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The Use of Mushrooms in the Development of Functional Foods, Drugs, and Nutraceuticals

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5.1 Introduction

It is so determined by Nature that right from the beginning everyone's life, at least for a certain period of time, depends on the strict fulfillment of Hippocrates' advice: "Let food be thy medicine and medicine be thy food" (Milner 2002). In this context, the use of mushrooms to improve health represents an important cultural heritage as they have been used since time immemorial as a source of highly tasty/nutritional foods and medicinal preparations according to traditional ecological knowledge transmitted through the generations by the greatest early civilizations (Pereira *et al*. 2012; Stamets 2002; Wasser 2010a; Wasser & Weis 1999). Sometimes the health benefits of their use were so impressive that ancient people converted the result observed into long-lived stories of mushroom magic (Hobbs 2000). Thus, experiences of ethnomycological uses of mushrooms deserve a modern evaluation.

Although for most people mushrooms are still considered as one of the curiosities of Nature, by combining tradition and new information, edible and medicinal mushrooms are now attracting more attention. Looking at the health-related issues of the new millennium, the driving forces for this upsurge of interest in mushrooms include aging, projections of the global burden of cancer and chronic noncommunicable diseases (e.g. cardiovascular diseases, diabetes, obesity, and neurodegenerative disorders, among others), with cancer being the main cause of death around the world in the last few years, and pandemic diseases like acquired immune deficiency syndrome (AIDS). A cost-analysis carried out at Harvard University suggested that if current health trends are not addressed, the costs to medical services associated to chronic nontransmissible

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diseases will rise to US$47 trillion in the next 20 years (Bloom *et al*. 2011). In consequence, there is an increase in consumers' interest in modifying lifestyles, particularly through a health-promoting and/or disease-preventing diet (Chang & Wasser 2012; Keservani *et al*. 2010; Mahabir & Pathak 2013; Shahidi 2012).

Mushrooms are emerging as a vital component of the human diet and several comprehensive reviews of their nutritional value have been presented (Chang & Buswell 2003; Kalač 2013; Khan & Tania 2012; Ulziijargal & Mau 2011) (see also Chapter 3). Thus, mushrooms have become attractive as a functional food and as a source of drugs and nutraceuticals (Chang 2009; Ferreira *et al*. 2009; Patel *et al*. 2012) and world production in 2012 was 30 million tons (Wasser 2014). Mushrooms as functional food and nutraceuticals (dietary supplements) can help in the intervention of subhealth states and may prevent the full-blown consequences of life-threatening diseases (Vikineswary & Chang 2013).

Several mushroom species are known to possess medicinal value and some are already being used for such purposes. Of the known mushroom species, approximately 700 are considered to be safe with medicinal properties (Wasser 2010a). Pharmacological effects have been demonstrated for many traditionally used mushrooms, including species from genera *Ganoderma*, *Lentinus (Lentinula)*, *Agaricus*, *Auricularia, Flammulina*, *Grifola*, *Hericium*, *Pleurotus*, *Trametes (Coriolus)*, *Schizophyllum*, *Lactarius*, *Phellinus*, *Cordyceps*, *Inonotus*, *Inocybe*, *Tremella*, and *Russula* (Lindequist *et al*. 2005; Patel & Goyal 2012; Stamets 2002; Vikineswary & Chang 2013). In this wonderful world, *Ganoderma*, mushroom of immortality, has been considered as king of medicinal mushrooms, followed by *Lentinula* and others, including *Pleurotus* (Patel *et al*. 2012).

Fruiting bodies as well as mushroom mycelia have a broad range of bioactive properties (see Chapter 4). Mushrooms are thought to exert approximately 130 pharmacological functions such as antitumor, immunomodulatory, antigenotoxic, antioxidant, antiinflammatory, hypocholesterolemic, antihypertensive, antiplatelet-aggregating, antihyperglycemic, antimicrobial, and antiviral activities (Lindequist 2013; Patel *et al*. 2012; Paterson & Lima 2014). Many controlled studies have investigated this long list of medicinal actions, thus upgrading mushrooms to today's world of evidence-based medicine (Wasser 2014).

Mushrooms are natural bioreactors for the production of compounds with human interest for biotechnological applications (Ferreira *et al*. 2010; Pereira *et al*. 2012). The bioactive molecules comprise high molecular weight compounds, mainly polysaccharides, and low molecular weight secondary metabolites (de Silva *et al*. 2013). Polysaccharides (especially β-glucans) are the best known and most potent mushroom-derived substances, with antitumor and immunomodulatory effects, thus acting as biological response modifiers (BRMs) by improving the host immune system (Chan *et al*. 2009; Chen & Seviour 2007; Wasser 2002; Zhang *et al*. 2007). The vast structural diversity of mycochemicals (phenolic compounds, terpenes, lactones, steroids, alkaloids, among others) provides unique opportunities for discovering new drugs that target and modulate molecular and biochemical signal transduction pathways (Chang & Wasser 2012; Patel & Goyal 2012; Zaidman *et al*. 2005). Some species possess a variety of bioactive compounds and therefore may be able to produce enhanced pharmacological effects. The best example is *Ganoderma lucidum* (Curtis) P. Karst., which contains not only more than 120 different triterpenes but also polysaccharides, proteins and other bioactive molecules (Wasser 2010b).

Owing to this plethora of useful bioactive compounds, mushrooms represent a growing segment of today's pharmaceutical industry. Better insight into the different roles of multiple active compounds and the mechanisms underlying their biological action will accelerate commercial production of pharmaceuticals for therapeutic applications. Asian countries have a head start in the study of medicinal mushrooms compared to the rest of the world, and Western medicine still has a lot to learn from Eastern practices (Paterson & Lima 2014). As presented later in this chapter, several immunoceutical polysaccharides have been developed for clinical and commercial purposes in Japan, Korea, and China. For instance, the Chinese pharmacopeia lists more than 100 mushroom species for medicinal use, and fungal polysaccharide extracts have been used for over three decades as an adjuvant to cancer radio- and chemotherapy (El Enshasy & Hatti-Kaul 2013; Kidd 2000; Martel *et al*. 2014).

Ongoing research projects are aiming to promote mushrooms as a new generation of "biotherapeutics" (Patel & Goyal 2012). Given that only about 10% of mushroom biodiversity has been studied so far (see Chapter 2), and few of them have been characterized with regard to health benefits, it is likely that new active compounds will be discovered in the future (Hawksworth 2012). Particularly in tropical areas, 22–55% (in some cases up to 73%) of mushroom species have not yet been described (Bass & Richards 2011).

Medicinal mushroom science has been recognized as a successful multidisciplinary new branch of science which has experienced great progress in the last 30 years. As a consequence, around 400 clinical trials have been performed to evaluate the effects of medicinal mushrooms in various diseases and more than 50 000 scientific studies and 15 000 patents on medicinal mushrooms have been produced so far (Wasser 2014).

This chapter will summarize the available information and reflect the present state of mushroom use for developing functional foods, drugs, and nutraceuticals. These prospects are expected to provide new avenues for upgrading mushrooms from functional food to translational mushroom medicine.

5.2 A Window into the "Garden" of a Novel Class of Products

The Chinese have an ancient saying which highlights the concept that medicine and food have a common origin. At the intersection between food, nutrition, and medicine and encouraged by growing concerns about the impact of diet on health and efforts to achieve "optimal nutrition," a rich "garden" of terms has emerged, for many of which there are no absolute definitions accepted by the scientific community. In this section, we will try to open a window into this puzzle in order to provide a comprehensive perspective on the contemporary uses of mushrooms in the context of this book.

Most mushroom-derived preparations find use not as pharmaceuticals ("real" medicines) but rather as a novel class of products with different names: food supplements, tonics, functional foods, nutraceuticals, phytochemicals, mycochemicals, biochemopreventives, and designer foods (Chang 2009; Wasser & Akavia 2008). Our starting point will be the functional foods and nutraceuticals, a growing field in food science seeking alternatives to improve personal health and reduce healthcare costs. According to the International Life Sciences Institute of North America (ILSI), functional foods are "foods that by virtue of physiologically active food components consumed as part of

the usual diet provide health benefits and/or reduce the risk of chronic diseases beyond basic nutritional functions" (Coles 2013). Such foods range from traditional foods possessing demonstrated physiological benefits as well as processed foods, e.g. fortified with added or concentrated ingredients to functional levels (Betoret *et al*. 2011; Prakash *et al*. 2014).

The term "nutraceutical" was coined from "nutrition" and "pharmaceutical" in 1989 by Dr Stephen DeFelice and is defined as "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease." Based on this definition, a functional food would be a kind of nutraceutical (Keservani *et al*. 2010) and in some countries the two terms are used interchangeably.

In the case of mushrooms, the terms "nutraceutical" and "functional food" are synonymous (Chang & Buswell 1996, 2003). In the general context of this book, including wild edible plants and nuts, we will discuss "mushroom nutraceuticals" in correspondence with the Health Canada definition describing them as products isolated or purified from foods generally sold in "pharmaceutical forms" of pills, capsules, and liquids, not usually associated with food. A nutraceutical is demonstrated to have a physiological benefit or provide protection against chronic disease (Mahabir & Pathak 2013). Thus, nutraceuticals could be found in many products emerging as "dietary supplements," comprising ingredients obtained from food, plants, and mushrooms (fungi) that are taken without further modification, separately from foods for their presumed health-enhancing benefits. Therefore, they may be classified as a category between foods and drugs (Wasser & Akavia 2008).

"Phytochemicals" are specific types of nutraceuticals and comprise the naturally occurring, biologically active compounds found in plants which have capabilities of inhibiting various diseases, as part of the antioxidant defense molecules among other physiological actions on the human body. Important phytochemicals are secondary metabolites such as phenolic compounds, sterols, and alkaloids. Phrases like "chemopreventive agents" are sometimes used to describe phytochemicals thought to reduce risk for certain types of cancer (Jabeen *et al*. 2014). Analogically, "mycochemicals" refers to the untapped metabolites from mushroom fungi that can be used as nutraceuticals and as new life-saving drugs (Patel *et al*. 2012). Similar to "phytopharmaceuticals," the resulting drugs should be considered as "mushroom pharmaceuticals" (Lindequist 2013).

