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Review Article

A Review on Health Benefits of Medicinal Mushroom *Agaricus Subrufescens*

MAURYA MANGLA*, CHANDRA SURESH, SAHU SHACHI

Department of Pharmacology, Pranveer Singh Institute of Technology, Bhauti-Kanpur 208020, India

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ABSTRACT

Medicinal mushrooms have currently become an important issue due to their various therapeutic properties. Of these, *Agaricus subrufescens,* formerly named *A. blazei* Murrill, has been used as dietary mushroom and to formulate nutraceuticals and functional foods. Since its discovery in 1893, this mushroom has been cultivated throughout the world, especially in Brazil where several strains of A. subrufescens have been developed. This mushroom is rich in the immunomodulating polysaccharides, *β*-glucans, and has been shown to have antitumor, anti-infection, and antiallergic/-asthmatic properties in mouse models, in addition to anti-inflammatory effects in inflammatory bowel disease patients. This article presents up-to-date information on this mushroom including its taxonomy and health promoting benefits. Medicinal properties of A. subrufescens are emphasized in several studies which are reviewed here.

INTRODUCTION

The uses of mushrooms as nutritional or therapeutic bases were sometimes daily or even decisive in human progress. They usually exhibit special fragrance and texture [1]. *Agaricus subrufescens* (incorrectly known as *Agaricus blazei*, *Agaricus blazei Murrill*, *Agaricus brasiliensis*, *Agaricus sylvaticus* or *Agaricus rufotegulis*) is a species of mushroom, commonly known as Almond mushroom, Mushroom of the Sun, God´s Mushroom, Mushroom of Life, Royal Sun Agaricus, Jisongrong or Himematsutake (Japanese:, "princess matsutake") and by a number of other names [2,3]. Agaricus subrufescens is a choice edible, with a somewhat sweet taste and fragrance of almonds. This mushroom is also well known as a medicinal mushroom, for its purported medicinal properties, due to research which indicates it may stimulate the immune system [4]. Higher fungi have been used by mankind for millennia. Firstly, they are used as part of regular diet for their nutritional value, completing population food intake. They contain minerals, vitamins & nutritive such as proteins, polysaccharides & have low fat content. Secondary, mushrooms fruiting bodies are also appreciated for delicacy. Thirdly, higher fungi are used for medicinal purpose. Their pharmacological action and therapeutic interest in promoting human health are also known for thousands of years. This mushroom has been used as a medicinal food for the prevention of cancer, diabetes, hyperlipidemia, arteriosclerosis, and chronic hepatitis and is known to impact putatively the immune system [5]. Potential effective compounds of this mushroom can be isolated either from their fruiting bodies, or even from pure culture of mycelia and culture broth filtrate.

Agaricus subrufescens often occurs in domesticated or semi disturbed habitats, including leaf piles. It has been recognized occasionally growing “wild” outside northeastern North America, for example in California [6], Israel, Taiwan [7] and Hawaii, where it grows under forest trees.

The aim of this present paper is to summarize the available information on A. subrufescens, including its taxonomy, phylogeny, distribution, various health benefits and current status of scientific research.

CHARACTERISTICS AND TAXONOMY

A. subrufescens Peck is a gilled fungus belonging to the family of the Agaricaceae in the order Agaricales [9] within the phylum Basidiomycota. It is a saprobe and inhabits rotting leaves often at the borders between forests and parks. The

**Author for Correspondence:*
Email: manglamaurya13@gmail.com

basidiomata morphology of this species is variable. Sporocarps can be robust or gracile, both due to the genotype and environmental influences [3]. The cap is 20–70 mm broad in button stage and 60–150 mm broad in mature stage hemispherical to convex to plano-convex shape and fleshy. The surface is dry and covered by fibrillose squamulose hairs. The pileus color is somewhat variable and sensible to the light, ranging from brownish-gold, reddish brown, purple brown to brownish orange, more or less pale and sometimes completely white. The basidiospores are chocolate brown (5 ·4 lm) [9, 10]. The specimens from Thailand differ from those previously reported in terms of size, sturdiness and length of stipe, which were larger, more virgate and fragile than those found in America; their cap color is more reddish than those of the European taxa. The stipe is also highly variable which could be short and firm or more slender and virgate [3]. However, the cap shape and lamellae color were in concordance with those reported by Firenzuoli et al. (2008) from Brazil. The small cottony floccules beneath the remnant partial viel are a consistent character of the species. This important feature is also found in Thai taxa. The morphological variability of this species may be influenced by different climates and ecosystem.

