Determination of Chemical Antioxidants and Phenolic Compounds in the Brazilian Mushroom *Agaricus sylvaticus*

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ABSTRACT

Agaricus sylvaticus mushroom has been widely studied because of its high nutritional value and medicinal properties. The objective of this study was to evaluate the antioxidant potential of both alcoholic and aqueous extracts of Agaricus sylvaticus and quantify their total polyphenol content. The antioxidant activity was performed by the 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity and total polyphenol content was assessed by colorimetric method. Observation also noted the great antioxidant potential of aqueous, alcoholic and ethereal extracts (14.6%, 75.6% and 14.6%, respectively) of the Agaricus sylvaticus mushroom, highlighting the alcoholic extract, which demonstrates the extraordinary benefits of this mushroom in the diet, since antioxidants prevent premature ageing and various types of cancer.

Keywords: *Agaricus sylvaticus*, antioxidants, medicinal fungus, mushroom, phenolic compounds

Determinación de los Antioxidantes Químicos y los Compuestos Fenólicos y el Champiñón Brasileño *Agaricus sylvaticus*

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RESUMEN

El champiñón brasileño – la llamada seta del sol Agaricus sylvaticus *– ha sido ampliamente estudiada debido a su alto valor nutritivo y sus propiedades medicinales. El objetivo de este estudio fue evaluar el potencial antioxidante de los extractos acuosos, alcohólicos y etéreos de* Agaricus sylvaticus, *y cuantificar su contenido total de polifenoles. La actividad antioxidante fue realizada por la capacidad atrapadora del radical 2,2-difenil-1-picrilhidracilo, y el contenido total de polifenoles fue evaluado mediante colorimetría. La observación también puso de manifiesto el gran potencial antioxidante de los extractos acuosos, alcohólicos y etéreos (14.6%, 75.6% y 14.6%, respectivamente) del champiñón* Agaricus sylvaticus, *destacando el extracto alcohólico, lo cual demuestra los extraordinarios beneficios de este hongo comestible en la dieta, ya que los antioxidantes previenen el envejecimiento prematuro así como diversos tipos de cáncer.*

Palabras claves: *Agaricus sylvaticus*, antioxidantes, hongo medicinal, compuestos fenólicos, champiñón del sol

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INTRODUCTION

Appropriate nutrition is essential to maintaining health, contributing to risk reduction of disease and also the restoration of homeostasis in cases of illness. Through nutrition, it is possible to promote recovery, rehabilitation, detoxification and repair of cells, providing greater vitality to organs and tissues (1).

For more than two thousand years, natural products have been used empirically in the treatment of various diseases such as cancer. Mushrooms are fungi known from ancient times when man used them as a food of high nutritional and therapeutic value (2). Mushrooms have high genetic diversity that represents a source of protein essential to human health (1).

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Despite the great biodiversity of fungi existing in Brazil and the great potential still to be explored, there are few data related to their antioxidant activity (3). That activity is very important because antioxidants have the ability to sequester free radicals harmful to human health (4).

Antioxidants are able to slow down oxidation rate, inhibiting free radicals and preventing the onset of diseases, thus contributing to greater longevity; making essential the balance between free radicals and the antioxidant defence system (5). Among the various classes of naturally occurring antioxidants, phenolic compounds have received much attention in recent years, especially by inhibiting *in vitro* lipid peroxidation and lipooxygenase (6).

As the human antioxidant defence system is not complete without dietary antioxidants (7), the main way of getting antioxidants in the body is the ingestion of compounds with this activity. The main dietary antioxidants are some vitamins, carotenoids and phenolic compounds (8).

The *A sylvaticus* mushroom has nutritional, antimutagenic, antitumour, antiviral, antithrombotic, hypocholesterolaemic, hypolipidaemic properties and antioxidant activities that are related to the presence of esters, oleic and linoleic acid, proteins, enzymes, vitamins and polysaccharides (9, 10).

Study of the characteristics and effects of the medicinal *A sylvaticus* mushroom is relevant in the context of public health, given that the population has used it as a nutritional supplement, either in dry form, capsules or as tea (11). It is suggested that dietary supplementation with *A sylvaticus* fungus is able to promote beneficial effects on energy metabolism, blood pressure, biochemical parameters and enzyme activities (12), and improve the quality of life of patients with colorectal cancer in the postsurgical phase (13). Based on the numerous benefits provided by this mushroom, the objective of this study was to evaluate the antioxidant potential and the amount of total polyphenols in ethereal, alcoholic and aqueous extracts obtained from it.