In the mushroom science community, the term "nutriceutical" is also an accepted definition emerging from the recognition of numerous biological activities of mushroom products. A "mushroom nutriceutical" is a refined/partially refined extract or dried biomass from either the mycelium or fruiting body of a mushroom, which is consumed in the form of capsules or tablets as a dietary supplement (DS) (not a food) and has potential therapeutic applications (Chang & Buswell 1996, 2003; Chang & Miles 2004). According to Wasser and Akavia (2008), mushroom-based products can serve as a diverse and superior class of dietary supplements. Regular intake of medicinal mushroom preparations may enhance the immune response of the human body, thereby increasing resistance to disease. Acting as immunopotentiators, these mushroom preparations modify host biological responses and therefore, they are also known as biological response modifiers (BRMs) (Chang 2009; Wasser 2014; Wasser & Weis 1999). Moreover, several classes of mushroom bioactive substances having immunotherapeutic efficacy when taken orally can be considered as immunoceuticals (Kidd 2000; Petrova *et al*. 2005).

Although our garden seems like an intricate labyrinth, the truth is that edible and medicinal mushrooms as well as mushroom products have definitively arrived (Chang & Wasser 2012). The next questions are:

- How can humans use mushrooms as innovative resources for a healthy lifestyle and in preventive and curative medicine?
- What defines a particular use?
- Are mushroom products "magic" like the foods of "Alice in Wonderland"?

5.3 Main Uses of Edible Medicinal Mushrooms in the Age of Human Health Crises

It is well known that we live in an age of human health crises. This is where the role of edible and medicinal mushrooms with their products has become important (Chang & Buswell 2003; Cheung 2008). Nowadays, interest in biotechnological cultivation of basidiomycete mushrooms is related to the increasing demand for mushroom-based biotech products in the pharmaceutical, food, and cosmetic industries (Badalyan 2014). The physiological functions of mushrooms can be described by the pyramid model suggested by Chang and Wasser (2012). In this model, human health may be divided into three states: health, subhealth, and illness. Mushrooms themselves can be used as a food to promote a healthy state; pure refined products can be used as medicine for ill health, and crude extract products can be used as dietary supplements (nutraceutical for our purpose) for a subhealthy state, as well as for both healthy and ill states.

Thus, mushrooms are not only food but are the raw material for development of functional food and dietary supplements (nutraceuticals). Mushrooms as functional food can help in the early intervention of subhealthy states and may prevent the consequences of life-threatening diseases. The ideal strategy is subhealthy intervention and prevention rather than cure of chronic nontransmissible diseases by reverting to traditional knowledge as a source of chemopreventive food and nutraceuticals. Further, the quality of life of those who are on lifelong therapeutic drugs may be enhanced by using functional molecules from mushrooms (Vikineswary & Chang 2013). When used as drugs, mushroom products can supplement other treatments and complement modern medicine (Chang & Wasser 2012; Wasser 2014).

Between 80% and 85% of mushroom products are taken from fruit bodies either collected in the wild or grown commercially, and the resulting products are considerably diverse and unpredictable. Only 15% of all products are based on extracts from mycelia and a small percentage are obtained from culture filtrates (Barros *et al*. 2007; Lindequist *et al*. 2005). One main prerequisite to using mushrooms as drugs, nutraceuticals or for other purposes is its continuous production in high amounts and at standardized quality. In addition, safety of mushrooms and their products should be verified and proven as thoroughly as possible (Chang & Wasser 2012). In the opinion of Chang (2001), mycelial products are the "wave of the future" because they ensure standardized quality and year-round production. Thus, submerged liquid fermentation can provide more uniform and reproducible biomass and may provide valuable medicinal products (Suárez & Nieto 2013). However, fruiting bodies obtained under good manufacturing

practice (GMP) can also be used in the formulation of consistent and safe mushroom products such as functional foods, nutraceuticals, and biologically active compounds (Morris *et al*. 2014a).

As mentioned above, the range of human states in which mushroom-derived products can be used is broad. Therefore, in this section, an attempt will be made to dissect and distinguish the importance and uses of mushrooms as part of a modern healthy lifestyle by passing from cuisine to clinical applications.

5.3.1 Mushrooms as Functional Foods: A Paradigm of Integrating Tradition and Novelty

In agreement with the notion that prevention is better than cure, functional foods based on medicinal mushrooms have gained popularity for their high nutritive and medicinal values (Chang & Miles 2004; Mane *et al*. 2014; O'Neil *et al*. 2013). Generally, edible mushrooms possess all three desired properties of food: nutrition (see Chapter 3), taste, and physiological functions (Chang & Buswell 2003; Chang & Wasser 2012).

Over a 15-year period (1997–2012), the global per capita consumption of mushrooms increased from about 1 kg/year to over 4 kg/year, with *Agaricus*, *Pleurotus*, *Lentinula*, *Auricularia*, and *Flammulina*, the so-called "high five," accounting for 85% of the world's mushroom supply (Royse 2014). Commercial cultivated mushrooms are readily available fresh, frozen or canned and they are useful and versatile ingredients that can easily be added to many dishes such as pizzas, casseroles, and salads (Stamets 2002). For example, in Japan fresh and dried shiitake (*Lentinus edodes* (Berk) Singer) is used in medicinal mushroom dishes – "Yakuzen." These dishes can be prepared in many ways: boiled, grilled, skewered, or on aluminum foil with different types of seasoning. Concentrates, obtained from whole fruiting bodies or powdered mushrooms, are used as drinks (Wasser 2010c). Mane *et al*. (2014) reported an improvement in nutritional quality and therapeutic properties of meal items through the addition of fresh or fried oyster mushroom *Pleurotus sajor-caju* (Fr.) Singer without affecting its acceptability.

It is important to note the potential relevance of new species of culinary-medicinal mushrooms cultivated recently at commercial scale, e.g. *Flammulina velutipes* (Curt.: Fr.) P. Karst., *Tremella* spp., *Coprinus comatus* (O.F.Mull.: Fr.) Pers., *Hypsizygus* spp., *Dictyophora* spp., and *Hericium erinaceus* (Bull.: Fr.) Pers. among others (Chang & Wasser 2012). Wild mushrooms, for example, the nutritional and chemical (antioxidant) inventory of Portuguese edible mushrooms in different habitats (Pereira *et al*. 2012), also deserve interest for the development of functional foods.

Several mushrooms are helpful in human ailments because they possess many typical pharmacological features, such as metabolic activation, bioregulation (maintenance of homeostasis and immune balance), prevention/control of intoxication, decreasing cholesterol levels, as antioxidants with rejuvenating and energy-boosting properties, and their role in the prevention and improvement of life-threatening diseases such as cancer, neurodegenerative disorders, diabetes, and metabolic syndrome (Lindequist *et al*. 2005; Patel *et al*. 2012; Roupas *et al*. 2012). In view of these properties, mushrooms have been considered as "the new superfood" or "the choicest food of nutritionists" (Mane *et al*. 2014).

Much more research is needed on the bioactive components in mushrooms to determine their biological responses in humans. Promising evidence suggests that ergothioneine, vitamin D, β-glucan, and selenium offer positive effects for immune function, intestine function, and weight management (Feeney *et al*. 2014). Information about the proximate composition and energy as well as mushroom mycochemicals is of great interest as both fruiting bodies and mycelia could be used as functional foods and/or as a source of functional ingredients. Thus, the benefits of mushrooms in human nutrition are growing as more research is undertaken to validate traditional claims.

5.3.1.1 Proven Functional Properties

Improvement of Digestive Function Mushrooms contain dietary fibers, including β-glucans, chitin, and heteropolysaccharides (pectinous substances, hemicelluloses, polyuronides, etc.), as much as 10–50% in the dried matter (Wasser & Weis 1999). Benefits of insoluble dietary fiber include reduction of bowel transit time, prevention of constipation, and reduction in risk of colorectal cancer. Concerning soluble dietary fibers and especially β-(1,3),(1,6)-D-glucans, health benefits include lowering of blood cholesterol, reducing hyperglycemia and hyperinsulinemia in relation to the control of diabetes mellitus, reduction of risk factors for degenerative diseases such as cardiovascular disease, cancer, hypertension, and promotion of the growth of beneficial gut microflora (as a prebiotic) (Jacobs *et al*. 2009; Laroche & Michaud 2007).

Constipation is one of the most prevalent gastrointestinal complaints and high fiber intake is recommended as an initial therapy. Ear mushrooms (*Auricularia*) are known to have higher fiber content (by 50%) than other mushroom varieties. In patients with functional constipation, fiber supplements using ear mushrooms have been shown to significantly improve constipation-related symptoms without serious side-effects (Kim *et al*. 2004).

Synytsya *et al*. (2009) reported that the fruit bodies of *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. and *P. eryngii* (DC.) Quél. contain significant amounts of β-glucans, which are components of both insoluble and soluble dietary fibers. The stems are a better source of insoluble dietary fibers and glucans than the gastronomically attractive pilei, and therefore the stems can be used for the preparation of biologically active polysaccharides utilizable as functional foods. Mushroom polysaccharides can stimulate the growth of colon microorganisms, e.g. acting as prebiotics. Potential prebiotic activity of glucan extracts L1 (water soluble) and L2 (alkali soluble) isolated from stems of *P. ostreatus* and *P. eryngii* was tested using probiotic strains of *Lactobacillus*, *Bifidobacterium*, and *Enterococcus*. These probiotics showed different growth characteristics dependent on extract used and strain specificity. This exploitation of fruit body extracts extends the use of *P. ostreatus* and *P. eryngii* for human health.

Interactions between the host and its microbiota are increasingly recognized to be critical for health. Rapid and reproducible changes in human gut microbiota were evidenced in an interventional randomized clinical trial conducted with healthy volunteers treated for 14 days with a *Trametes versicolor* (L.: Fr.) Lloyd extract at doses of 1200 mg, three times daily (Beth Israel Deaconess Medical Center, NCT 01414010, http://clinicaltrials.gov/ct2/results?term=mushroom).