Figure 1: Agaricus subrufescens.
(A)Basidiocarp, (B) Schaeffer's cross-reaction on pileus

Agaricus subrufescens was first described by the American botanist Charles Horton Peck in 1893 [11]. During the late 19th and early 20th century, it was cultivated for the table in the eastern United States [3].It was discovered again in Brazil during the 1970s, and misidentified as *Agaricus blazei* Murrill, a species originally described from Florida. It was soon marketed for its purported medicinal properties under various names, including *ABM* (for Agaricus blazei Murill), *Cogumelo do Sol* (mushroom of the sun), *Cogumelo de Deus* (mushroom of God), *Cogumelo de Vida* (mushroom of life), *Himematsutake*, *Royal Sun Agaricus*, *Mandelpilz*, and *Almond Mushroom*.

In 2002, Didukh and Wasser correctly rejected the name *A. blazei* for this species, but unfortunately called the Brazilian fungus *A.brasiliensis* [2] a name that had already been used for a different species, *Agaricus brasiliensis* Fr. (1830). Richard Kerrigan undertook genetic and interfertility testing on several fungal strains and showed that samples of the Brazilian strains called *A. blazei* and *A. brasiliensis* were genetically similar to, and interfertile with, North American populations of *Agaricus subrufescens*. These tests also found European samples called *A. rufotegulis* to be of the same species. Because *A. subrufescens* is the oldest name, it has taxonomical priority.

CHEMICAL COMPOSITION

The average composition of mushrooms is normally 90% water, 2-40% protein, 2-8% fat, 1-55% carbohydrate, 3-32% fibber, yielding 8-10% ash [12]. Several categories of molecules are supposed to be involved in beneficial effects and most of the molecules categories found in *A. subrufescens (A. blazei* Murill) are common to the entire fungal kingdom. Active compounds found in *A. blazei* are listed in Table 1. The active compounds isolated from *A. subrufescens* are believed to be mainly polysaccharides [13-16] β-glucan [17-19, 22] and glucomannan [20-22] or riboglucan [24] (Table 1). Additionally, in *A. blazei,* a protein (or glycoprotein), Glucan protein complex, a lectin [23, 24] originally found associated to β (2, 25) -glucan, were characterized and claimed to be anti-tumor (Table 1). Recently, it has been shown that *A. blazei* sp do contain aromatic hydrazines, especially agaritine [26] (Table 1) and its derivatives [27] More recently, blazein a steroid derivative found in *A. blazei (A. subrufescens)*, was shown to kill Human lung cancer LU99 cells but not normal Human lymphocyte [28]. Agartine extracted from *A. blazei* was also showed to kill leukemic cell in vitro. Its cytotoxicity is triggered cell apoptosis with an IC50 in the range of 2.7 to 16 µg /ml depending on the cell line [29, 30]. Agartine is found at similar concentration in both *A. blazei* and *A. bisporus* (approximately 1.8 mg/g dry weight) [27].

Blazein (named after the synonym A. blazei) is a steroid derivative found in A. subrufescens with an in vitro anti-cancer activity on human lung cancer LU99 cells without affecting normal human lymphocytes [29].

Table 1: Bioactive compound from Agaricus subrufescens

Bioactive Compounds	Main Potential Effects	Reference
Polysaccharides		
β-(1,6)-glucan	Immuno-active involved in anti tumour effect and/or prevention; antimicrobial	[13], [14], [15], [16]
α-(1,4)-; β-(1,6)-glucan		[14]
α-(1,6)-; α-(1,4)-glucan		[17], [18]
β-(1,6)-; β-(1,3)-glucan		[19]
β-(1,6)-; α-(1,3)-glucan		[19]
Glucomannan		[19]
β-(1,2)- β-(1,3)-glucomannan		[20], [21], [22], [15]
Glucan-Protein complex	Oncogenesis prevention	[14], [17], [18]
Lectin	Anti-tumour	[23]
Riboglucan	Anti-tumour	[24]
Ergosterol	Anti-tumour	[5]
Sodium pyroglutamate	Anti-tumour	[31]
RNA-protein complex	Anti-tumour	[32]
Agaritine	Anti-tumour	[26]
Blazein	Anti-tumour	[28]

