

# Natural Products from *Agaricales* Medicinal Mushrooms: Biology, Nutritional Properties, and Pharmacological Effects on Cancer

## *Produtos Naturais dos Cogumelos Medicinais Agaricales: Biologia, Propriedades Nutricionais e Efeitos Farmacológicos no Câncer*

Maria Rita Carvalho Garbi Novaes<sup>1</sup>, Luiz Carlos Garcez Novaes<sup>2</sup>, Vanessa Cunha Taveira<sup>3</sup>

### **Abstract**

Various studies have focused on adjuvant treatment alternatives for cancer patients to improve their quality of life with minimum adverse reactions. The current study aims to evaluate the nutritional activity and pharmacological effects of nutrients present in *Agaricales* mushrooms, using a systematic review of articles indexed in Medline, Database, NCBI, Lilacs, and Cochrane. Studies on the nutritional and pharmaceutical effects of dietary supplementation with *Agaricales* mushrooms have shown improved prognosis for cancer patients. Data involving extensive review of action mechanisms, in particular beta-glucans, ergosterol, terpenes, lectin, arginine, and protein-glucans, have shown effects on immune system modulation, tumor growth inhibition, and the ability to elicit cellular response, in particular cytokine expression and production. In a significant number of cases these substances can have positive effects on patients' quality of life, if specific parameters are considered, such as immune system stimulation, tumor growth inhibition, and metastasis. Randomized controlled clinical trials are needed to establish criteria for administering the proper doses of *Agaricales* mushrooms as complementary therapy in cancer patients.

**Key words:** *Agaricales*, Cancer, Dietary supplement

<sup>1</sup>Institute of Human Nutrition and Health Sciences - FEPECS / University of Brasília, Brazil

<sup>2</sup>Biotechnology and Genomic Science Researcher, Catholic University of Brasília, Brazil

<sup>3</sup>University of Brasília, Brazil

*Correspondence Address:* Maria Rita Novaes, PhD - Professor of Human Nutrition and Health Sciences, FEPECS / Universidade de Brasília. SHIS QI 09 - conjunto 06 - casa 14 - Lago Sul - Brasília (DF), Brasil - CEP: 71.675.030. *E-mail:* ritanovaes@ig.com.br

## INTRODUCTION

For millennia, mushrooms have been used for human consumption due to their organoleptic characteristics, as flavorful foods, and as medicinal substances. They are widely sold as nutritional supplements and touted as being beneficial to human health<sup>1,2</sup>.

Four species of edible mushrooms belonging to the *Agaricales* order have been cultivated most frequently for human consumption: *Agaricus bisporus* or *A. brunescens*, (Agaricaceae), *Lentinus edodes* (Tricholomataceae), and *Pleurotus ostreatus* (Pleurotaceae), not cultivated in Brazil, but common in the Orient. Agaricaceae is the mushroom family with the largest number of cultivated edible species, the most common being: *A. bisporus*, *A. campestris*, and *A. placomyces*<sup>3</sup>.

Empirical use of *Agaricales* has been reported as dietary supplementation for the treatment of cancer and other diseases, with successful results. Various researchers have investigated the pharmacological effects of these mushrooms. *Lentinus edodes*, *Grifola frondosa*, *A. blazei*, and *A. sylvaticus*, known in Brazil as Cogumelo do Sol®, are mushrooms from the *Agaricales* family that are known for their therapeutic properties. Other mushroom species belonging to the Basidiomycetes class but not to the *Agaricales* family are also used because of their medicinal properties, including: *Ganoderma lucidum* (*Amphylophorales* family), known in Brazil as king mushroom and in China as Ling Zhi, and *Auricularia auricular-jude* (Auriculariaceae). Within the Ascomycete order, *Cordyceps sinensis* is the most important species, considering its therapeutic effects<sup>4</sup>.

Well-documented research on *Agaricales* mushrooms has led to the isolation and biochemical characterization of their pharmacologically active substances, such as beta-glucans, ergosterol, terpenes, arginine and other immunomodulating amino acids, lectin, and other substances<sup>5</sup>.