Antioxidant Properties Mushrooms packed with a wide array of bioactive components are excellent antioxidants and antiinflammatory agents which may help to prevent the occurrence and aid the treatment of chronic diseases including heart disease and various cancers (Vikineswary & Chang 2013). Primary metabolites, including enzymes such as glucose oxidase, superoxide dismutase, peroxidases, and laccases, may prevent oxidative stress (Chang & Wasser 2012; Wasser 2010a). In addition, some common widely consumed edible mushrooms have been found to possess antioxidant activity (see Chapter 4), which is well correlated with their total phenolic content. Phenolics can act as free radical inhibitors (chain breakers), peroxide decomposers, metal inactivators or oxygen scavengers and thus delay food spoilage and oxidative damage in the human body (Asatiani *et al*. 2010).

The ability of preparations from *Pleurotus ostreatus*, *Agaricus bisporus* (J. Lge) Imbach and *Ganoderma lucidum* (Curt.: Fr.) P. Karst to prevent oxidative damage of DNA has been established (Jose *et al*. 2002).

Palacios *et al*. (2011) investigated the antioxidant properties of eight types of edible mushrooms (*Agaricus bisporus*, *Boletus edulis* Bull., *Calocybe gambosa* (Fr.) Donk, *Cantharellus cibarius* Fr., *Craterellus cornucopioides* (L.) Pers., *Hygrophorus marzuolus*, *Lactarius deliciosus* (L.) Gray, and *Pleurotus ostreatus*). Homogentisic acid was the free phenolic acid significantly present in all mushrooms although the content varied considerably among the analyzed species. Flavonoids, such as myricetin and catechin, were also detected in the mushrooms studied. The antioxidant properties were evaluated by monitoring linoleic acid autoxidation, and all the species showed inhibition, with *C. cibarius* being the most effective (74% inhibition) and *A. bisporus* the species with lowest antioxidant activity (10% inhibition).

The oyster mushroom, *P. ostreatus*, has potent antioxidant activity by virtue of its scavenging hydroxyl and superoxide radicals, inhibiting lipid peroxidation, reducing power on ferric ions, and chelating ferrous ions. *P. ostreatus* also exhibits good *in vivo* antioxidant activity by reducing lipid peroxidation and enhancing the activities of enzymatic and the levels of nonenzymatic antioxidants. The antioxidant principles identified, such as ascorbic acid, α-tocopherol, β-carotene and flavonoid compounds (rutin and chrysin), possibly contributed to the observed effects (Jayakumar *et al*. 2011). Phenolic compounds were detected in five extracts obtained from fruit bodies of *Pleurotus* spp., obtained with solvents of different polarity; however, the highest levels were found in polar extracts (water and ethanol) with values of 138.4 and 86.37 mg/100 g dry base, respectively (Beltrán *et al*. 2013).

In addition to their total phenolic content, the antioxidant activity of mushrooms was also found to be due to their polysaccharide content. Khan *et al*. (2014) evaluated the antioxidant (lipid peroxidation inhibition) and functional (swelling power, fat binding, foaming, and emulsifying properties) properties of β-glucans extracted from edible mushrooms *A. bisporus*, *P. ostreatus*, and *Coprinus atramentarius* (Bull.) Fr. The glucan from *C. atramentarius* showed better antioxidant and functional properties compared to those from *A. bisporus* and *P. ostreatus*. Fungal pigment melanin also possesses antioxidant, immune-modulating, antimutagenic, and radioprotective properties (Badalyan 2014).

Selenium has also received increasing attention as a possible cancer preventive trace mineral, possibly through antioxidant protection and/or increased immune function. Mushrooms accumulate selenium based on their growing medium and

provide more selenium than other foods in the fruit and vegetable group (Sadler 2003). Using the vacuum impregnation technique, Cortés *et al*. (2007) developed a product with functional characteristics by means of fortification of *P. ostreatus* mushroom with calcium, selenium, and ascorbic acid. Fortification levels for Ca and Se of 7.3% and 42.3% of the Daily Recommended Intake (DRI)/100 g of fresh mushroom, respectively, were obtained. At the beginning of storage at 4 °C, the ascorbic acid content was 40% of the DRI/100 g of fresh mushroom. In another study, Mao *et al*. (2014) purified and evaluated the antioxidant activities of selenium-containing proteins and polysaccharides in the Royal Sun mushroom, *Agaricus brasiliensis* S.

The antioxidant properties displayed by edible mushrooms as functional foods are also closely associated with their antimutagenic, antigenotoxic, radioprotective, and antiaging effects. Moreover, Naveen and Anilakumar (2014) reported that the antifatigue property of *A. bisporus* was supported through decreased levels of lipid peroxidation in tissue and also proposed the development of a fermented yogurt product using an *A. bisporus* extract.

It is important to highlight that mushrooms are generally cooked or processed into various culinary dishes industrially or at home. Cooking processes bring about a number of changes in their physical characteristics and chemical composition, including an effect on antioxidant activity. Arora (2014) stated that, in general, frying does not affect antioxidant activity but boiling and microwave cooking deplete the radical scavenging ability of *A. bisporus*, *Calocybe indica*, *Volvariella volvacea* (Bull.: Fr.) Sing, *Lentinula edodes*, and *P. ostreatus*.

Improvement of Blood Lipid Profile and Lower Risk of Cardiovascular Disorders Mushrooms may be able to improve cardiovascular disease risk through their ability to reduce blood cholesterol levels. The results of numerous studies indicate that mushrooms are a valuable source of statins (Endo 2004), which inhibit the activity of the key enzyme in cholesterol synthesis, hydroxyl-methyl-glutaryl-CoA reductase (HMG-CoA reductase). The best known edible higher basidiomycetes for potential production of lovastatin are species of the genus *Pleurotus* and the highest content was found in the fruiting bodies of *P. ostreatus* (Gunde-Cimerman & Plemenitas 2001).

It is known that shiitake mushroom (*L. edodes*) is able to lower blood cholesterol and lipids in animals and humans via a factor known as eritadenine (also called "lentinacin" or "lentysine"). Apparently, eritadenine reduces serum cholesterol in mice, not by inhibition of cholesterol biosynthesis but by acceleration of the excretion of ingested cholesterol and its metabolic decomposition. For many patients (60 years of age or older) with hyperlipidemia, consuming fresh shiitake mushroom (90 g/day for seven days) led to a decrease in total cholesterol blood level by 9–12% and triglyceride level by 6–7% (Hobbs 2000). Although feeding studies with humans have indicated positive effects, further research is needed.

In addition to the improvement in blood lipid profile, the cardioprotective role of mushrooms is also related to their antithrombotic activity (antiaggregatory action on blood platelets), including nucleic acid components of *L. edodes* (Kabir & Kimura 1989) and a blood pressure-lowering effect (*e.g.* cardioactive proteins of *V. volvaceae* (Yao *et al*. 1998) and antihypertensive angiotensin I-converting enzyme inhibitory peptides from *Pleurotus cystidiosus* O.K. Mill. and *Pleurotus cornucopiae* (Paulet)

Rolland (Ching *et al*. 2011)). A new glycoprotein (Fraction SX) obtained from *Grifola frondosa* (Dicks.) Gray (Maitake) helps to maintain healthy cardiovascular function (Zhuang & Wasser 2004).

In China, more than 40 patents use *Tremella* as the base for food products. It can be made into a mushroom tea with the health-promoting functions of nourishing the kidneys, preventing coagulation, lowering blood pressure and prolonging life, and is a multifunctional nutrient liquid that lowers fat and cholesterol levels in blood, prevents cancer, and increases the number of leukocytes. A unique feature of *Tremella* mushrooms is that its most often mentioned medicinal properties depend on glucuronoxylomannans contained in fruiting bodies, or those produced in pure culture conditions. In particular, the hypocholesterolemic actions may be attributable to the high molecular weight anionic charged polysaccharides, involving the suppression of cholesterol absorption from the digestive tract (Reshetnikov *et al*. 2000). These bioactive materials may be beneficial for applications in the medicinal food industry.

Improvement of Glucose Homeostasis and Antidiabetic Effect Some protective effects of mushrooms as functional foods have been investigated, *in vitro* and *in vivo*, while some clinical trials have confirmed their therapeutic implications as an effective alternative treatment for type 2 diabetes mellitus (Deepalakshmi & Mirunalini 2014). This effect appears to be mediated via mushroom polysaccharides (possibly both α- and β-glucans) via a direct interaction with insulin receptors on target tissues, although this mechanism remains to be confirmed (Roupas *et al*. 2012).

A randomized, double-blinded, and placebo-controlled clinical trial (n = 72) showed that *A. blazei* Murill supplementation in combination with metformin and gliclazide improved insulin resistance in these subjects. An increase in adiponectin concentration after *A. blazei* extract consumption for 12 weeks may be the relevant mechanism (Hsu *et al*. 2007).

Jayasuriya *et al*. (2012) reported that long-term consumption of *P. ostreatus* and *P. cystidiosus* as a functional food appears to be effective for glycemic control. The study evaluated the effect of a suspension, made with powdered mushrooms, on the fasting and postprandial serum glucose levels in healthy volunteers at a dose of 50 mg/kg body weight, followed by a glucose load. Reductions in the fasting serum glucose levels for *P. ostreatus* and *P. cystidiosus* groups were 6.1% and 6.4%, respectively and the postprandial glucose reductions were 16.4% and 12.1%. Antihyperglycemic activity was demonstrated with a water-soluble polysaccharide from *P. citrinopileatus* fermentation broth. The polysaccharide was effective in lowering blood glucose levels in diabetic rats (Hu *et al*. 2006). Additionally, the *in vitro* and *in vivo* antidiabetic activity of *Calocybe indica* suggests its therapeutic potential for the prevention and control of diabetes as an easily accessible source of a natural antidiabetic functional food (Rajeswari & Krishnakumari 2013).

