether the extract of *G. lucidum* has estrogen-like activity and a preventative effect on bone loss in calcium deficient ovariectomized rats. Our findings indicated that the ethanol extract of *G. lucidum* does, in fact, protect against the bone loss caused by estrogen deficiency. The beneficial effect of *G. lucidum* may be mediated, at least in part, by its estrogenic activity.

The effect of the extract of G. lucidum on the proliferation of MCF-7 cells. The MCF-7 cell is a well-established *in vitro* system characterized by its estrogen responsiveness through the expression of the estrogen receptor. E2 exhibited a strong proliferation-stimulating activity of about 145% in comparison with the control cells. The ethanol extract of *G. lucidum* exerted a proliferation-stimulation activity of more than 200% at 0.1 μg/ml. However, at a high concentration of more than 10 μg/ml, the ethanol extract lost the activity. The exact mechanism involved in the dose-dependent change in activity remains unclear. However, its phenomena might be caused by its cytotoxicity, at least in part. Additionally, a recent report indicated that the *G. lucidum* extract can induce cell cycle arrest and apoptosis in MCF-7. The concentration of the ethanol extracts for the maximal stimulation effect was 0.1 μg/ml. In contrast, the water extract, which was prepared from *G. lucidum* after extraction with ethanol, did not show any activity.

The effect of G. lucidum on the body and uterine weight of ovariectomized rats. Rat ovariectomies have been regarded as a model for studying postmenopausal osteoporosis. All animals gained weight during the course of the experiment, yielding increases of 63.9% (Ovx), 45.4% (Ovx + E2), 62.6% (Ovx + GH), and 54.2% (Ovx + GL) in the initial body weight. The uterine weight was markedly increased in the E2 treatment compared to the Ovx group. However, the Ovx + GH and Ovx + GL groups showed a slightly higher uterine weight than the Ovx group, respectively, which was far lower than that of the Ovx + E2 group.

The effect of *G. lucidum* on serum osteocalcin. Compared with the Ovx controls, the treatment of Ovx rats with the ethanol extracts of *G. lucidum* resulted in a 75.9% ($P<0.05$) and 79.3% ($P<0.05$) reduction in serum osteocalcin in the GH and GL groups, respectively. Also, E2 treatment showed a 61.6% reduction in serum osteocalcin.

The effect of G. lucidum on the bone density of the ovariectomized rat. By the pQCT analysis of the right femur, significant protection of the trabecular

bone density was observed in the Ovx rats treated with E2 and the ethanol extract of *G. lucidum* at all doses. The trabecular bone density was higher by 34.6% in the E2-treated group, 21.0% in the GH-treated group, and 38.8% in the GL-treated group compared with the Ovx controls.

In summary, our results suggest that the ethanol extracts of *G. lucidum* have a bone-protecting effect, without exhibiting a substantial effect on the uterus in Ovx rats. The beneficial effects of the ethanol extract of *G. lucidum* on bone may be mediated, at least partly, by the estrogen-like activity. The intake of *G. lucidum* may be useful in preventing the bone loss caused by estrogen deficiency.

Anti-Tumor Activities of Medicinal Mushrooms

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Medicinal mushrooms have attracted extensive attention in health promotion and are used as supplements for disease treatment. They may serve as food supplements and as drugs, as an alternative means for disease treatment. This is particularly evident in the area of cancer and chronic disease treatment. The development of medicinal mushroom-based drugs is still one of the major routes leading to therapeutics for various diseases. Well known medicinal mushrooms include *Ganoderma lucidum* and *Trametes versicolor*. *Ganoderma lucidum* is a favorite remedy in Oriental medicine for centuries.

To investigate how medicinal mushrooms function, we have recently developed different cancer cell models to study the effects of *G. lucidum* and demonstrated that the extract of this species inhibits tumor cell adhesion (Wu et al., Enzyme Microbial Technol, 2006, 40, 32-41) and induces tumor cell death (Xie et al., Enzyme Microbial Technol, 2006, 40, 177-185). We also demonstrated that preparations of the lipid-soluble fraction of *G. lucidum* induced tumor cell death (La Pierre et al., Cancer Res, 2007, in press). Using these models, we report here the roles of another medicinal mushroom, *Trametes versicolor,* in affecting tumor cell adhesion and cell growth, and compared the effects of *Ganoderma lucidum* and *Trametes versicolor* on mediating tumor cell activities. *Trametes versicolor* is widely prescribed for prophylaxis and treatment of cancers. Pre-clinical and clinical trials have demonstrated that the water extracts of *T. versicolor* display a wide range of biological activities. Double-blind random clinical trials have shown significant improvement in different types of symptoms of cancer patients since *T. versicolor* has protective effects against tumors, and it reduces side effects of chemo- and radiotherapies. We hypothesize that the polysaccharides and some small molecules of *T. versicolor* may be able to reduce tumor cell adhesion and induce tumor cell death. As well, *Ganoderma lucidum* also contains some small molecules with similar functions. The objective of this study is to isolate and analyze the bioactivities of the small molecules in *G. lucidum* and *T. versicolor*. We have extracted the water-soluble fraction of *T. versicolor*, which was subjected to further fractionations with different molecular weight. We examined whether different fractions affected cancer cell adhesion, proliferation, and cell death. Malignant human breast carcinoma cells, maintained as monolayer cultures on tissue culture plates, and human lymphoma, maintained as a suspension culture that could partially adhere to the tissue culture plates, were used in our studies. We found that while the total extract of *T. versicolor* inhibited tumor cell adhesion and cell growth, the fractions with the smallest molecules (<20 kDa) exhibited the highest activity, and the fraction with the largest molecules (>50 kDa) had lowest activity.