Mushrooms are slowly being incorporated into Western medicine. Legally sold in Japan as dietary supplements: Krestin®, a polysaccharide peptide extracted from *Trametes* (or *Coriolus* or *Polyporus*) versicolor (Basidiomycete, *Amphylophorales* order, Coriolaceae family) known as Kawaratake, Yun Zhi, or turkey tail<sup>6</sup>; Schizophyllan® (PolyC), from the fungus *Schizophyllum commune* (*Agaricales* order, Schizophylalceae family), also known as Suehirotake<sup>7</sup> and Lentinan®, extracted from Shiitake<sup>8</sup>. All of these products are marketed in Japan as medicines, primarily indicated for cancer treatment: Krestin (PSK)® for breast, digestive tract, and lung cancer; Schizophyllan® for uterine cervical cancer; and Lentinan® for gastric cancer.

In Brazil, beta-glucans, substances extracted from the non-basidiomycete yeast *Saccharomyces cerevisiae* are being

produced and marketed by Hebron Laboratory for intravenous use, also indicated as immunomodulators.

The aim of this study was to evaluate the nutritional activity and pharmacological effects of nutrients present in *Agaricales* mushrooms, conducting a systematic review of published articles found in the following databases: Medline, Database, NCBI, Lilacs, and Cochrane.

## THE BIOLOGY OF PHARMACOLOGICALLY RELEVANT AGARICALES

The taxonomic route of *Agaricales* order is: Eucariota (super-kingdom), fungi (Kingdom) Metazoa (group), Basidiomycota (phylum), Hymenomycetes (class), Homobasidiomycetes (sub-class), and *Agaricales* (order). Agaricaceae is a family from the *Agaricales* order with numerous important species<sup>3</sup>.

*Agaricales* are considered cosmopolitan fungi. They grow easily in a wide variety of habitats, from the Arctic to the Tropics. While some are limited to specific areas, others grow in geographically dispersed areas. In ecologically defined areas, mushrooms have preferences for specific substrates. The wide variety of habitats colonized by these fungi and substrates they extract from the soil shows that *Agaricales* order includes saprophytic, symbiotic, and parasitic species<sup>9</sup>. Chemical substances existing in mushrooms may change according to the soil and climate of the region in which they grow. Knowledge of *Agaricales* morphology is fundamentally important in the taxonomy of these basidiomycetes and for understanding their physiological and phylogenetic aspects. Morphology is studied at four levels: macroscopic, microscopic, ultra-structure, and molecular biology.

Macro and microscopic morphological characteristics are the first parameters used for species classification, while ultra-structure and molecular biology have been used for phylogenetics. DNA genetic analysis contributes to the taxonomic classification of *Agaricales* fungi<sup>10</sup>.

The general characteristics of *Agaricales* fungi involve the production of a fruiting body commonly known as a mushroom. In these fruiting bodies or basidiocarps, unicellular basidia or basidiospores are produced, and they are therefore classified as holobasidiomycetes. The cellular walls of mycelia and fruiting bodies are important sources of beta-glucans, having a stratified structure consisting of a fibrous layer, proteins, beta-glucans, associated with chitin beta-glucan protein, and plasmatic membrane<sup>9</sup>.

## NUTRITIONAL ASPECTS OF AGARICALES MUSHROOMS

Mushrooms show a high protein content. As compared to beef (with about 14.8% dry weight protein), some *Agaricales species* have 22.5%. Qualitative analyses have demonstrated that mushrooms have eight essential amino acids in addition to non-essential ones<sup>1</sup>.

Compared to traditional sources of animal protein considered low in cholesterol, such as fish and poultry, mushrooms have even lower amounts of cholesterol and other saturated fats. The amount of dry weight fat varies from 0.7% to 9.7%. Total mushroom fat consists of lipids including free fatty acids, mono, di, and triglycerides, sterols, terpenes, and phospholipids with lectin<sup>11</sup>.

Total carbohydrate as a proportion of dry weight varies from 51% (*Volvariella volvacea*) to 88% (*Auricularia* species)<sup>1</sup>. Glycides are present in beta-glucan chains in the cell wall and cytoplasm.

The fiber has a high molecular weight, is excreted practically undigested and unabsorbed, and contains chitin (N-acetyl-glucosamine polymer, a cell wall component in most fungi), heteropolysaccharides (pectin, hemicellulose), and beta-glucans, which are abundantly present in mushrooms. The percentage of dry weight fiber in these mushrooms varies from 4% (*Flamulina velutipes*) to 20% (*Auricularia species*)<sup>1,12</sup>.