Other results indicated that *Tremella mesenterica* Schaeff. (fruiting bodies, submerged culture biomass and tremellastin, an acidic glucuronoxylomannan polysaccharide) might be developed as a potential oral hypoglycemic agent or functional food for diabetic patients and those with high risk for diabetes mellitus (Lo *et al*. 2006). *Tremella* constitutes the major part of functional foods, having pronounced medicinal

properties, with existing patents for hyperglycemia suppressants in the form of food or drink (Reshetnikov *et al*. 2000).

Mushroom β-glucans, as soluble dietary fiber, have been gaining interest as a food ingredient due to their beneficial role in maintaining blood sugar balance via blood sugar lowering effects, elevation of plasma insulin levels, and the enhancement of cellular insulin sensitivity; they also have been shown to help in dyslipidemia, obesity, and metabolic syndrome (El Khoury *et al*. 2012). Research into mushroom antiobesity potential conducted in men and women who were overweight or obese (n = 73) revealed a significant loss in body weight, body mass index (BMI), and waist circumference during the six months of the trial in those consuming the mushroom diet (substitution of 8 oz (227 g) of fresh mushrooms for 8 oz of meat three times/week) compared with baseline (Poddar *et al*. 2013).

Enhancement of Immune Function and Lower Risk of Certain Tumors Edible mushrooms with functional properties have long been suggested to possess immunomodulatory effects (Lindequist 2013; Wasser & Weis 1999). It was stated in the *Ri Yong Ben Cao* (1620), written by Wu-Rui of the Ming dynasty, that "shiitake accelerates vital energy, wards off hunger, cures colds, and defeats body fluid energy" (Wasser 2010c). Many of these effects are related to the immune system and recent investigations have found evidence of the health promotion abilities associated with mushroom consumption, including antiviral, antibacterial, antifungal, and antiparasitic effects (Tejera *et al*. 2013).

Many, if not all, basidiomycete mushrooms contain biologically active polysaccharides in fruit bodies, cultured mycelium, and culture broth. Polysaccharides are the most potent mushroom-derived substances with antitumor/immunomodulating properties. These polysaccharides are of different chemical composition, with most belonging to the group of β-glucans having β-(1,3) linkages in the main chain and additional β-(1,6) branches needed for their antitumor action. Most of the clinical evidence for immunomodulating and antitumor activities comes from the commercial polysaccharides, such as lentinan (from *L. edodes*), PSK (krestin) (from *Trametes versicolor*), and schizophyllan (from *Schyzophyllum commune* Fr.: Fr.) (Chang & Wasser 2012; El Enshasy & Hatti-Kaul 2013; Wasser 2002). The use of these mushroom polysaccharides as drugs will be discussed in section 5.3.3, and in this section the benefits of food products based on whole mushrooms or foods supplemented with β-glucans to support our immune system will be the focus of attention.

Fungi β-(1,3)-glucans are traditionally part of the Japanese diet, in which whole mushrooms are eaten. The consumption of fresh mushrooms was found to increase anti-β-glucan antibodies in the serum of humans; it was also suggested to provide better defense against pathogenic fungi (Ishibashi *et al*. 2005). In addition, dietary intakes of *A. bisporus* (fresh) and *L. edodes* (dried) mushrooms and green tea combine to reduce the risk of breast cancer in Chinese women (Zhang *et al*. 2009). Although many patents have been published claiming immunopotentiator effects of β-glucans in functional foods, in some cases β-glucan is incorporated in such a low quantity that the real health benefit is difficult to determine (Laroche & Michaud 2007).

Two types of hydrogels of β-D-glucan, pleuran (from *P. ostreatus*) and lentinan, have been added to yogurts, natural, sweetened, flavored or with fruit, to increase their bioactivity. The application of both hydrogels to yogurts had no negative influence on

the sensory acceptability of the products and all samples maintained very good quality during the whole storage period. The regular daily consumption of such dairy products could contribute to the reduction of relapsing or chronic infectious as well as autoimmune and oncological diseases, especially in more risky age groups (children and older people) (Hozová *et al*. 2004).

Wild edible BaChu mushroom (*Helvella leucopus* Pers.), grown in Xinjiang Province, China, can be used in the treatment of leukocytopenia, and reduced immunity due to chronic hepatitis and radiochemotherapy. It also has a preventive role for AIDS. BaChu mushrooms are reported to enhance the phagocytosis ability of leukocytes, lymphocyte conversion ratio, and antibody titer (Meng *et al*. 2005). BaChu mushroom crude polysaccharides have been used in a processing technology for obtaining a beverage mixed with water and fresh juice. This juice recipe has more than 14 000 IU of vitamin A and over three times the vitamin C content of an apple (Hou *et al*. 2008).

Bioactivity analyses present a possible direction for developing reliable functional foods based on whole shiitake or food supplemented with isolated lentinan. The consumption of *L. edodes* has been associated with the proliferation, activation, and modification of memory and naive innate immune cell populations (Stanilka *et al*. 2013) and it modulates human immune function by altering cytokine secretion (Dai *et al*. 2013).

Nanotechnology has shown great potential for improving the extraction effectiveness of bioactive compounds in functional foods. For example, a new method was developed for nanoparticle extraction of water-soluble β-glucans from mushrooms (sparan, the β-D-glucan from *Sparassis crispa* (Wulfen) Fr., and phellian from *Phellinus linteus* (Berk. & M.A. Curtis) Teng). This "nanoknife" method could be used in producing β-glucans for the food, cosmetics, and pharmaceutical industries (Park *et al*. 2009). Nanotechnology applied to mushrooms also aims to enhance solubility, facilitate controlled release, improve bioavailability, and protect bioactive compounds during processing, storage, and distribution.

Neurogenerative Potential and Improvement of Neurodegenerative Diseases Studies have shown that consumption of *Hericium erinaceus* (lion's mane mushroom) is associated with neurite-stimulating activity through the induction of nerve growth factor (NGF) (*in vitro* and *in vivo*) by dilinoleoyl phosphatidylethanolamine (DLPE), hericenones C–H, and erinacines A–I. Preliminary human trials with *H. erinaceus* derivatives showed efficacy in patients with dementia in improving the Functional Independence Measure (FIM) score or retarding disease progression (Kawagishi & Zhuang 2008), while a double-blind, parallel-group, placebo-controlled trial with oral administration of *H. erinaceus* to 50–80-year-old Japanese men and women diagnosed with mild cognitive impairment reported significantly increased cognitive function scores compared to placebo during intake (Mori *et al*. 2009). Therefore, this mushroom has great potential to be developed as a functional food or nutraceutical for boosting brain and nerve health and for improvement of subhealth states related to aging and delaying neurodegeneration.

In sum, the consumption of whole edible-medicinal mushrooms or their bioactive ingredients as functional foods is a beneficial practice for preserving health. However, postlaunch monitoring is needed to establish whether functional foods are safe and effective under customary conditions of use and to assess their influence on the

effectiveness of drugs and patient compliance (Coles 2013). The development of new functional foods from mushrooms is increasingly challenging. It remains to be determined how often, how much and what species or mixture of species should be consumed to bring about a desired biological response (Vikineswary & Chang 2013).

5.3.2 Mushroom Nutraceuticals

The nutraceutical revolution leads into a new era of medicine and health, in which the food industry is expected to become a research-oriented sector similar to the pharmaceutical industry (DeFelice 1995). Nowadays, different mushroom-based healthcare commercial biotech products with preventive and curative effects are available and largely consumable in the world market as nutraceuticals (dietary supplements, DS). The market for DS from mushrooms is growing and is currently valued at more than US$18 billion (representing 10% of the general market for DS) and the demand for such products is expected to increase (Wasser 2014). For example, Aloha Medicinals Inc. (Carson City, NV), with a monthly production of 400 000 kg of finished product (equivalent to 16 million bottles of dietary supplement) is considered the largest in the world (www.alohamedicinals.com).

Numerous studies have shown that certain mushroom DSs are effective in both preventing and treating subhealth status and specific life-threatening diseases owing to the synergistic action of bioactive molecules, when regularly consumed; even in high dosages (over 150 g of fresh mushroom), they demonstrate very low toxicity. Many mushrooms or mushroom preparations traditionally taken as treatments for specific conditions are now often marketed for use as prophylactic agents (Badalyan 2014; Chang & Wasser 2012).

Mushroom-derived products are neither food (functional food) nor pharmaceuticals (drugs), because the active ingredient of most products is not a single, chemically defined compound as used in conventional drug treatments. Therefore, they may be classified as a type of DS or traditional medicine, which is a category between food and drugs (Chang & Wasser 2012). Each one is commercialized as a DS, specifying that the purpose is not to treat, diagnose, cure or prevent any disease, and they have not been evaluated by the FDA. The main types of mushroom DS products available on the market today are:

- artificial cultivated fruit body powders, hot water or alcohol extracts of these, or the same extract concentrates and their mixtures
- dried and pulverized preparations or the combined substrate, mycelium, and mushroom primordial after inoculation of edible semisolid medium (usually grains)
- biomass or extracts from mycelium harvested from submerged liquid culture grown in a bioreactor
- naturally grown, dried mushroom fruit bodies in the form of galenic formulations like capsules or tablets
- spores and their extracts (Chang & Wasser 2012; Lindequist 2013; Llauradó *et al.* 2013; Morris *et al.* 2011; Wasser & Akavia 2008).

Data regarding the dosage to be used are controversial; the suggested dosages vary widely due to various forms and formulations. Although the fresh form can be a valuable dietary supplement, the quantities one would require for therapeutic doses are so great

that its consumption could cause digestive upset. According to traditional Chinese medicine, the standard dose of the mushroom dried fruiting bodies per day in different forms (tablets, capsules, liquid extracts, etc.) must be equivalent to about 100–150 g of fresh mushroom material. Numerous clinical trials have established that six capsules (three capsules two times per day or two capsules three times per day), of 500–1000 mg each (biomass or extracts), is the accepted dosage of mushroom preparations (Wasser 2014).