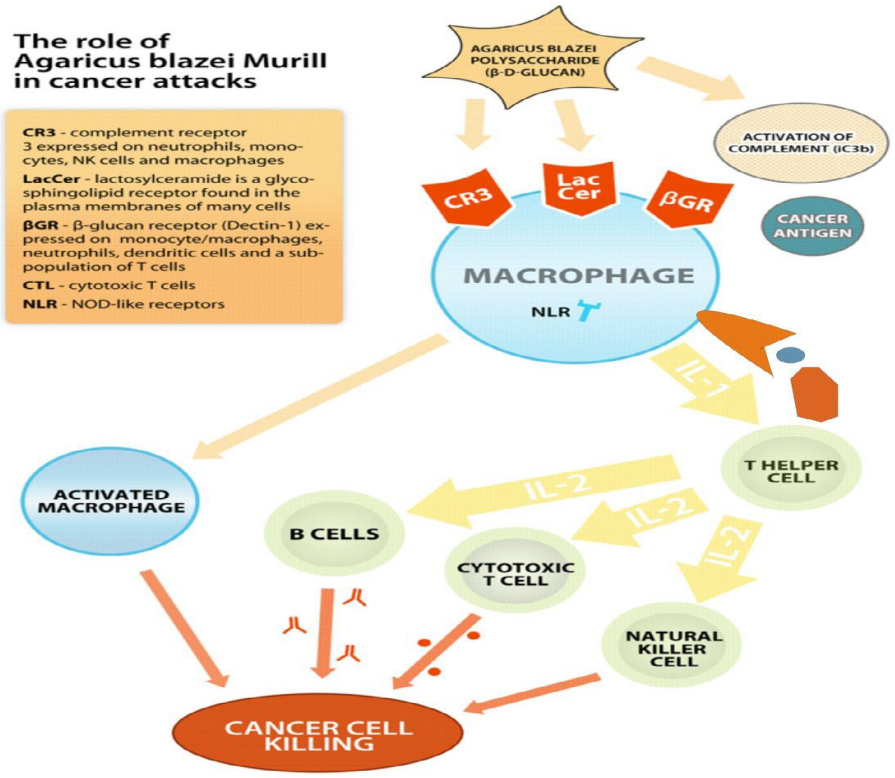

Figure 2: Role of agaricus blazei murill in cancer attacks

PHARMACEUTICAL PROPERTIES

A. subrufescens is a well-known medicinal mushroom used in many countries, and thus consumption of this mushroom is used as an alternative way to cure diseases. Various pharmaceutical activities have been found associated with A. subrufescens and researches to reveal the function of bioactive compounds are extensive. Recent studies have been performed in vitro and in vivo to confirm the mushrooms therapeutic properties [9]. Identification of (novel) immunomodulating bioactive compounds from the mushroom may also help in new treatments for patients suffering from cancer and immunodeficiency [33].

ANTI-CANCER AND TUMOR SUPPRESSIVE ACTIVITY

There have been many studies showing the anti-tumor activity of A. subrufescens [9]. In the last decade, many bioactive substances (Table 1) have been shown to exhibit the potential anti-tumor properties. The major anti-tumor substances from A. subrufescens are polysaccharide-enriched extracts and protein–bound polysaccharide complexes [34-36]. Mushroom polysaccharides prevent oncogenesis, show direct antitumor activity against various synergetic tumors, and prevent tumor metastasis. Their activity is especially beneficial when used in conjunction with chemotherapy. 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, neutrophiles, natural killer cells, dendritic cells, and chemical messengers (cytokines, such as interleukins, interferons, and colony stimulating factors) that trigger complementary and acute phase responses. Also, mushroom polysaccharides can be considered as multicytokine inducers able to induce gene expression of various immunomodulatory cytokines and cytokine receptors [13, 37-44].