Our results suggested that some small molecules in the *T. versicolor* preparation were important in reducing tumor cell adhesion and proliferation. To

test whether the small molecules in the preparation of *G. lucidum* were also important, the total extract of *G. lucidum* was subjected to dialysis to remove small molecules. We found that removal of small molecules reduced the effectiveness of *G. lucidum* extract on reducing tumor cell adhesion and proliferation. Furthermore, when the extract was passed through Sephadex columns, its biological activities decreased greatly. These results further confirmed that small molecules are important in maintaining the biological activities of *G. lucidum* on tumor cell adhesion and proliferation. To corroborate our results, we have purified a number of known small molecules using HPLC, and tested the effect of Ganoderic acid C on the inhibition of tumor cell growth. To our surprise, relatively high concentrations of Ganoderic acid C were needed in order to achieve clear evidence of the molecule in inhibiting tumor cell growth.

This result suggests that a mixture of the small molecules may be essential to retain sufficient biological activities. Our results shed some light on the development of better quality of *G. lucidum* and *T. versicolor* products for the therapeutic purpose of treating cancers.

The Potential Use of Limulus G Test Assay for the Evaluation of Immunomodulatory Activity of *Ganoderma* Polysaccharides

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Polysaccharides are believed to be the key ingredient contributing to *Ganoderma* mushrooms immune-enhancement actions. Therefore, the total polysaccharide content is popularly used as a crucial index to monitor the quality of *Ganoderma* products. However, there are more than two hundred identified *Ganoderma* polysaccharides, and not all of them bearing activities. The immunomodulatory activity of *Ganoderma* is revealed by its active polysaccharide content but not in its general polysaccharide content. Unfortunately, up to now, there is no convincing method available for the specific detection of bioactive polysaccharide quantities.

Generally, beta-1,3-glucans are thought to be the core structure in numerous polysaccharides with immunomodulatory actions. This type of polysaccharide also appears in the extracts from *Ganoderma* fruiting bodies, mycelia, and spores and can be quantified by using Limulus G test assay. Therefore, we hypothesize that the content of beta-1,3-glucan in *Ganoderma* polysaccharides might relate to their immunomodulatory activity and consequently the *Ganoderma* polysaccharide immunomodulatory activity can be evaluated by using Limulus G test assay.

Since the macrophages play an important role at the initial stage of immune defense as well as in the immune net reaction, we first analyzed the correlation between beta-1,3-glucan contents and the macrophage-stimulation activities. Polysaccharide samples, which were extracted from fourteen strains belonging to *Ganoderma lucidum, G. oerstedii, G. resinaceum, G. subamboinense,* and *G. resinaceum* were studied. Limulus G test assay and phenol-sulfuric acid method were used to detect the beta-1,3-glucan contents and total polysaccharide contents, respectively. Phagocytic activity and nitric oxide (NO) production by mouse macrophage cell line Raw 264.7 were used as two key indexes for macrophage activation after stimulation with various polysaccharide extracts and assayed by using neutral red assay and Griess reaction, respectively.

The results showed that the total polysaccharide contents varied from 34.30% to 62.23% in all tested samples, and the beta-1,3-glucan contents in polysaccharides varied from 0.004% to 5.020%. The glucan contents had a greater variance compared to polysaccharide contents, and they were not correlated to each other. All fourteen samples stimulated macrophage activation and consequently increased the phagocytic activity and NO production. Linear correlation analysis revealed that the phagocytic activity and NO production were generally in proportion to the beta-1,3-glucan contents. The correlation coefficients (R) are

0.606 and 0.537, respectively, and are all statistically significant ($p < 0.01$, $p < 0.001$). Conversely, such a correlation was not found between total polysaccharide contents and macrophage phagocytic activity and NO production (R = 0.033 and 0.001, $p > 0.05$).

Our data demonstrate that the beta-1,3-glucan content detected by using Limulus G test is a better index than total polysaccharide contents and can be potentially used for evaluation of the quality of *Ganoderma* mushroom products.

Antitubercular Activity of Metabolites of Higher Basidiomycetes

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Tuberculosis is a serious problem in many parts of the world. According to recent data it affects about one-third of the world population and is responsible for 2 million deaths per year. Very little is known about antitubercular activity of higher Basidiomycetes. Here we report our recent work on *Cerena unicolor,* a polypore with significant antimycobacterial activity. To the best of our knowledge the chemical composition of *C. unicolor* has not been studied before.