We illustrate this with shiitake mushroom, which is prescribed in various forms. It may be ingested as a sugar-coated tablet, capsule, concentrate, powdered extract, syrup, tea, or wine. Tablets are usually made from a dried water extract of the mycelia or fruiting bodies because drying concentrates the lentinan and other active principles. Standardized extracts are also available, and they are preferred because the amount of lentinan present is certified and clearly stated on the bottle. The standard dose of the dried fruiting body in tea or in mushroom dishes is given as 6–16 g, equivalent to approximately 60–160 g of fresh fruiting bodies. The dosage, usually in the form of a 2 g tablet, is 2–4 tablets/day (Stamets 2002; Wasser 2010c).

A brief overview of mushroom nutraceutical products is provided in Table 5.1.

We can conclude that the diversity of mushroom DSs with respect to composition/formulation items (combination of components containing in biomass, extracts or isolated fractions of different mushroom species in one preparation or only one species, combination of mushroom substances with other herbal products or pure nutraceuticals such as vitamins and minerals, etc.) is enormous. Most of these mushroom DSs containing polysaccharides function as immunomodulators. The physiological constitution of host defense mechanisms is improved, which restores homeostasis, thereby enhancing resistance to disease and in some cases causing regression. For example, products developed from biotechnologically cultivated mycelia of edible mushrooms *Hericium erinaceus* and *Tremella* spp. in combination with other natural substances possess antioxidant and immune-stimulating activity, and regulate the level of blood lipids and sugar (Khan *et al*. 2013; Standish *et al*. 2008).

In developing productive research programs for nutraceuticals, it is important to build a hierarchy of evidence for individual supplements, including understanding the essentials of product characterization (purity, active ingredients, and potential mechanisms of action), basic clinical chemistry, and subsequent rigorous testing in the setting of clinical studies. Multiple lines of investigation can then be coordinated for enhancing the knowledge base on a product, with the goal of informing practitioners and the public on safety and efficacy of DS use (Hopp & Meyers 2010). The growing DS industry has prompted the need for international governance in establishing regulatory and standard benchmarks for the expanding world market. The scientific validation of mushroom products can help boost their credibility (Wasser & Akavia 2008).

Where should functional foods and nutraceuticals (FFN) be positioned in current guidelines as treatments for lifestyle-related diseases? FFN, similar to pharmaceutical agents, contain bioactive substances that target and modulate biological processes that foster the development of disease. FFN are likely to prove useful in both alleviating and preventing human diseases. Thus, the gap that currently exists between FFN research and the medical community needs to be closed such that FFN can be implemented into clinical guidelines for chronic nontransmissible diseases throughout all stages of therapy.

Table 5.1 Overview of some mushroom nutraceutical products and their health effects.

Product	Content	Observations
Aloha Medicinals Inc. (www.alohamedicinals.com)		
Organic *Cordyceps sinensis*™ (525 mg)	*C. sinensis alohaensis* hybrid strain (US and international patents)	50/50 mixture of hybrid *Cordyceps* and CS-4 *Cordyceps*. This product is often combined with *Agaricus blazei*. *Tru-Cordyceps*™
Immune-Assist™ Critical Care Formula (500–mg)	*A. blazei:* 58.5% β-(1,3)-(1,6)-D-glucan; *C. sinensis:* 30% β-glucan and deoxiadenosine and other nucleosides; *G. frondosa:* 28% β-glucan (fraction D); *L. edodes:* 40% β-glucan (lentinan) and α-glucan (KS-2); *C. versicolor:* 40% β-glucan (including polysaccharides P and K); *G. lucidum:* 40% β-(1-3)-(1-6)-D-glucan and triterpenoids	This product has proven a significant reduction of the adverse effects induced by radio- and chemotherapy in clinical trials, including appetite loss, nausea, low energy status, among others.
Immune-Assist 24/7 (500 mg)	*A. blazei, C. sinensis, G. frondosa, L. edodes, C. versicolor, G. lucidum* (similar to the former formulation) plus hybrid *Cordyceps* and a green tea-derived substance	This formula has proven to be useful in HIV/AIDS patients after clinical trials Dosage: 3 tabs/day with meals
GanoSuper™	Concentrated Reishi extracts. Made from four different strains of Reishi – Black, White, Red and Purple	A concentrated extract for people who want a fully water-soluble form of Reishi for use in their coffee or tea. It is manufactured so as to make it fully water soluble so opened capsules can be dissolved directly into the coffee or other hot drink
Levolar Forte™ (750 mg)	Extract of *C. sinensis*, CS4 (from *C. sinensis*), fraction D of *G. frondosa*, extract of *Coprinus comatus*, full-spectrum *Cordyceps sinensis*, cinnamon extracts, and biotin	Specifically designed for compensating the symptoms of diabetes mellitus and fragile X syndrome Dosage: 4 tablets/day for 2 weeks
Pharmaceutical Mushrooms (www.nwbotanicals.org)		
Eighth Element™ (500 or 600 mg)	*Cordyceps sinensis*	Increase in cellular energy in about 28.8% Dosage: 2 capsules/day
Maitake (500 mg)	*Grifola frondosa* (contains a diversity of β-glucans)	Potent immunomodulating effect. It stimulates T cell production and is recommended for immunodeficiencies

(*Continued*)

Table 5.1 (Continued)

Product	Content	Observations
Purica-Immune FX (250 mg)	*A. blazei, C. sinensis, G. frondosa*, *L. edodes*, *C. versicolor*, *G. lucidum*, Nutricol™ (bioflavonoid concentrate)	Rich in β-glucans, potent immunopotentiators, and antioxidant bioflavonoids
Hep-Assist (500 mg)	Hot water extracts and ethanol precipitates of *L. edodes, A. blazei, G. frondosa, C. versicolor, G. lucidum*, and two *C. sinensis* extracts (one from mycelium and other from the culture broth)	The concentrated mixture of 200 β-glucans and nucleosides from 6 different species of mushrooms turns this formula into a valuable adjuvant product in the treatment of hepatitis B and C
Zhejiang Fangge Pharmaceutical and Healthcare Products Co. Ltd. (http://mushroom.en.alibaba.com)		
China's largest edible and medicinal mushroom processing enterprise. The company supplies mushroom powders, extracts (polysaccharides), supplements, and finished products (capsules and tea bags) from: *Grifola frondosa; Lentinus edodes, Ganoderma lucidum; Agaricus blazei; Cordyceps sinensis; Hericium erinaceus; Coriolus versicolor; Poria cocos; Polyporus umbellatus; Pleurotus ostreatus; Flammulina velutipes; Coprinus comatus, Pleurotus citrinopileatus; Agrocybe aegerita; Agaricus bisporus; Tremella fuciformis; Auricularia auricula; Marasmius androsaceus; Phellinus igniarius; Phaeoporus obliquus;Antrodia cinamomea; Auricularia polytricha*		
FineCo. Ltd. (www.fineco.net)		
Fine-Agaricus® Gold	Highly concentrated micropowder; active ingredients, protein-bound polysaccharides (100% *Agaricus* mushroom polysaccharides)	Effective against several cancers by enhancing the immune system. It has a powerful balancing effect on many physiological functions and has been effective for treating chronic diseases
Fine-Mesima P®	Micropulverized powder of dried *Phellinus linteus* mushroom. Contains *P. linteus* polysaccharide 50%, dextrin 50%	Information not available
Mushroom Wisdom (www.mushroomwisdom.com/products.php)		
Super Reishi	Contains both hot water and alcohol concentrated extracts to achieve the maximum range of beneficial constituents (β-glucans and terpenes); also enhanced with immune-boosting Maitake D-Fraction®	Believed to balance and support the body systems, including heart, lung, liver, nerve, and brain function Dosage: 4 tablets daily or 2 tablets twice a day
Breast-Mate®	*Phellinus linteus* PL-Fraction™ 1000 mg; Maitake PSX-Fraction® containing 18% glycoprotein SX-fraction 160 mg; broccoli sprout extract (4:1) 100 mg; green tea extract (50% polyphenols) 100 mg; vitamin D_3 800 IU	PL-Fraction™ possesses potent activity in maintaining healthy breast cells. Breast-Mate® also contains synergistic ingredients (SX-Fraction®, green tea extract, broccoli extract) Dosage: 4 tablets daily or 2 tablets twice a day

Table 5.1 (Continued)

Product	Content	Observations
Mushroom Emperors™	*A. blazei* Murill fruiting body 120 mg; *C. sinensis* mycelium powder 120 mg; *Hericium erinaceus* fruiting body 120 mg; *G. frondosa* fruiting body 120 mg; *L. edodes* fruiting body 120 mg; *Tremella fuciformis* fruiting body 120 mg; Maitake TD-Fraction® (10% D-fraction 40 mg); Maitake PSX-Fraction® (18% glycoprotein SX-fraction 40 mg; Lion's Mane Amycenone® (hericenones 0.5%, amyloban 6%, 40 mg); *P. linteus* extract PL-Fraction™ 40 mg; *Inonotus obliquus* extract 40 mg; *C. versicolor* extract 40 mg; *Poria cocos* extract 40 mg; *G. lucidum* double extract 40 mg; vitamin C 80 mg	Mushroom Emperors™ brings together 6 holistic mushroom powders with 8 concentrated extracts, including proprietary extracts (D-fraction, SX-fraction, and amycenone) to create a synergistic blend to help promote overall health and vitality Direction for use: 4 tablets daily or 2 tablets twice a day
Product 4life (www.tienda4life.mx/web/Productos.aspx)		
Transfer Factor Plus® Tri-Factor® Formula	*L. edodes, G. frondosa, Cordyceps,* β-glucans, hexaphosphate inositol, β-sitosterol, and an extract of olive leaves	Provides an optimal level of immune support, i.e. the activity of NK cells can be increased to 437%. Also benefits the cardiovascular system

By synthesizing the benefits of both food and medicine, nutraceuticals are expanding into a wide range of areas, competing against such basic items as raw fruit and vegetables and, in some cases, cutting-edge pharmaceuticals (DeFelice 1995).