Mushrooms are sources of the water-soluble vitamins thiamine, riboflavin, niacin, biotin, and ascorbic acid, although the amounts differ between species. Ascorbic acid is easily oxidized and may be absent in some samples according to the mushroom's developmental stage and the method used in the formulation. Mushrooms are also sources of fat-soluble vitamins, especially vitamin D<sup>13</sup>.

Mushrooms contain large quantities of minerals, especially phosphorus, sodium, calcium, and potassium. Heavy metals like lead, mercury, and copper can also be found in small amounts. Because excessive quantities of these metals are harmful, the chemical properties of the water used in the cultivation process should be carefully monitored<sup>4</sup>.

## EFFECTS OF AGARICALES MUSHROOMS ON TUMORS

Studies concerning the anti-neoplastic effects of *Agaricales* fungi on tumors induced in animals have evaluated their immunomodulating role in genetically altered cells, their cytostatic effect on tumor growth, and the effect on tumor-induced vascular proliferation.

Studies *in vitro* with *Agaricus blazei*, *A. blazei* Murill, and *A. bisporus* using extracts and opsonized particles derived from mycelia, fruiting bodies, and aqueous extracts have been tested in different cancer cell culture lines. These studies have shown inhibition of tumor activity and cell proliferation, stimulation of NK cell function, and other immunological parameters such as the secretion of IgA, IgM, and IgE and improvement of monocyte and macrophage function (Table 1).

## CLINICAL RESEARCH

Although the results of clinical trials have not shown complete agreement, most studies suggest that these fungi

have favorable effects in cancer treatment (Table 2). Several effects such as immunomodulation enhancement, tumor growth reduction by cytostatic effect, and inhibition of tumor vascularization are due to the different action mechanisms of *Agaricales* fungi.

Estrogen production *in situ* is the main factor in breast cancer in postmenopausal women. Aromatase (estrogen synthetase) is a P450 enzyme complex that converts androgens into estrogens. Aromatase activity occurs in tumors and may play a more predominant role in cell proliferation than in circulating estradiol. Studies on enzyme kinetics have demonstrated mixed inhibition, suggesting the presence of multiple inhibitors or more than one inhibitory mechanism. According to Grube *et al.*<sup>6</sup>: "Aromatase activity and cell proliferation were measured using MCF-7aro, an aromatase-transfected breast cancer cell line. Phytochemical compounds in the mushroom aqueous extract inhibited aromatase activity and proliferation of MCF-7aro cells. These results suggest that diets high in mushrooms may regulate aromatase activity and function in chemoprevention in postmenopausal women by reducing the *in situ* production of estrogen."

Shimizu *et al.*<sup>14</sup> conducted a clinical trial including 56 patients, all with middle to late stage cancer. Patients were in chemotherapy and radiotherapy. Thirty were allocated to the experimental group and 26 to the control group. In the experimental group, patients were treated with polysaccharide tablets 3 times/day, with 4 tablets per dose (total 6 grams/day) starting one week before chemotherapy and radiotherapy. Controls received Polyactin-A (polysaccharide isolated from hemolytic streptococcus alpha culture) 30mg/day, starting a week before radiotherapy and chemotherapy. At two months, the control group showed little change in the digestive tract reaction and had decreased the number of leucocytes as compared to the experimental group. The researchers concluded that polysaccharides can relieve toxic reactions caused by conventional therapies, improve nonspecific immunity and IgA secretion, stimulate macrophage and monocyte function, increase cellular immunity (natural killer cells, LAK cells, and Th/Ts cells), and present improved immunomodulating effects as compared to Polyactin-A.

Another clinical trial evaluated the effects of *A. blazei* in 20 patients with acute non-lymphocytic leukemia, divided into two groups. The experimental group received *A. blazei* (20g 3 times/day), and the control group received placebo. All 20 patients were receiving chemotherapy. Tumors were in remission and the erythrocyte/granulocyte ratio recovered to normal levels within 7-8 days in the experimental group as compared to the placebo group<sup>6</sup>.