METHODS

A sample of the dehydrated *A sylvaticus* mushroom was obtained from a producer in Minas Gerais state. The sample remained stored at room temperature until the time of analysis. First, the mushroom was processed in a Willey mill type, Model TE-648 (Tecnal, Piracicaba, Brazil) in order to obtain higher extraction of its components. All the analyses were performed at the Laboratory of Food Biochemistry, Pharmacy School, Universidade Federal de Goiás (UFG).

The antioxidant potential of *A sylvaticus* mushroom was determined following the methodology used by Borguini (14). The entire experiment was conducted using aluminium foil to reduce any possibility of interference of light in the sample. The ether, alcoholic and aqueous extracts of the mushroom were obtained. First, the ether extract was from the initial dilution of 2.5 g mushroom ground in 50 mL of ethyl ether. From the non-filtered residue and therefore not ether-soluble, the alcoholic extract was obtained with the addition of ethanol at a ratio of 1:20 (residue weight:volume of alcohol). Finally, the aqueous extract was obtained from the addition of water to the non-filtered residue from the previous step, also adding distilled water at a ratio of 1:20 (residue weight:water volume).

In the experiment, butylated hydroxytoluene (BHT) was used as standard antioxidant and 2,2-diphenyl-1-picrylhydrazyl (DPPH) as oxidant. The antioxidant activity of mushroom extracts was determined by DPPH described by Brand-Williams *et al* (15). The DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule and, in this way, is reduced in the presence of an antioxidant.

To evaluate the antioxidant activity, the extracts were reacted with the stable DPPH radical in ethanol solution. According to Neves *et al* (16), in radical form, DPPH has a characteristic absorption at 517 nm, which disappears after reduction by hydrogen pulled from an antioxidant compound. The reduction of DPPH radical was measured by reading absorbance at 517 nm in a spectrophotometer, model SP-220, (Biospectro, Brazil) at intervals of 0, 1, 2, 3, 4, 5, 10, 15 and 20 minutes of reaction. The values observed in the spectrophotometer were converted to a percentage scale, where 0% indicates no inhibition by the production of free radicals and 100% indicates complete inhibition of them.

The antioxidant activity was expressed according to Equation 1, described below (17, 18):

$$AA\% = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100]/Abs_{control}\} \quad (1)$$

The concentration of total polyphenols was determined by the colorimetric method (15), using the Folin-Ciocalteu, which is based on the reduction of phosphomolybdic and phosphotungstic acids in alkaline solution. The blue colour produced by reduction of the Folin-Ciocalteu phenol is measured spectrophotometrically at a wavelength of 765 nm. For quantification of total polyphenols of the sample, a standard curve of gallic acid solution at concentrations of 0.01 mg/mL to 0.06 mg/mL was used. A correlation coefficient (R^2), resulting $R^2 = 0.99775$ to the level significance of 5% was calculated. The result was expressed as milligrams of gallic acid equivalents per gram of extract (mg/g).

The analysis of total polyphenols was performed in triplicate, from the use of ether extracts, alcoholic and aqueous sample, the same concentration used for the standard solution of gallic acid previously reported. The readings were taken in a spectrophotometer, model SP-220 (Biospectro, Brazil) to 750 nm.

RESULTS

The aqueous, ethanolic and ethereal extracts of *A sylvaticus* mushroom showed the DPPH inhibition percentage of 14.6%, 75.6% and 14.6%, respectively. The value obtained for the synthetic antioxidant (BHT), used in this study for comparison, was 80.06%.

The antioxidant effect of aqueous, ethanol and ether of the mushroom *A sylvaticus* is shown in the Figure by the

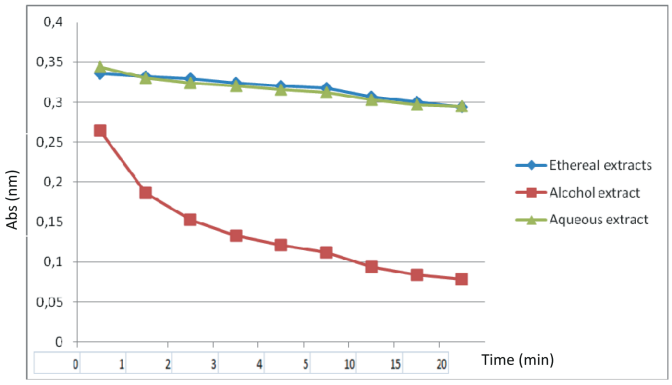

Figure: Antioxidant potential of ethereal, alcoholic and aqueous extracts of the *A sylvaticus* mushroom.