The anti-tumor mechanisms of A. subrufescens extracts have been shown by oral administration in different laboratory mice models such as Sarcoma 180-implanted bearing mice [5], Meth-A fibrosarcoma tumor-bearing mice [34, 23, 19, 35, 17, 5] and BALB/c nu/nu mice implanted leukemic model [45, 46] extracted the polysaccharide complex from A. subrufescens and characterized a glucan–protein complex by FTIR, 13C NMR and 1H NMR spectroscopy. The extract exhibits both of α and β glycosidic linkage of glucans. The result indicates that β-glucans are the predominant structures as compared to α-glucans. Moreover, it also can increase the antitumor activity of other chemotherapeutics.[18]
A new class of antitumor medicinal mushroom drugs has been called biological response modifiers (BRMs). The application of BRMs has become the new kind of cancer treatment together with surgery, chemotherapy, and radiotherapy [13, 47-49].

ANTI-GENOTOXICITY ACTIVITIES

Some in vitro and in vivo genotoxic studies (e.g. DNA damage and aberration tests) have been performed on chemical carcinogenesis models. In a in vitro study by Angeli et al., [50] the authors suggested that -glucan present in *A. subrufescens* has no genotoxic or mutagenic effect, but protects the damaged DNA (Deoxyribonucleic acid) caused by benzopyrene in test protocols. Results indicate that the beta-glucan works through a link with benzopyrene by capturing free radicals during their activation. To investigate the possible antimutagenic effect of *A. subrufescens* in vivo, Delmanto et al. [51] evaluated its effect on clastogenicity induced by Cyclophosphamide (CP) in mice, using the micronucleus test in bone marrow (MNPCE) and in peripheral blood (MNRET). Male Swiss mice were treated with CP (25 or 50mg/kg i.p.) or with CP plus mushroom solution at three different temperatures: 4, 21, and 60 degrees C. Aqueous solution of a mixture from various lineages of the mushroom inhibited induction of micronuclei by CP in bone marrow and in peripheral blood of mice. In contrast to the mixture of lineages, a single isolated lineage did not lead to a reduction of CP-induced MN frequencies in either bone marrow or blood cells of mice. The results suggest that under certain circumstances these mushrooms exhibit antimutagenic activities that might contribute to an anticarcinogenic effect.

BIOLOGICAL ACTIVITIES ON THE IMMUNE SYSTEM

The immunostimulants and immunomodulatory activity of both mycelial and fruiting bodies of A. subrufescens using water and ethanol extracts have been demonstrated in many in vitro experiments, although not always the results are concordant, but sometimes contradictory. Water extracts of the mycelial culture and fruiting bodies such as fractions B-4, B-5 obtained from ethanol precipitation (respectively 44% and 50%) of fruiting bodies, markedly induced TNF production and IL-8 of macrophages derived from rat bone-marrow [52]. Other extracts containing lignin-based derivatives have shown the induction of TNF-g, IL-8 and nitric oxide secretion by macrophages [53], anti-viral activity of different viruses [54], and direct anticancer activity [55]; so lignin derivatives apparently have more different and important pharmacological activities. Fine particles of *A. subrufescens* fruiting body and mycelium, respectively, prepared by mechanical disruption, activated the human complement system via the alternative pathway in human serum, is another proof of its activity in enhancing natural immunity in bacterial infections [56]. From these studies it is

clear that the A. subrufescens fractions act on many different biological receptors of the immune system.

CYTOKINE INDUCTION

A. subrufescens is shown to stimulate cytokine production, such as interleukin-12 [57], interferon-c and natural killer (NK) activity. The water-soluble extracts (proteo-glucans) from crude A. subrufescens up regulate the in vitro maturation of dendritic cells [58, 59]. Ex vivo experiment on total heparinised blood of volunteers and in vitro experiment show that the monocytes-derived dendritic cells from peripheral blood mononuclear cells produce an increase level of cytokine and chemokines. The most abundant cytokines after A. subrufescens stimulation were mainly pro-inflammatory cytokine and chemocytokines IL-8, G-CSF, TNF-α, IL-1β, IL-6, IL 17 and MIP-1β.