**Table 1.** *In vivo* studies

Reference	Fungi	Substance	Tumor/Dosage	Results
Ito et al., 1986 <sup>11</sup>	<i>Agaricus blazei</i> (Iwade 101) "Himematsutake"	Protein-polysaccharide complex ATOM*	Sarcoma 180mg/kg 10mg/kg e 20mg/kg/daily	ATOM has no cytotoxic effect in tumoral cells <i>in vitro</i>
Fujimya et al., 1999 <sup>5</sup>	<i>Agaricus blazei</i>	F-III-2B (Polissaccharide of <i>Agaricus blazei</i> Murill) with or without 5-Fluorouracil	Meth-A tumoral cells F-III-2B 10mg/kg/day x 30	Development of implanted tumor was strongly inhibited by the combination of F-III and 5-FU
Irazoqui et al., 2000 <sup>9</sup>	<i>Agaricus blazei</i>	(1-4)- $\alpha$ -D-glucan; (1-6)- $\beta$ -D-glucan	Primary phase of systemic tumor in Balb/c rats	Infiltration of NK cells with antitumoral effect and inhibition of tumoral growth with apoptosis induction
Irazoqui et al., 2000 <sup>9</sup>	<i>Agaricus blazei</i> "Himematsutake"	(1-3)- $\beta$ -D-glucan; FA-1- $\alpha$ - $\alpha$ , acidic (1-6) (1-4)- $\alpha$ -D-glucan; FA-1- $\alpha$ - $\beta$ , acidic (1-6) (1-4)- $\alpha$ -D-glucan e FA-2- $\beta$ - $\beta$ , acidic RNA-protein complex	Sarcoma 180 in rats	Fractions have important tumoricidal action
Ito et al., 1986 <sup>11</sup>	<i>Agaricus blazei</i> (Iwade 101) "Himematsutake"	Protein-polysaccharide complex ATOM (antitumor organic substance Mie) prepared from micelium culture	Ehrlich ascitic carcinoma; 50mg/kg a 100mg/kg/day carcinoma 42 Shionagi 50mg/kg to 100mg/kg/day	Increase in macrophage number and the proportion of complement (C3) in rats treated with ATOM
Ito et al., 1994 <sup>12</sup>	<i>Agaricus blazei</i>	Soluble fraction of <i>Agaricus blazei</i> (alfa-1.6 complex and alfa 1.4 glucan)	Sarcoma 180	Tumoral reduction (p<0.05)
Jenneman et al., 2000 <sup>13</sup>	<i>Agaricus blazei</i>	<i>Agaricus blazei</i> Murill fruting bodies extracts	Meth-A tumoral cells 5mg at 3, 4, 5 day in right flank	Regression of right tumor and inhibition of growing of left tumor
Novaes et al., 2005 <sup>19</sup>	<i>Agaricus blazei</i>	(1,4)- $\beta$ -glucan (1,6)-glucan	Meth-A tumoral cells	- Tumoral growth inhibition - Increase in immunosuppressive acidic protein (IAP) levels indicating activation of granulocytes
Itoh et al., 1994 <sup>12</sup>	<i>Agaricus blazei</i>	Low molecular weight fraction 3 (LM-3), with alfa-1.4-glucan- $\beta$ -1.6- glucan complex; A11; ATF	Meth-A tumoral cells	- A11 LM3 e ATF fractions showed <i>in vitro</i> selective cytotoxic action in Meth-A tumoral cells with no toxic effect on normal cells - Increase in immunosuppressive acidic protein (IAP) in serum of rats with LM-3 indicating the