*The antioxidant potential of the *A sylvaticus* mushroom was observed from spectrophotometric analysis of three extracts from the sample, and its action was compared with the standard synthetic antioxidant, BHT, using DPPH as the oxidant.

decrease of absorbance observed at 0, 1, 2, 3, 4, 5, 10, 15 and 20 minutes. The mean percentage of total polyphenol extracts, ethereal and ethanolic mushroom *A sylvaticus* are shown in the Table. Regarding the antioxidant activity,

Table: Amount of polyphenol extracts of ethereal, alcoholic and aqueous extracts of *A Sylvaticus* mushroom

Polyphenols (%)	Ethereal extract	Ethanolic extract	Aqueous extract
	4.11 ± 1.40	9.42 ± 2.45	0.98 ± 0.31

*The Folin-Ciocalteou reagent was used in a spectrophotometer at 750 nm.
** We calculated the mean and standard deviation of the results obtained for each extract analysed.

results showed that the alcoholic extract of the *A sylvaticus* mushroom has great antioxidant potential (75.6%), suggesting that most antioxidant compounds present in this mushroom can be more easily diluted in alcohol. As for the aqueous and ethereal fractions, they showed reduced antioxidant potential (14.6% each), when compared to the alcoholic fraction, since they had less ability in kidnapping the DPPH radical after 20 minutes of reaction.

DISCUSSION

Lately, the interest in the study of phenolic compounds has increased greatly, mainly due to the ability of these antioxidant substances in kidnapping free radicals, which are harmful to human health (4).

Comparing the results of this study to the results reported by Percário *et al* (19) for the mushroom in liquid suspension (50%), the aqueous fraction of this study obtained reduced antioxidant potential (14.6%), which can be explained by the fact that the antioxidant components had already been extracted by ether and by alcohol before the analysis of the antioxidant in aqueous extract.

The biological properties of phenolic compounds are related to the antioxidant activity each phenol exerts on a given medium. The activity of antioxidants, in turn, depends on their chemical structure and it can be determined by the action of the molecule as a reducing agent, represented by the rate of inactivation of free radical reactivity with other antioxidants and metal chelation potential (20).

Epidemiological studies revealed correlation between the increased consumption of phenolic compounds with antioxidant activity (21) and reduced risk of cardiovascular disease as well as certain types of cancer (20). Phenolic compounds appear to be the main components responsible for antioxidant activity of extracts from mushrooms (22). According to Tsai *et al* (23), the genus Agaricus mushrooms may have antioxidant properties associated with its high concentration of tocopherols.

Polyphenols make a heterogeneous group, composed of several classes of substances with antioxidant capacity, among which phenolic acids and flavonoids stand out. The antioxidant activity of polyphenols is mainly due to its reducing properties, whose intensity of antioxidant activity exhibited by these phytochemicals is notably different since it fundamentally depends on the number and position of hydroxyl groups present in the molecule (24).

In this study, it was noticed that the alcoholic extract concentrates the biggest amount of polyphenols (9.42 mg/100 g), followed by the ethereal extract (4.11 mg/100 g), and aqueous extract (0.98 mg/100 g). The use of ethanol made possible the extraction of a higher content of polyphenols, as the alcoholic extract of the sample *A sylvaticus* mushroom exhibited higher total phenolic content when compared to the aqueous and ethereal, which have lower levels of these constituents.

The significant antioxidant capacity, but the low total polyphenol extracts in ether, alcoholic and aqueous extracts indicate that antioxidants other than polyphenols are the bioactive compounds of the *A sylvaticus* mushroom.

Aiming at evaluating the antioxidant capacity of the *A sylvaticus* mushroom in different forms of preparation (liquid suspension, fresh, dry and tablets), Percário *et al* (19) evaluated the ability of samples to inhibit *in vitro* the formation of free radicals by 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) [ABTS] over a period of 90 seconds, resulting in decreased absorbance at 600 nm. The authors observed excellent antioxidant activity (%) in all forms of preparation of *A sylvaticus* at concentrations of 1 mg sample. It must be emphasized that the temperatures used in the preparation of the samples were 60 °C for the dried mushroom and liquid suspension, since high temperatures can inactivate most molecules with antioxidant properties. According to the authors, these molecules are easily degraded when exposed to industrial processes, which makes their antioxidant capacity reduced. According to Barros *et al* (25), the cooking

processes are responsible for the reduction of nutrients with antioxidant capabilities in several mushrooms analysed in Portugal.