The inhibition of pro-inflammatory cytokines appears to be dose dependant [60]. This is in contradiction with most of the tests made in murine models with different A. blazei extracts. This contradiction can be explained only by the capacity of certain glucans to cross the murine intestinal barriers. Ex vivo experiment with healthy volunteers heparinised blood controversially showed stimulation of all pro-inflammatory cytokines tested as expected. More recently, a clinical test with patients suffering of inflammatory bowel disease (IBD) inflammatory disease of colon and intestine has been conducted [59]. Patients with Chron's disease (CD) widely regarded as an autoimmune disease, and ulcerative colitis (UC) were exposed orally to A. blazei enriched mixture (AndoSan). After 12 days ingestion, the A blazei extract promotes in these patients anti-inflammatory effects with no side effect.

IMMUNOLOGIC INTERVENTION

The specific mechanisms that contribute to an enhanced state of immunity remain partially understood. Recent insights in two rapidly expanding fields, the cytokine mediated homeostasis of mature lymphocytes by cytokines, such as interleukins and autoreactive T cells by $CD4^+CD25^+$ regulatory T cells, provide the foundation for what might be occurring. Recent advances in immunology have demonstrated the importance of local interactions between antigen presenting cells and effector cells such as natural killer cells and T-lymphocytes for an effective immune reaction against tumors [61]. Interferons stimulate such interactions, while interleukins play a central role in the activation of NK cells and T-lymphocytes. Interferons were investigated as potential anticancer agents because of their antiproliferative and cytotoxic effects, their ability to activate specific components of the immune system and their relatively modest toxicities. Increasing biological evidence supports the hypothesis that tumor-generated chemokines provide more than simply angiogenic signals. Tumor-derived chemokines may potentially act as inhibitors of anti-tumor immune responses as well as autocrine growth factors for the tumor. All these chemokines activating activities of A. subrufescens remain to be completely evaluated both in animal model and in real clinical practice. However, immunologically active glucans are (1-3)-β-D-linked glucose polymers, which occur as a primary component in the cell walls of bacteria and fungi or are secreted extracellularly by various fungi, and actually seem the most important active substance.

EFFECTS ON INFECTION AND ALLERGY

There are a few studies in the anti-allergic test of A. subrufescens extract. Hetland et al found that an AbM-based extract (AndoSan,), also containing the medicinal *Basidiomycetes* mushrooms *Hericium erinaceum*(15%) and *Grifola frondosa* (3%), given orally increased survival from bacterial sepsis in mice inoculated i.p. a day afterward with pneumococci [62] or fecal bacteria [63]. The mixed mushroom extract also protected against IgE-mediated allergy in a mouse model when given p.o. either before or after ovalbumin s.c. sensitization of the animals [64]. In supernatants of cultured spleen cells from the sacrificed A. subrufescens treated mice, there was an increased T-helper cell response relative to the allergy-inducing Th2 response. The observation fits with the reduced specific serum IgE levels in these animals and shows that also adaptive immunity is engaged by the mushroom. Since the original Th1/Th2 dichotomy [65] says that the antitumor and anti-infection Th1 response is inversely related to the Th2 response, the spleen cell finding above also helps explain the concomitant antiallergic, antitumor, and anti infection effects of A. subrufescens.

A. subrufescens extract has also shown to have an antidiabetic activity in diabetic rats [58, 66, 67]. Purified β-glucan from this mushroom was shown to have anti-hyperglycemic, anti-hyper

triglyceridemic, anti-hypercholesterolemic and anti-arteriosclerotic activities indicating overall anti-diabetic activity [58, 66] which was also confirmed in β-glucan removed semi purified fractions of A. subrufescens [67]. An ethyl acetate fraction of hot-water extract from this fungus was tested on Streptozotocin-induced diabetic rats. Hypoglycemic action indicated the fungus could be useful in the treatment of diabetes mellitus [67].

COMPARISON OF A. SUBRUFESCENS EXTRACTS

There are many different A. subrufescens extracts available. Glucans, one of the most active and beneficial components of A. subrufescens, are known to augment immune responses against viral and bacterial infections, as well as against cancer [68]. It is important to point out that not all available A. subrufescens extracts give the same result because they are prepared by using different mushroom strains and in accordance to different protocols. Latest reports show that there are various compositions of beta-glucans in A. subrufescens extracts [69] and the concentration of active ingredients in each component depends on the methods of extraction [67, 52] and on the substrate (rotting woods) they are grown on.