Reference	Fungi	Substance	Tumor/Dosage	Results
See et al., 2002 <sup>29</sup>	<i>Agaricus blazei</i>	Aqueous extracts of micelium and fruiting bodies	- Macrofages derivate from mice bone marrow - 25-50µl	- induced secretion of TNF-alfa by macrophage derivate from rats bone marrow - components of <i>Agaricus blazei</i> which activated macrophages resulted in induction of cytokine and NO secretion <i>in vitro</i>
Nanba, 1995 <sup>21</sup>	<i>Agaricus blazei</i>	<i>Agaricus blazei</i> lipid fraction (ergosterol)	Sarcoma 180 400mg/kg e 800mg/kg orally for 20 days Intraperitoneally for 5 days - 5mg/kg, 10mg/kg e 20mg/kg	- Inhibited tumor induced neovascularization. Ergosterol is supposed to be the active substance
Takeshi et al., 2001 <sup>20</sup>	<i>Agaricus blazei</i>	- F10-0-a-beta FA-1-a-beta FA-2-b-beta (water soluble polysaccharides)	Sarcoma 180 in rats	These fractions presented important antitumoral action.
Ebina et al., 1998 <sup>4</sup>	<i>Agaricus blazei</i>	<i>Agaricus blazei</i> (Ammonium oxalate solution and insoluble ethanol) 2-ATF (beta-1, 6- D-polyglucose)	1.1- intratumoral: 0.1 a 2.5mg 1.2- oral: 0.1 mg a 2.5mg 2.1- 5,0 mg intratumoral 2.2- oral 4.5mg (4 days before inoculation)	1.1- tumoral reduction (p<0.05 vs. Control saline; n=7) 1.2- no regression of tumor (p>0.05 vs. Control) 2.1- regression of tumor growth compared to non treated fraction 1 2.2- significant reduction (p<0.05 vs. All other treatments; n=7)
Barbisan et al., 2003 <sup>1</sup>	<i>Agaricus blazei</i>	- <i>Agaricus blazei</i> - NaCl 0.9%	Hepatotoxicity induced by DEN in male Wistar rats <i>A. blazei</i> . Animals of placebo group received NaCl 0.9% solutions	- After 48 hours the animals were sacrificed. It has been suggested that treatment with <i>A. blazei</i> has effect on hepatocarcinogenic process
Shimizu et al., 2002 <sup>14</sup>	<i>Agaricus blazei</i> "Himematsutake"	Saline solution (0.9% de NaCl) in groups 1 and 6 of adult Wistar rats - Aqueous extract of <i>Agaricus blazei</i>	Hepatic carcinogenesis - Saline solution (0.9% de NaCl) in groups 1 e 6 of adult Wistar rats - Aqueous extract of <i>Agaricus blazei</i> (1.2mg/ml; 5.6mg/ml; 11.5mg/ml orally for 6 weeks of treatment	Treatment with aqueous extract of <i>Agaricus blazei</i> after initial stage of hepatic carcinogenesis did not present any effect against the development of GST-P- positive liver foci (liver preneoplastic lesion) induced by DEN (dietilnitrosamina)

(\*) ATOM=Antitumor Organic Substance Mie extracted from mycelia culture

**Table 2.** Clinical trials

Reference	Mushroom specie	Substance	Dosage	Tumor	Results
Grube et al., 2001 <sup>6</sup>	<i>Agaricus bisporus</i>	White fruiting bodies	2.5µL, 5µL or 10µL lyophilized extract solubilized in culture media (10X <sub>CM</sub> )/mL de cellules (5mL/well)	Breast cancer	- Suppression of aromatase activity <i>in situ</i> in a dose dependent manner - Inhibition of HCF-7aro cells proliferation
See et al., 2002 <sup>29</sup>	<i>Agaricus blazei</i> Murill	1) <i>Agaricus blazei</i> Murill tea 2) Transfer Factor Plus 3) IMUPlus 4) intravenous and oral ascorbic acid 5) Imunomodulador Mix, nitrogenated soy extract and <i>Andrographis Paniculata</i>	1) 10mg/day 2) 3 tablets 3 times daily 3) 40mg/day 4) 50 a 100 mg/day intravenous e 1-2 mg/day orally 5) 500mg twice a day	1 Urinary bladder 5 breast 2 prostate 2 lung 3 colon 2 lymphomas 1 ovary 1 gastric and 1 osteosarcoma	- Enhancement of NK cells function and other immunological parameters and hemoglobin of PBMC or plasma in late stage cancer patients
Mizuno et al., 1990 <sup>18</sup>	- <i>Agaricus blazei</i> - <i>Lentinus edodes</i> - <i>Grifola frondosa</i> - <i>Ganoderma lucidum</i> - <i>Coriolus versicolor</i> - <i>Cordyceps sinensis mycelium</i>	- Polysaccharides mixture of 6 medicinal mushrooms in tablets with de 500mg - Polyactin-A	4 tablets each time, 3 times a day (total 6g/dia) of mushrooms mixture - 10 mg, each time of Polyactin-A, 3 times daily (total 30 mg/day)	Gastric carcinoma, hepatic carcinoma, lung carcinoma, large intestines carcinoma, nasopharyngeal carcinoma	- little changes in digestive tract reaction after chemotherapy or radiotherapy in patients of control group and in this group the enhancement of total number of white blood cells was less expressive than in the patients of experimental group - Non specific immunity increased after treatment. The increase in IgA secretion and improvement of NK cells and monocytes activity were also observed

## MUSHROOM SUBSTANCES WITH PHARMACOLOGICAL EFFECTS

### *Beta-glucans*

Different physico-chemical parameters such as solubility, primary structure, stereochemistry, molecular weight, and branching, all play an important role in determining the potential immune activities of polysaccharides. Glucans have a three-dimensional structure with side chains branching off the backbone structure of linear glucose molecules. These secondary structures confer the biological function and immune activity of glucans, thus allowing interaction with immune system receptors.