For our studies the fruiting bodies of *C. unicolor* were collected in fall of 2005 from Southern Ontario in Canada, Grenada in Mississippi, and from Norway. Fresh fruiting bodies were extracted exhaustively with 95% ethanol. This extract has been subjected to bioassay-guided fractionation using flash column chromatography with a gradient of hexane/ethyl acetate for elution. All fractions and pure compounds isolated were subjected to *M. tuberculosis* assays at the laboratories of the Institute for Tuberculosis Research of the University of Illinois at Chicago.

The crude extract of *C. unicolor* was active against *M. tuberculosis* strain H37Ra with IC_{50} and MIC values of 39.6 and 5.7 μg/mL, respectively. In a toxicity test using Vero cells, crude extract of *C. unicolor* gave a lower TC_{50} value (39.6 μg/mL) than the control drug rifampicin (TC_{50} 97.8 μg/mL). Bioassay-guided fractionation of crude ethanolic extract of *C. unicolor* resulted in obtaining several fractions with anti-TB activity from which the active component has been identified as ergosterol-5α, 8α-peroxide (**1**) with a MIC value of 1.0 μg/mL. The structure of ergosterol-5α, 8α-peroxide (**1**) was confirmed by NMR and X-ray crystallography. To study the structure-activity relationship, semi-synthesis of six derivatives (**2–7**) of ergosterol peroxide (**1**) were designed and compounds obtained tested against *M. tuberculosis* (Fig. 1). The results obtained by us and other researchers have shown the crucial importance of the presence of the 3β-hydroxyl group and the lipophilic side chain at carbon atom C-17.

This work is the first report of anti-TB activity of *C. unicolor* and its relation to the presence of ergosterol peroxide (**1**). In order to understand better the structural requirements for anti-TB activity in this system we designed the synthesis of several ergosterol peroxide derivatives. Six analogs (**2–7**) with various changes in the functional groups of ergosterol peroxide molecule were prepared (Fig. 1). Results of the test against *M. tuberculosis* have shown reduced potency as compared to parent molecule (**1**). It is important, however, to notice that according to our and other researchers' studies the presence of 3β-hydroxyl group and the side chain at C-17 are of crucial importance in retaining activity. Lipophilicity of the side chain plays an important role in the anti-TB activity. The presence of an endoperoxide moiety in the molecule is not crucial for activity, but its reduction in ergosterol peroxide significantly diminished antitubercular activity. Further studies to understand the mechanism of interaction of ergosterol peroxide with *M. tuberculosis* are needed.

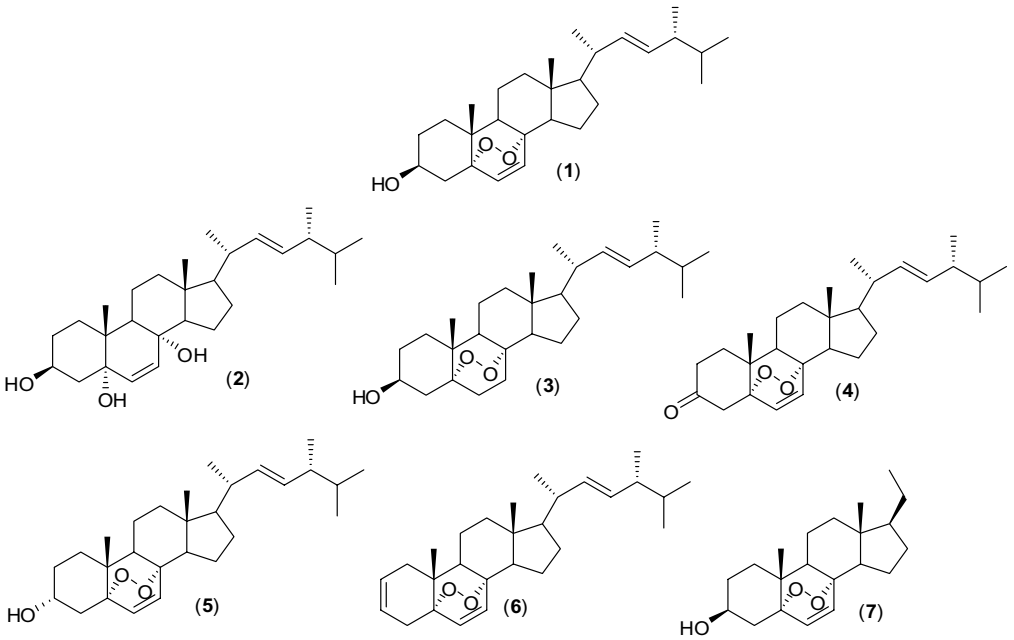

FIGURE 1. Ergosterol peroxide and ergosterol peroxide derivatives of *Cerena unicolor.*