5.3.3 Mushrooms as a Significant Source of Drugs: Lessons from Wasser's Discovery Pathway

According to current categories of botanical products, medicinal mushrooms can serve as "botanical drugs" or "real drugs." Botanical drugs are complex extracts to be used for treatment of disease and they are clinically evaluated for safety and efficacy just like conventional drugs, but this process can be expedited because of the history of safe human use. Botanical drugs are highly but not completely characterized and are produced under the same strictly regulated conditions as conventional pharmaceuticals. Drugs (prescription drugs or over-the-counter drugs) require the most rigorous testing, including three phases of clinical testing, to ensure safety and efficacy, and close scrutiny by the FDA and/or EFSA (Chang & Wasser 2012).

Öztürk *et al.* (2014) reported on mushroom species which were studied for their chemistry and biological activities in the last two decades. In general, the authors covered 24 types of polysaccharides including β-glucans and other complexes from 13 mushroom species; 259 terpenoid compounds including seven monoterpenes, 19 sesquiterpenes, 54 diterpenes, and 179 triterpenes from 29 mushroom species; 59 steroid compounds from 10 mushroom species; 41 phenolic compounds from

13 mushroom species; and 42 alkaloid compounds from 13 species. Therefore, it is important to develop a knowledge base for individual products, which will provide direction for further clinical investigations.

What steps should we follow to discover a myco-compound with potential as a drug? Wasser (2010a) proposed the Drug Discovery Pathway, which was specially prepared for the development of mushroom pharmaceuticals. This pathway includes nine steps:

- mushroom cultivation and biomass production
- biomass extraction
- screening of mushroom extracts
- effect of selected extracts on a target of interest
- chemical fractionation of selected extracts
- elucidation of active fractions (compounds), mechanism of action, and potency
- effect on animal models
- preclinical drug development
- clinical drug development.

Wasser's Drug Discovery Pathway gives a step-by-step guide and each phase provides recommendations for successful development of mushroom drugs, from the test tube of a mushroom collection to final clinical applications. The pathway will also open new avenues in this "central highway" because there are concerns to solve and questions to answer. Future biotechnological development, the application of modern high-tech screening, the OMICs sciences such as genomics and proteomics, research on validated animal models, and the accurate assessment of clinical values of the candidate drug are directions for approval of mushroom products as drugs. Although Wasser's Pathway is valid for any mushroom drug candidate, in particular, it is intended to play a pivotal role in discovering the potential of low molecular weight metabolites for their use as drugs, i.e. targeting cancer.

Out of the huge diversity of activities, the most frequently sought for the majority of mushrooms is antitumor/immunomodulating activity. Those compounds able to stimulate the biological response of immune cells are being pursued for the treatment of cancer, immunodeficiencies (i.e. to protect AIDS patients against opportunistic infections) or for immunosuppression following drug treatment or surgical procedures. They are also sought for combined therapies with antibiotics and as adjuvants for vaccines (Lull *et al.* 2005; Wasser 2014). Polysaccharides are the most potent mushroom-derived substances with antitumor/immunomodulating properties (El Enshasy & Hatti-Kaul 2013; Mizuno 1999; Wasser 2002). Mushroom polysaccharides occur mostly as glucans, some of which are linked by β-(1-3),(1-6) glycosidic bonds and α-(1-3) glycosidic bonds, but many are true heteroglycans. Historically, hot water-soluble fractions (decoctions and essences) from medicinal mushrooms, i.e. mostly polysaccharides, were used as medicine in the Far East (Hobbs 2000).

Polysaccharides demonstrating remarkable antitumor and immunomodulating activity *in vivo* have been isolated from various species of mushrooms belonging to the Auriculariales, Tremellales, Polyporales, Gasteromycetideae, and Agaricomycetideae. The number of polysaccharides extracted from the fruiting body or cultured mycelium of each species is strongly dependent on the method of fractionation used, but in general, the total amount of polysaccharides is higher in fruiting bodies (Wasser 2002). In addition to their immune regulation potential, polysaccharides are useful biologically

active ingredients for pharmaceutical use, such as for antiradiation, anti-blood coagulation, anti-HIV, and hypoglycemic activities (Shenbhagaraman *et al*. 2012).

One of the first reports on antitumor activities of hot water extracts from fruiting bodies of mushrooms belonging to the family Polyporaceae (Aphyllophoromycetideae) and a few other families was published by Ikekawa *et al*. (1969), demonstrating a host-mediated effect against grafted cancer, such as sarcoma 180 in mice. After this, the first three major drugs were developed and commercialized from medicinal mushrooms; the three were polysaccharides, specifically β-glucans (krestin (PSK) and polysaccharide-peptide (PSP)) from cultured mycelia of *Trametes versicolor*, lentinan from fruiting bodies of *Lentinus edodes*, and schizophyllan (SPG, sonifilan, sizofiran) from liquid cultured broth of *Schizophyllum commune*. In addition, more than 100 types of polysaccharides with biological activities have been isolated from the fruiting body and mycelia of *Ganoderma lucidum* (e.g. ganoderan, GLPS) (Wasser 2010b). Among the most studied mushroom polysaccharides in Japan, China, Korea, Russia, and the US for immunomodulating/antitumor activities, we can mention grifolan or GRN, D- and MD-fractions (from *Grifola frondosa*), PL (from *Phellinus linteus*), PG101 (from *Lentinus lepideus* (Fr.) Fr.), CA1 β-glucan fraction and SCG (from *Sparassis crispa*), and befungin (from *Inonotus obliquus* Pers. (Fr.) Boud. et Sing.) (Chen & Seviour 2007; Kidd 2000; Lull *et al*. 2005; Zhang *et al*. 2011).

Mushroom polysaccharides are among the emerging new agents that could directly support or enhance immunotherapy, and their safety in use is important in biomedical science. More than 50 mushroom species have yielded potential immunoceuticals that exhibit anticancer activity *in vitro* or in animal models and of these, only a few have been investigated in human cancers. The β-D-glucans or β-D-glucans linked to proteins are currently the most promising class of immunoceuticals, displaying stronger immunoenhancing activity than the corresponding free glucans (Kidd 2000; Petrova *et al*. 2005; Vannucci *et al*. 2013; Wasser 2014).

A number of mushroom immunoceuticals polysaccharides have proceeded through phase I, II, and III clinical trials. Lentinan (*L. edodes*), PSK and PSP (*T. versicolor*) have been used in clinical trials with hundreds of cancer patients (stomach, colorectal, esophageal, lung, breast, nasopharyngeal, and leukemia). Other compounds have only been assessed with a relatively small number of patients and in many cases, the standards of these trials may not meet the current Western regulatory requirements, although significant improvements in quality of life and survival of patients are reported (Paterson & Lima 2014). A number of Chinese patents on the medicinal application of lentinan administered orally (Sun & Wei 2007) or intravenously (Ma & Wang 2007) have been published. The effect of lentinan in prolonging life has been observed, especially in those with gastric and colorectal carcinoma, and this polysaccharide has been approved for clinical use in Japan for many years and is manufactured by several pharmaceutical companies (Zhang *et al*. 2011). Schizophyllan has also exerted beneficial activity for patients with head and neck cancers, recurrent gastric cancer, stage 2 cervical cancer, and advanced cervical carcinoma (Hobbs 2000).

PSK and PSP from *T. versicolor* have controlled various carcinomas in human clinical trials. In Japanese trials undertaken since 1970, PSK significantly extended survival at five years or beyond in stomach, colorectal, esophagus, nasopharyngeal, and lung (nonsmall cell types) cancers, and in a HLA B40-positive breast cancer subset. PSP was subjected to phase II and III trials in China. It significantly improved quality of

life and enhanced immune status in 70–97% of patients with stomach, esophagus, lung, ovary, and cervix cancers. PSK and PSP boosted immune cell production, ameliorated chemotherapy symptoms, and enhanced tumor infiltration by dendritic and cytotoxic T cells. Their high tolerability, proven benefits to survival and quality of life, and compatibility with chemotherapy and radiation therapy make them well suited for cancer management regimens (Kidd 2000).

In clinical studies, *G. lucidum* products have been widely used as a single agent or in combination with other herbal medicines or chemotherapeutic drugs, mainly in Asian countries. However, randomized, placebo-controlled and multicenter clinical studies using *G. lucidum* alone have rarely been reported. In one randomized, placebo-controlled clinical study, 143 patients with advanced previously treated cancer were given an oral *G. lucidum* polysaccharide extract (Ganopoly) of 1800 mg three times daily for 12 weeks. The prostate-specific antigen (PSA) levels in the five prostate cancer patients were reduced significantly, indicating that Ganopoly may have an adjunct role in the treatment of patients with advanced cancer although objective responses were not observed (Gao *et al*. 2002). A polysaccharide injection formulated from *G. lucidum* has been also developed (Jiang *et al*. 2014).

Although the maitake D-fraction is a relatively new compound, the claims of benefit are encouraging. There are a number of clinical trials in breast, prostate, lung, liver, and gastric cancers under way in the US and Japan, and several US physicians have reported good results with maitake D-fraction. Grifolan-D accomplished (>95%) cell death of prostate cancer cells *in vivo* and hindered metastatic progress, increased NK cell activity, and maintained the elevated levels of cytotoxicity for more than one year (Kodama *et al*. 2003).

Much recent research has been carried out on *Pleurotus* spp. crude extracts and isolated compounds such as polysaccharides, proteins, and other substances that possess antitumor and immunostimulatory activities (Gregori *et al*. 2007). Antitumor effects have been shown on different human tumor cell lines. From these results, POPS-1, a water-soluble polysaccharide from the fruiting bodies of *P. ostreatus*, has been considered as a potential candidate for developing a novel low-toxicity antitumor agent (Tong *et al*. 2009). A hot water mycelial extract from *Pleurotus* spp. (76.8% polysaccharides) exerted *in vitro* antiproliferative activity against human NB4 leukemia cells through apoptosis induction and cell cycle arrest in the G_2/M phase (Morris *et al*. 2014b). In light of its effects on macrophage phagocytosis and the hematopoiesis response of mice that would otherwise remain damaged by radiation and chemotherapy substances, this extract could be considered as a candidate for radio- and chemoprotective therapy (Llauradó *et al*. 2015; Morris *et al*. 2003). Used as an immunoceutical, *Pleurotus* fruiting body powder (55% polysaccharides) given orally for seven days (1000 mg/kg) to cyclophosphamide-treated mice potentiated the cellular immune response and the lymphoproliferative-stimulating index (Llauradó *et al*. 2013). Thus, *Pleurotus*-based products could be promising for clinical immunotherapy applications.