Percário *et al* (19) researched different molecules with antioxidant capacity in *A sylvaticus*, and found results of 72 mg/g for β-glucan in the liquid suspension and 14.1 mg/g in tablet form. For flavonoids, values of 0.88 mg/g were found in liquid suspension and 0.63 mg/g in tablet form. For total phenols, values of 0.1 mg/g were found in the liquid suspension and 3.4 mg/g for tablets. The authors suggested that the antioxidant activity of mushroom *A sylvaticus* is by virtue of the number of molecules present, not for a specific component.

In a study performed by Silva *et al* (3), the antioxidant potential of different extracts of the mushroom *Agaricus blazei* was evaluated by the DPPH method. The authors also observed a higher antioxidant activity (28.9%) in methanol: aqueous extract (1:1), with extraction time of six hours. In results presented in the present work for *A sylvaticus*, the best antioxidant activity was observed in the alcoholic fraction (75.6%), which shows that components with antioxidant properties of this mushroom are more easily soluble in alcohol.

It was observed that some authors used the mushroom extracts under analysis as ingredients of some foods in order to find out the antioxidant effect in the processed product. Silva *et al* (3) added the methanol:aqueous extract (1:1) to soybean oil and obtained good results, since it showed a protective effect (20.4 hours of oxidative stability) and the activity of the extract of *A blazei* was more efficient than the synthetic antioxidant BHT (100 mg/kg) and less efficient than tert-Butylhydroquinone [TBHQ] (50 mg/kg).

Silva *et al* (3), evaluating the mushroom *A blazei*, had a concentration of 15 mg/g of total phenolic compounds in methanol:aqueous extract (1:1). The content of total phenolic compounds exhibited by the *A blazei* was also assessed by Tsai *et al* (23), who obtained 5.67 mg/g of phenolic compounds in the aqueous extract of this mushroom. In this study, the values of total polyphenols were lower. The alcoholic extract of the mushroom *A sylvaticus* had 9.42 mg/100g of phenolic compounds. The ethereal and aqueous extracts showed 4.11 mg/100g and 0.98 mg/100g, respectively.

In a study conducted by Cruz *et al* (2), the authors found positive results in tests for pharmacognostic tannins, flavonoid glycosides and essential oils, indicating the antioxidant capacity of *A sylvaticus.*

Chemical studies have revealed that the high concentration of nutrients and active ingredients in mushrooms is directly related to the type of lineage used, which requires specific conditions or several factors, such as: i) nutritional factors (substances essential for development: carbon, nitrogen, vitamins and minerals); ii) abiotic factors (moisture content of compost and cover, temperature, light, oxygen, chemicals in the air, CO_2); iii) biotic (virus, bacteria, actinomycetes, fungi, nematodes, insects, mites and genetic); iv) genetic factors (natural or artificial); and v) factors of processing [harvest, drying/dehydration and storage] (26).

According to Neves *et al* (16), the market demand for functional foods has grown considerably; the consumer expects to reduce spending on various diseases that affect the population. During the last decade of the twentieth century, consumers in western countries have shown great interest in functional foods, including in this category all food products or ingredients, whether conventional or not, capable of providing health benefits. Among the benefits of eating *A sylvaticus* mushroom are the nutritional and antioxidant properties (10), which is why this is considered an excellent functional food.

The relevance of *A sylvaticus* research in Brazil, as a developing country, is to increase this medicinal mushroom production and processing. The results show that *A sylvaticus* fungus has a great antioxidant potential that can prove that this mushroom can be used as a functional food, being a supporting actor for combating cancer. This way, Brazil producers can expand the therapeutics mushrooms' market, leading to benefits to many parts of the world.

CONCLUSIONS

Through the results obtained in this work, we can conclude that the *A sylvaticus* mushroom is an excellent source of antioxidants. Its great antioxidant potential was observed, particularly in alcoholic extract when compared to concentrations obtained in aqueous and ethereal extracts. This demonstrates the extraordinary benefits of this mushroom as preventive medicine, inasmuch as antioxidants fight free radicals produced in various metabolic situations mainly as a consequence of countless diseases.