Beta-glucans are polysaccharides. They can present glucopyranosidic bonds in 1-3, 1-4, and 1-6 carbon atoms. The branching side chains can be fructose, mannose, xylose, galactose, amino acids, and polypeptide chains. Beta-glucans are components of cell walls of hyphae and mycelia found in yeasts, bacteria, mushrooms, algae, and cereals, each having a different chemical structure. Beta 1,3 glucan derived from bacteria and algae is linear. Mushroom and yeast beta-glucans are branched with  $\beta$ 1,6 and  $\beta$ 1,3 enhancing their ability to bind and stimulate macrophages (Figure 1).

Besides the primary structure, beta-glucans with 1-3 bond present secondary and tertiary structures, which form triple helix and multimers supporting cell structure<sup>16</sup>.

When beta-glucans are administered orally or during the purification process if prepared for intravenous administration, a number of fragmentations of multimeric chains occur. Each fragmented component binds to different beta-glucan receptors. Binding between receptors on the macrophage surface is responsible for stimulating the immune system.

Macrophage stimulation and immunomodulating effects are due to beta-1,3 glucan, with a molecular weight of 6500 Daltons<sup>17</sup>. The 1-3- beta-glucan has

several effects on the immune system, including an increase in cellular and humoral immunity, macrophage phagocytic and chemiostatic activity, monocyte count, depuration of antigens, and cytolytic activity on human tumoral cells *in vitro*<sup>18</sup>.

Beta-D-glucans function as pseudo-antigens in the immune system activation. Antigen is phagocytosed by thymus-dependent cells that are suppressor and auxiliary lymphocytes, and bursa-dependent cells, the antibody-secreting plasmocytes. They also activate Th-1 cells and thymus-dependent cells that stimulate T-lymphocytes to secrete interleukins. These interleukins stimulate natural killer cells, responsible for the destruction of neoplastic cells. In the presence of antigen, CD-8 lymphocytes acquire higher specific cytotoxicity, contributing to the process of cell destruction<sup>19</sup>.

Among products containing beta-D-glucans extracted from medicinal mushrooms, Lentinan® and Schizophyllan® are available on the market in Japan<sup>15</sup>.

### *Lectin*

Lectin is found in the lipid fraction of *Agaricales* extracts. Phospholipids are found in all living organisms. In animals lectin is an important component of nerve tissue, including the brain. Lectin is generally synthesized by an association of stearic, palmitic, or oleic acids linked to phosphoric acid colinic ester. Lectin acid contains palmitic, stearic, palmitoleic, oleic, linolenic, linoleic, and aradonic acids, in addition to 20 to 22 other fatty acids.

Yu et al.<sup>7</sup> have described the antitumor effects of fatty acids in the lipid fraction of *Agaricales* fungi. Some authors attribute this action to oleic acid<sup>20</sup>. According to Kimura, the inhibitory action of oleic acid on the growth of LHC tumors may be due to tumor-induced angiogenesis inhibition<sup>20,21</sup>.

Parslew (1999)<sup>6</sup> observed that besides the effects on





Beta Glucan Type	Structure	Description
Bacterial		Linear $\beta$ 1,3-glucan (Curdlan)
Fungal		Short $\beta$ 1,6 branched $\beta$ 1,3-glucan (i.e. Schizophyllan)
Yeast		Long $\beta$ 1,6 branched $\beta$ 1,3-glucan (Beta Glucan, Betafectin™)
Cereal		Linear $\beta$ 1,3/ $\beta$ 1,4-glucan (i.e. oats, barley, rye)

Figure 1. Beta-glucan structure

tumor, lectin from *A. bisporus* fungi inhibits cell proliferation, a potentially useful property in the treatment of psoriasis.