There are plenty of clinical studies proving the cancer inhibitory effects of other mushrooms such as *Inonotus obliquus*, *Phellinus linteus*, *Flammulina velutipes*, *Cordyceps sinensis* (Berk.) Sacc., etc (Wasser 2014). For example, studies conducted for antitumor activities at the National Cancer Center (Japan) demonstrated that extracts containing polysaccharides and glycoproteins prepared from *Hypsizygus marmoreus*

and *F. velutipes* showed positive effects on the cachexia of advanced cancer patients. These extracts had better effects than methyl-acetoxy-progesterone in clinical response, performance status, and quality of life (Ikekawa 2005). Befungin (a multi-compound preparation containing 50% of β-(1-3),(1-6) glucans, terpenes, phenols, steroids, organic acids, and microelements) obtained from *Inonotus obliquus* was approved as an antitumor drug in Russia and reportedly successful in treating breast, lung, cervical, and stomach cancers (Badalyan 2014).

Mushroom immunoceuticals act primarily by augmenting all the key pathways of host immunity, both innate and adaptive, and signaling cascades. Due to a high potential for structural variability, polysaccharides have the necessary flexibility to affect the precise regulatory mechanisms of various cell–cell interactions (Wasser & Weis 1999). The antitumor action of polysaccharides requires an intact T cell component; their activity is mediated through a thymus-dependent immune mechanism. They activate cytotoxic macrophages, monocytes, neutrophils, natural killer (NK) cells, dendritic cells (DCs), B cells, and chemical messengers (cytokines, such as interleukins, interferons, and colony-stimulating factors) that trigger complement and acute-phase responses. Also, mushroom polysaccharides induce gene expression of various immunomodulatory cytokines and cytokine receptors (Lull *et al.* 2005; El Enshasy & Hatti-Kaul 2013; Zhang *et al.* 2007). The first step of action of these metabolites is their recognition by certain receptors located on different immune cells and activation of signal transduction pathways. It has been clarified that several β-glucan receptors mediate these activities, such as complement receptor 3 (CR3, αM β2-integrin, CD11b/CD18), lactosylceramide, glycosphingolipid, scavenger receptors, dectin-1, TLR-2, and TLR-4 (Brown *et al.* 2007; Li *et al.* 2011; Moradali *et al.* 2007).

In sum, a new class of antitumor and immunomodulating medicinal mushroom drugs (the biological response modifiers (BRMs)) is emerging in the clinical scene. The application of BRMs as a special type of immunotherapy to target and eliminate cancer cells could represent a new kind of cancer treatment together with surgery, chemotherapy, and radiotherapy (Mizuno 1999; Wasser 2002, 2014). Findings suggest that some mushrooms work in synergy with commercial anticancer drugs as an effective tool for treating drug-resistant cancers. Antitumor monoclonal antibodies in conjunction with β-glucans have been considered as a novel anticancer immunotherapy against GD2 ganglioside, G250 protein, and CD20 protein, respectively in experimental neuroblastoma, carcinoma, and $CD20^{+}$ lymphoma (Vannucci *et al.* 2013; Xiang *et al.* 2012). Mushroom β-glucans might also have synergistic effects with monoclonal antibodies used in cancer treatment similar to yeast β-glucans.

More than 30 mushroom extracts and fungal compounds are currently being investigated in clinical trials by the National Institutes of Health in the US. Table 5.2 lists some of these clinical trials with mushroom polysaccharides or polysaccharide-rich extracts/powders. The addition of new areas of application, apart from the immunological use in oncology, opens interesting perspectives and makes the study of β-D-glucans a prospective field of research. For example, β-D-glucans also appear suitable for use in nanomedicine for preparation of nanocarriers for drug or biological molecule delivery (Soto *et al.* 2012).

In addition to high molecular weight polysaccharides, another anticipated application of mushroom species is concerned with the active pool of secondary metabolites with low molecular weight (phenolic acids, flavonoids, terpenoids, lactones, quinones, steroids,

Table 5.2 Selection of recent clinical trials conducted with polysaccharide-rich mushroom-derived preparations.

Official title	Intervention	Subjects	Purpose
Immune Benefits from Mushroom Consumption (University of Florida/ Mushroom Council) Last updated: December 2, 2013	Dietary supplement: 3 or 6 ounces (around 28 g) daily for 4 weeks	52 healthy patients	To determine whether consuming shiitake polysaccharide-rich mushroom is effective in enhancing the function of γ δ T cells
A Translational Breast Cancer Prevention Trial of Mushroom Powder in Postmenopausal Breast Cancer Survivors (City of Hope Medical Center/National Cancer Institute) (NCI) Last updated: June 5, 2014	Drug: white button mushroom extract Dose escalation beginning at 5 g/ day, then 8, 10 up to 13 g/day	16 females with breast cancer, 21 years and older	To show that a wholefood extract of white button mushrooms can inhibit aromatase-induced estrogen biosynthesis in women who are breast cancer survivors
Does Maitake Mushroom Extract Enhance Hematopoiesis in Myelodysplastic Patients? A Phase II Trial (Memorial Sloan-Kettering Cancer Center/ Yukiguni Company) Last updated: September 3, 2014	Patients will receive maitake mushroom extract orally 3 mg/kg twice daily for 3 months	43 myelodysplastic patients, age 18 or older	To see whether maitake improves the hematopoietic response, in particular, neutrophil count and function, in myelodysplastic patients
Efficacy and Safety of Cauliflower Mushroom Extract on Promotion of Immunity (Chonbuk National University Hospital) Last updated: November 26, 2012	Phase II and III Dietary supplement: cauliflower mushroom extract (1 g/day), for 12 weeks	60 males and females, 30 years to 65 years	To evaluate the efficacy and safety of cauliflower mushroom extract on promotion of immunity (IL-10, IFN- γ, TNF-α, and blood cell counts)
Phase Ib of Mushroom Powder in Biochemically Recurrent Prostate Cancer (City of Hope Medical Center/National Cancer Institute) (NCI) Last updated: October 9, 2014	Drug: white button mushroom extract. Dosages: 4, 6, 8, 10, 12, and 14 g/day	36 male patients	To study the side-effects and best dose of white button mushroom extract in treating patients with recurrent prostate cancer after local therapy
A Randomized, Parallel, Double-blind, Placebo-controlled, Pilot Clinical Study on the Effects of Yunzhi as Dietary Supplement in 60 Adult Patients Undergoing Adjuvant/ Neoadjuvant Chemotherapy for Breast Cancer (Hospital Clinic of Barcelona) Last updated: December 14, 2010	Dietary supplement: Yunzhi extract from *Coriolus versicolor* 3.5 g/day	60 women patients with diagnosis of breast cancer, 18 years and older	To assess the effects of the traditional Yunzhi mushroom, as adjuvant in the treatment of patients with breast cancer

Table 5.2 (Continued)

Official title	Intervention	Subjects	Purpose
Use of the Medicinal Mushroom *Agaricus blazei* Murill in Addition to High Dose Chemotherapy in Patients With Multiple Myeloma (Ullevaal University Hospital) Last updated: February 22, 2014	Phase II Intake of 60 mL *A. blazei* daily in addition to chemotherapy. Commercial name: AndoSan™	39 patients scheduled to undergo high-dose chemotherapy with autologous stem cell support for multiple myeloma	To assess the effects of *Agaricus* extract (AndoSan™) in addition to chemotherapy on cytokine levels as well as treatment response and quality of life of patients with multiple myeloma

Adapted from: http://clinicaltrials.gov/ct2/results?term=mushroom

and alkaloids) that have antitumor, antimicrobial, and antiviral properties. The scientific investigation of these compounds has gained momentum in recent years because they are simpler chemically and equivalent to existing fungal-based pharmaceuticals, such as penicillin and cephalosporins (Patel & Goyal 2012; Paterson & Lima 2014).

Mushroom terpenoids (tri- and sesquiterpenes) have cytotoxic, antibacterial, antifungal, hypocholesterolemic, hypoglycemic, hypotensive, and antioxidant effects (Badalyan 2014). About 400 bioactive molecules have been isolated from *Ganoderma* species: *G. lucidum*, *G. applanatum* (Pers.) Pat., and *G. tsugae* Murrill. Among them, lanostane-type triterpenoids are promising candidates for the development of antitumor drugs (Fatmawati *et al*. 2013). Ganoderic acids, ganoderenic acids, ganodermic acids, applanoxidic acids, ganoderals, ganoderols, lucidone, ganodermanontriol, and ganodermanondiol are some of the basidiomycetous triterpenoids. In spite of the fact that many triterpenoids have been discovered in mushrooms, few studies have been done to elucidate the mode of action of their anticancer and immunomodulating effects. The research performed on *G. lucidum* has shown that such triterpenoids could activate the NF-kB pathway and modulate Ras/Erk, c-myc, CREB protein, and mitogen-activated protein kinases, leading to other immune activations against tumor cells (Calviño *et al*. 2010; Moradali *et al*. 2007; Petrova *et al*. 2008).

Hispolon, an active phenolic compound extracted from *Phellinus* spp., is known to possess potent antineoplastic properties and to potentiate the cytotoxicity of chemotherapeutic agents. Hispolon induces epidermoid and gastric cancer cell apoptosis and, regardless of p53 status, it inhibited breast and bladder cancer cell growth. Aa crucial role of hispolon in ubiquitination and downregulation of MDM2 (the protooncogene inhibiting the tumor suppressor function of p53) was reported, suggesting this phenolic compound as an attractive therapeutic strategy in breast, gastric, and bladder cancers (Chen *et al*. 2008; Lu *et al*. 2009).