A scientific study compared the efficacy of the leading products available in the market, revealing extracts with the strongest anti-infection properties. In order to evaluate antibacterial efficacy of A. subrufescens extracts, five A. subrufescens products described as A, B, C, D, E were compared in a mouse model for deadly pneumococcal (Gram-positive) sepsis. The day before the sepsis was induced, the mice received similar volumes of A. subrufescens extracts orally: Extract described as A, which was a mixture of 82% A. subrufescens, 15% Hericium erinaceum and 3% Grifola frondosa, all members of the Basidiomycetes mushroom family, appeared to be the only one which had a statistically significant protective effect [68]. Extract A was the most effective in reducing the number of bacteria in the blood of the infected mice and increasing the survival rate of the animals. In another study, using Gram-negative bacteria in mice, the efficacy of A. subrufescens extract in decreasing bacteremia and increasing survival rate was confirmed [70]. One may speculate whether this is due to the presence of additional biological components and synergies with the two other Basidiomycetes mushrooms ingredients as well as the protocols for cultivation and processing methods [68]. Extract A currently represents the AndoSan™ product, which appeared to be the only one that gave a significant protection to overcome deadly bacterial sepsis. Therefore, this particular mushroom extract was chosen for studies in other animal models and clinical studies (allergy, HCV infection, IBD, multiple myeloma, colon cancer). Hence, it may be a choice for prevention or treatment of different illnesses as an adjuvant.

TOXICOLOGICAL PROBLEMS

Many researchers have studied *Agaricus subrufescens*, as well as other medicinal mushrooms for close to 50 years. Although eating cultivated mushrooms or their extracts is thought to provide medicinal and other benefits, there are few studies on the safety of this practice. *Agaricus subrufescens has* been consumed by human for decades and has not been reported to be directly toxic or carcinogenic to humans. Nevertheless, we need to be cautious and consider if there are any deleterious effects from eating A. subrufescens. The content of poisonous microelements in fruiting bodies of this fungus has been reported. The content of Cobalt (Co), Molybdenum (Mo), Selenium (Se), Vanadium (V) are under detectable limits which are about 0.1–0.2 mg/kg of dry matter. However, the detectable amount of cadmium (Cd) is about 2–17 mg/kg of dry matter. The major concern over the use of A. subrufescens is the problem of the hydrazine content. A. subrufescens has been studied and assessed for possible side-effects of agaritine and its derivatives [26, 27] as these are suspected to be genotoxic and possible carcinogenic or tumorigenic agents. This molecule is thought to be capable of binding to the DNA of organs after administration to mice models [72]. The genotoxicity of agaritine is however, very limited. The cumulative lifespan of cancer risk obtained from agaritine consumption is probably estimated at $10^{(-5)}$. The beneficial or toxicological effect of a bioactive substance often depends on its concentration; it will occasionally have beneficial properties at low concentrations but could be toxic at high concentrations. However, to ensure the safety of long-term using of A. subrufescens, further research may be required to find the optimal method to remove or reduce the content of agaritine in A. subrufescens extracts [73]. Further toxicology studies still need to completely confirm the safety of the use of A. subrufescens.

CONCLUSIONS

The aim of present review is to outline past and current major therapeutic interest and pharmacology of medicinal mushroom and their use in human healthcare. The broad spectrum of biological activities from mushrooms suggest further screening and research in that promising field of health care substances. They not only cure but have also important prophylactic properties. Mushroom metabolites defining new generation of pharmacologically active compound, should definitely help fill some of the weaknesses of current therapeutic arsenal and develop it against present and future therapeutic challenges. This mushroom with high medicinal value; used successfully in cancer therapy, chronic inflammation, diabetes, infections (HIV, fungus, bacteria), immune system disorder and possess anti-oxidant properties. Safety issues in particular should focus on the toxicity and carcinogenicity of agaritine and its derivatives in this mushroom. It would be beneficial to the success of A. subrufescens if it was shown not to give rise to any toxicological effects. A. subrufescens should be an excellent future alternative medicine.

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