### **Ergosterol**

Ergosterol or provitamin D2 is found in the lipid fraction of *Agaricales* extracts and is an important substrate in vitamin D biosynthesis.

According to Takaku et al., when rats with sarcoma 180 were treated with lipid fraction extracted from *A. blazei*, tumor growth was delayed, while the authors did not observe side effects like the decrease in thymus and spleen volume and lymphocyte count that commonly occur as a consequence of chemotherapy. The active ingredient responsible for these effects is believed to be ergosterol, which has no direct in vitro cytotoxic effect on sarcoma 180 cells, although it can inhibit tumor-induced neovascularization<sup>19</sup>.

A study in vivo focused on the effect of ergosterol in Lewis hepatic carcinoma (LHC) cell lines. The administration of ergosterol in the intraperitoneal cavity inhibited tumor-induced neovascularization, suggesting that either ergosterol or its metabolites might be involved in this action<sup>19</sup>.

Ergosterol is a precursor of ergocalciferol. Fujimiyia et al.<sup>22</sup> demonstrated that colecalciferol inhibits angiogenesis. According to these authors, the fact that ergosterol is metabolized to ergocalciferol corroborates evidence of the pharmacological effects of angiogenesis inhibition.

### **Terpenes**

Triterpenoids and ganoderic acids (alpha, gamma, zeta, and others) are the most commonly found terpenoids in *Agaricales* and Amphylophorales (*Ganoderma lucidum*) and display antitumor activity<sup>22</sup>.

Terpenoids (isoprenoids and terpenes) are widely used as flavor and odor enhancers. Their extraction is generally performed by evaporation or distillation of essential oils present in resin and other plant cell structures.

Terpenoids have complex molecular structures and are able to adopt different cyclic or polycyclic conformations, thus comprising numerous stereoisomeric and enantiomeric forms.

Terpenes are classified according to the number of isoprene units, as follows: hemiterpenoids, C5; monoterpenoids, C10; sesquiterpenoids, C15; diterpenoids, C20; triterpenoids, C30; and carotenoids, C40.

In plants, the role of terpenoids is related to antitumor action in *Agaricales* fungi. Monoterpenes, diterpenes, and sesquiterpenes have many different roles. Triterpenes and other derivatives including steroids have a wide variety of functions such as: protection against

herbivores; antimitotic activity; induction of seed germination; and inhibition of root growth. Cholesterol, vitamins A, D, and E, and sex hormones (estradiol and testosterone) are especially important triterpenes. Steroids with C27 and C29 belong to the terpene group but are not true terpenes, since they are synthesized from the same precursor squalene, which has 30 carbon atoms in its structure.

Considering their antitumor activity, triterpenes are the most important group among the terpenoids. The mechanism of antitumor action in triterpenes extracted from *Agaricales* fungi is related to the inhibition of tumor-induced angiogenesis<sup>23,24</sup>.

### **Protein-Glucans**

The covalent bindings of peptides or amino acids in the neutral or acid form, with long branched glucan chains form the protein-glucans<sup>16</sup>. The polysaccharide protein complex known as ATOM is a type polysaccharide linked to peptide.

PSP have higher opsonizing action compared to isolated polysaccharides and act as better epitopes. Krestin® PSK has been prescribed for oral use. Its active substance is beta-glucan linked protein, extracted from mushrooms<sup>15</sup>. When administered orally, peptide-linked polysaccharides display better absorption as compared to non-linked polysaccharides<sup>6</sup>.

### **Immunomodulating Amino Acids**

Arginine is one of the most abundant amino acids present in *Agaricales*. In animal and clinical experiments, arginine enhances immunity through several mechanisms. This amino acid is related to the release of growth hormone and stimulates the production of nitric oxide, hydroxyproline, cytokines, and polyamines<sup>7, 25</sup>.

According to Cho-Chung et al. (1980), an in vitro cancer cell culture presented growth inhibition after daily supplementation with 1mg/ml of arginine. This amino acid, acting with cyclic AMP, resulted in complete interruption of cell replication after two days of treatment. According to the results, dibutyl cyclic AMP and arginine inhibit breast tumor growth, suggesting a positive effect by these components in breast cancer therapy<sup>26</sup>.