As for low molecular weight mushroom compounds, only a minute fraction have proceeded to a higher level of clinical evaluation. In this group, irofulven (6-hydroxymethylacylfulvene), a novel synthetic antitumor agent derived from the sesquiterpene illudin S of *Omphalotus olearius* (DC.) Singer, has been one of the most extensively studied. Phase II clinical trials were performed in different tumors (advanced melanoma, advanced renal cell carcinoma, metastatic colorectal cancer, and recurrent or

persistent endometrial carcinoma), but unfortunately irofulven demonstrated minimal to no significant antitumor activity in these trials (Zaidman *et al*. 2005). There are still ongoing phase II clinical trials by MGI Pharma in recurrent ovarian cancer, hormone-refractory prostate cancer, and recurrent malignant glioma (http://adisinsight.springer.com/drugs/800006987; Sborov *et al*. 2015).

As mentioned above, low molecular weight mushroom metabolites exhibit an extraordinary diversity but their investigation in clinical trials and use as drugs is currently scarce. Table 5.3 presents an overview of some compounds whose pharmacological activities have been tested at the preclinical level, in some cases with contradictory results depending on the model used, sample concentration, etc. Overall, *in vivo* activity studies are limited when compared with *in vitro* studies. The compound quantity of natural products might be one reason for screening biological activities *in vivo*. Efforts should be made to find new sources for anticancer drugs using low molecular weight mushroom metabolites that can inhibit or trigger specific responses, i.e. activating or inhibiting NF-κB, inhibiting protein and especially tyrosine kinases, aromatase and sulfatase, matrix metalloproteinases, cyclo-oxygenases, DNA topoisomerases and DNA polymerase, antiangiogenic substances, etc. (Chang & Wasser 2012; Patel & Goyal 2012; Petrova *et al*. 2008; Zaidman *et al*. 2005).

The available information about bioactive molecules of medicinal mushrooms suggests that these may be powerful sources from which to develop novel pharmaceutical products. It is hoped that as technology advances for the production of mushroom drugs, there will be increased clinical research to ensure their safety and efficacy, thus validating many claims made for the medicinal use of these products. As Chang and Miles (2004) stated, "Anecdotal accounts are interesting and may be useful, but scientific experimentation is essential."

5.4 Conclusion

There is no better time for mushroom products to emerge as judged by their positive impact on human quality of life. Recent basic and applied studies in mushroom metabolism, biotechnology, and clinical trials represent a large contribution to the expansion of mushroom potentialities for the development of functional foods, nutraceuticals, and novel drugs.

Mushroom functional foods represent an opportunity to obtain innovative products that would help to satisfy the demand that already exists. In addition, different mushroom formulations provide health-enhancing nutraceuticals for healthy and subhealthy people. Although not "magic" products like those of "Alice in Wonderland," based on the multiple biological properties of mushroom nutraceuticals, the view of Stephen DeFelice that "One good nutraceutical can wipe out the drugs" has gained momentum in recent years.

However, many of the bioactive properties attributed to mushroom functional foods and nutraceuticals are based on data obtained from *in vitro* and animal experiments (Vikineswary & Chang 2013). Well-designed and -conducted clinical trials and better insight into the mechanism underlying the biological action of mushrooms will accelerate commercial production of myco-pharmaceuticals. A more detailed chemical and biological characterization of both high and low molecular weight biologically active

Table 5.3 Overview of the pharmacological activity of some low molecular weight compounds from mushrooms in various *in vitro/in vivo* systems.

Mycochemical family	Examples of compounds	Mushroom	Pharmacological effect
Terpenoids	Irofulven (illudin's derivative)[1,6,8]	*Omphalotus illudens* *Suillus placidus*	Antitumor activity against human pancreatic carcinoma cell lines *in vitro* and *in vivo*, HT-29 and HCT-116 colorectal and A2780 ovarian carcinoma cells, head and neck, nonsmall cell lung, malignant glioma, colon, ovary and prostate cancer. Phase II clinical trials are ongoing
	Triterpene-enriched fraction WEES-G6 (especially ganoderic acid F)[1,7]	*Ganoderma lucidum*	Selective growth inhibition of Huh-7 human hepatoma cells. It caused a rapid decrease of PKC and the activation of JNK and p38 MAPK protein kinase signaling pathways. Inhibition of angiogenesis in an *in vivo* model
	7-oxo-ganoderic acid Z and 15-hydroxy-ganoderic acid S		Inhibition activity against HMG-CoA reductase and acyl CoA acyltransferase.
	Ganoderic acid C2[1,3,7]		Apoptosis induction in NB4 human leukemia cells. Effective against cell proliferation and colony formation in MCF-7 human breast adenocarcinoma cell line; mediated G1 cell cycle arrest
	Ganoderic acid X[1,7]		Inhibition of DNA synthesis in human hepatoma cell lines (Huh-7), inhibition of topoisomerase I and Iiα, activation of apoptosis and inhibition of protein kinases
	Lucidenic acid B[1,7]		Implicated in the inhibition of Erk on HepG-2 human liver cells, apoptosis
	Tricholomalides A, B and C[1,7]	*Tricholoma* spp.	Induction of neurite outgrowth in rat PC-12 cells
	Sarcodonin G[7]	*Sarcodon scabrosus*	Suppression of inflammation induced by TPA, activation of caspases-3 and -9 and increased Bax/Bcl-2 ratio, antiproliferative activity against HOC-21, HEC-1, U251-SP, MM-1CB, and HMV-1 human cancer cell lines.
	Eryngiolide A[7]	*Pleurotus eryngii*	Cytotoxic effects against Hela and HepG2 tumor lines by using MTT assay
Steroids	Ergosterol and ergosterol peroxide[6-8]	Multiple species	Increase serum 25(OH) vitamin D_2 levels (*in vivo* – humans). Antibreast cancer. Direct inhibition of angiogenesis induced by solid tumors. Inhibition of leukemic cells proliferation
	Blazein[6,8]	*Agaricus blazei*	Induction of apoptotic chromatin condensation in human lung cancer cells and stomach cancer cells
	Ergosta-4,6,8(14),22-tetraen-3-one (ergone)[5]	*Russula cyanoxantha*	Cytotoxic and antiproliferative activity towards HepG2 cells through apoptosis induction and G2/M cell cycle arrest

(*Continued*)

Table 5.3 (Continued)

Mycochemical family	Examples of compounds	Mushroom	Pharmacological effect
Nucleotide-derivatives	Cordycepin[2,4-6,8]	*Cordyceps militaris*	Inhibition of human leukemia cell growth by inducing apoptosis through a signaling cascade involving a ROS-mediated caspase pathway. It continues to be a potentially useful tool to identify therapeutic targets
	Clitocine[6,8]	*Leucopaxillus giganteus*	It targets Mcl-1 to induce drug-resistant human cancer cell apoptosis *in vitro* and tumor growth inhibition *in vivo*
Phenolic compounds	Hispolon[5,7]	*Phellinus* spp.	Inhibition of breast and bladder cancer cell growth, potentiation of cytotoxicity of chemotherapeutic agents used in the clinical management of gastric cancer
	Caffeic acid phenethyl ester (CAPE)[1,2,4]	*Agaricus bisporus, Lentinus edodes, Phellinus linteus, Marasmius oreades*	Specific cytotoxicity against tumor cells, shows NF-κB inhibitor activity, and can be a candidate for antitumor drugs, especially against breast cancer
	Genistein (an isoflavone)[1]	*Flammulina velutipes*	Modulates G2/M checkpoint and apoptosis induction and suppresses proliferation of p53 null human prostate carcinoma cells. Inhibition of several tyrosine kinases and topoisomerases. Also acts as antioxidant
Alkaloids	Norsesquiterpene alkaloid[7]	*Flammulina velutipes*	Cytotoxicity against human cervical carcinoma KB cells *in vitro* by using the MTT assay
	Isohericenone, isohericerin and erinacerin A[6-8]	*Hericium erinaceum*	Cytotoxic activity against HCT-15, SK-MEL-2, SK-OV-3, and A549
	Sinensine[7]	*Ganoderma sinense*	Biological activity in protecting H_2O_2 oxidation-induced injury on human umbilical vein **e**ndothelial **c**ells
Lactones	Clavilactones A, B and D (respectively CA, CB and CD) and two semisynthetic derivatives (diacetyl-CA and dimethyl-CA)[1]	*Clitocybe clavipes*	Inhibitory activity in kinase assays against the Ret/ptc1 and epidermal growth factor receptor (EGF-R) tyrosine kinases, weak inhibition activity when administered to mice bearing the ascitic A431 tumor

Sources: Zaidman *et al.* (2005)[1]; Petrova *et al.* (2008)[2]; Calviño *et al.* (2011)[3]; Chang & Wasser (2012)[4]; Patel & Goyal (2012)[5]; Roupas *et al.* (2012)[6]; Öztürk *et al.* (2014)[7]; Paterson & Lima (2014).[8]

MAPK, mitogen-activated protein kinase; MTT, (3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide; NF-κB, nuclear factor κB; PKC, protein kinase C; ROS, reactive oxygen species; TPA, 12-O-tetradecanoylphorbol 13-acetate.

compounds from different mushroom species appears necessary to better define the rationale for their application in anticancer therapies as well as in other pathologies. Glucan and proteoglycan immunoceuticals acting as biological response modifiers are effective immune boosters for individuals afflicted with cancer or impaired immunity and possess a unique clinical versatility. Interest in the investigation of new and powerful low molecular weight compounds has increased due to the wide range of their medicinal activities.

The target for the future should be to adopt regulations, standards, and practices from Western and Eastern medicine that have proven to be the most valuable in the quest for health benefits (Wasser 2014). Further sustainable research on the natural and genetic resources of edible and medicinal mushrooms using improved screening methods of OMICs sciences will assist future usage of their bioactive myco-compounds to develop unique health biotech products with a positive impact on human welfare. In sum, this chapter provides insights into the possible uses of mushrooms as functional foods, nutraceuticals, and drugs. The present status and future prospects suggest great potential for upgrading mushroom species from functional food to translational mushroom medicine.

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