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REFERENCES

1. Figueiredo VA, Silva CHC. A influência da alimentação como agente precursor, preventivo e redutor do câncer. Univ Ci Saúde 2001; **1:** 317–25.
2. Cruz MSA, Okamoto MKH, Wadt NSY. Avaliação farmacognóstica, antimicrobiana e a ação toxicológica do extrato de espécies de cogumelos (Agaricus). III Encontro de Iniciação Científica e Seminário Nacional de Pesquisa. O papel da pesquisa na produção do conhecimento. São Paulo: Uninove; 2007: 20–21.
3. Silva AC, Oliveira MC, Del Re PV, Jorge N. Use of mushroom extract as natural antioxidant in soybean oil. Ciênc Agrotec 2009; **33:** 1103–8.
4. Dorman HJD, Kosar M, Kahlo K, Holm Y, Hiltunen R. Antioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varieties, and cultivars. J Agric Food Chem 2003; **51:** 4563–9.
5. Ferreira LA, Matsubara LS. Free radicals: concepts, related diseases, defense system and oxidative stress. Rev Ass Med Bras 1997; **43:** 61–8.
6. Soares SE. Ácidos fenólicos como antioxidantes. Rev Nutr 2002; **15:** 71–81.

7. Pereira ALF, Vidal TF, Constant PBL. Antioxidantes alimentares: importância química e biológica. J Brazilian Soc Food Nutr 2009; **34:** 231–47.
8. Ratnam D, Ankola D, Bhardwaj V, Sahana D, Kumar M. Role of antioxidants in prophylaxis and therapy: a pharmaceutical perspective. J Control Release 2006; **113:** 189–207.
9. Furlani RPZ, Godoy HT. Valor nutricional de cogumelos comestíveis. Rev Inst Adolfo Lutz 2005; **64:** 149–54.
10. Hi EMB, Oliveira ARM, Wadt N, Bach EE. Extratos de cogumelo do sol previnem câncer induzido pelo pristane em ratos – uma visão bioquímica. Anais do III Simpósio Nacional Sobre Cogumelos Comestíveis; 2006.
11. Hi EMB, Azevedo MRA, Bach EE, Ogata TRP. Efeito protetor do extrato de Agaricus sylvaticus em fígado de ratos do tipo Wistar inoculado com Pristane. Saúde Coletiva 2008; **5:** 76–8.
12. Fortes RC, Novaes MRCG. The effects of Agaricus sylvaticus fungi dietary supplementation on the metabolism and blood pressure of patients with colorectal cancer during post surgical phase. Nutr Hosp 2011; **26:** 176–86.
13. Fortes RC, Recôva VL, Melo AL, Novaes MRCG. Alterações gastrointestinais em pacientes com câncer colorretal em ensaio clínico com fungos Agaricus sylvaticus. Rev Bras Coloproctol 2010; **30:** 45–54.
14. Borguini, RG. Antioxidant potential and physical-chemical characteristics of organic tomato (Lycopersicon esculentum) in comparison with conventional tomato [Thesis]. São Paulo: Universidade de São Paulo; 2006.
15. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Food Sci Technol 1995; **28:** 25–30.
16. Neves LC, Alencar SM, Carpes ST. Determinação da atividade antioxidante e do teor de compostos fenólicos e flavonóides totais em amostras de pólen apícola de Apis mellifera. Braz J Food Technol 2009; **8:** 107–10.
17. Mensor LL, Menezes FS, Leitão GG, Reis AS, Santos TC, Fit CS et al. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res 2001; **15:** 127–30.
18. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 1965; **16:** 144–58.
19. Percário S, Naufal AS, Gennaro MS, Gennaro JL. Antioxidant activity of edible mushroom blushing wood, Agaricus sylvaticus Schaeff. (Agaricomycetideae) in vitro. Int J Med Mushr 2009; **11:** 133–40.
20. Rice-Evans CA, Miller NJ, Paganga G. Structure antioxidant activity relationships of flavonoids and phenolic acids. Free Rad Bio Med 1996; **20:** 933–56.
21. Javanmardi J, Stushnoff C, Locke E, Vivanco JM. Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Food Chem 2003; **83:** 547–50.
22. Elmastas M, Isildak O, Turkekul I, Temur N. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. J Food Compos Anal 2007; **20:** 337–45.
23. Tsai S, Tsai H, Bad J. Antioxidant properties of Agaricus blazei, Agrocybe cylindracea and Boletus edulis. Lebensm Wiss Technol 2007; **40:** 1392–402.
24. Kaur C, Kapoor HC. Antioxidant activity and total phenolic content of some Asian vegetables. Int J Food Sci Technol 2002; **37:** 153–61.
25. Barros L, Baptista P, Correa MD, Mitchell JS, Ferreira ICFRJ. Antioxidant activity of Portuguese wild edible mushrooms. J Agric Food Chem 2007, **55:** 4781–8.
26. Urben AF. Morphological and physiological access of Agaricus blazei and A. sylvaticus. Biotecnologia Ciência e Desenvolvimento 2007; **37:** 76–9.