Arginine supplementation in patients with post-operative trauma, cancer, or burn increased the activation of T-lymphocytes and improved cellular immunity, although patients submitted to gastrointestinal surgery who were supplemented with intravenous arginine as a nitrogen source did not show an increase in lymphocyte proliferation. According to these authors, when arginine is administered alone it does not stimulate lymphocyte



activity, so concomitant administration of calories and other amino acids is necessary<sup>27</sup>.

### Gene Expression

Evidence indicates that fractions of medicinal mushroom extracts, especially from *Agaricus* fungi, may alter gene expression. Such fractions are considered multi-cytokine inducers and are able to induce gene expression of immunomodulating cytokine receptors<sup>15</sup>.

Chow (2003), evaluated the antiproliferative action of PSP extracted from *Coriolus versicolor*, a mushroom used in breast cancer treatment. MDA-MD-231 breast cancer cells were cultured *in vitro*, with and without PSP, for 7 days, and cell growth at 24, 72, 120 and 168 hours was measured with cell proliferation reagent (WST 1). Cells treated with PSP were found to have a significant reduction in cell proliferation when compared to the control group after 72 hours of incubation. Using the tunnel method, PSP was found to have a significant effect on apoptosis after 24 hours<sup>28</sup>.

Kuo et al.<sup>16</sup> tested the immunopharmacological action of *Agaricus blazei* Murill fractions AB-BDM-1 to AB-BDM-10 on the proliferation of peripheral blood mononuclear cells (PBMC). Their results indicated that proliferation was suppressed by fraction AB-BDM-2 after the activation of phytohemagglutinin (PHA). The proposed mechanism of action was not attributed to direct cytotoxicity. Cell cycle analysis indicated that AB-BDM-2 activated PBMCs in the transition from phase G to phase S. The growth of activated PBMCs and the production and expression of mRNA from IL-2, IL-4, IFN-gamma, and cyclin D were suppressed in a dose-dependent manner. No effects were observed on the production of nitric oxide or the induction of RNA nitric oxide synthetase levels in PBMCs stimulated by PHA<sup>28</sup>.

### Toxic Effects of *Agaricales* Fungi

No adverse effects of *Agaricales* fungi have been described in the specialized literature. According to experimental tumor models, the continuous administration of 10.5% and 2.5% *A. bisporus* in the diet of 6-week-old rats induced the formation of liver, stomach, and ovary tumors in some groups<sup>4</sup>.

## CONCLUSION

The *in vitro*, *in vivo*, and clinical trials presented in this evidence-based review suggest that dietary supplementation with *Agaricales* fungi may bring promising perspectives as adjuvant therapy to improve the prognosis of cancer patients.

To elucidate the regulatory action mechanism of

these fungi in cancer, it is of fundamental importance to evaluate the nutritional and therapeutic elements that stimulate substances capable of inhibiting tumor growth and providing beneficial effects on the immune system.

However, additional randomized clinical and placebo-controlled trials are needed to establish the criteria and benefits of adjuvant therapy with *Agaricales* fungi in cancer treatment.

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## Resumo

Vários estudos enfatizam os tratamentos alternativos adjuvantes para pacientes com câncer, com o intuito de melhorar a qualidade de vida com o mínimo de reações adversas. O presente estudo objetivou avaliar a atividade nutricional e os efeitos farmacológicos dos nutrientes presentes nos cogumelos *Agaricales*, por meio de uma revisão sistemática dos artigos indexados no Medline, Database, NCBI, Lilacs e Cochrane. Estudos sobre os efeitos nutricionais farmacêuticos da suplementação dietética com cogumelos *Agaricales* indicaram melhora no prognóstico de pacientes com câncer. Dados envolvendo extensa revisão dos mecanismos de ação, em particular beta-glucanas, ergosterol, terpenos, lecitina, arginina e proteínas-glucanas mostraram efeitos na modulação do sistema imune, inibição do crescimento tumoral e habilidade para elicitar resposta, especialmente expressão e produção de citocina. Em um número significativo de casos, essas substâncias têm um efeito positivo na qualidade de vida do paciente, em especial no estímulo ao sistema imune, inibição do crescimento tumoral e da metástase. São necessários ensaios clínicos randomizados a fim de estabelecer critérios para a administração de doses adequadas dos cogumelos *Agaricales* como terapia complementar em pacientes com câncer.

**Palavras-chave:** *Agaricales*, Câncer, Suplemento dietético