Determination of antioxidant and antimicrobial properties of *Agaricus bisporus* from Romanian markets

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Abstract The antioxidant action of ethanolic extract from *Agaricus bisporus* which is available in the supermarkets of Bucharest, Romania, was assessed by determining its reducing power and its radical scavenging activity. The determinations were made by analyzing the freeze-dried ethanolic extract. The radical scavenging activity was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), superoxide radical, nitric oxide radical and hydrogen peroxide scavenging assays. Total phenols, flavonoids, ascorbic acid, β-carotene and lycopene were also determined. The ethanolic extract from *A. bisporus* could be a natural antioxidant and antimicrobial source against the tested organisms, as demonstrated by the minimum inhibitory concentration values.

Keywords: mushroom, freeze dried, scavenging activity, MIC.

1. Introduction

Agaricus bisporus is well known by several names, such as the common mushroom, button mushroom, white mushroom and champignon mushroom. This mushroom may be cultivated with minimum effort and, therefore, it is most frequently found sold along with other market produce. It is traded all year round, registering the highest sales volumes among the edible mushrooms. The marketed mushrooms are known for their rich source of biologically active compounds (phenolic compounds, carotenoid compounds and ascorbic acid), which offer protection to the human body in a natural way [1]. Moreover, they contain proteins and nutritive fibers, as well as other vitamins necessary for the normal functioning of the human body.

By way of previous researches, it was noticed that *A. bisporus* has a significant antioxidant activity as compared with *Pleurotus sajor-caju, Volvariella volvaceae* or *Pleurotus ostreatus*. The most significant antimicrobial activity was observed in *Staphylococcus aureus* for ethyl acetate extracts [2]. Such studies demonstrate that, besides its use in the food industry, these mushrooms also have a therapeutic role, by integration thereof, in nutritional supplements. In the case of *A. bisporus*, at the phenols composition which prevails in most mushrooms, ergothioneine is added [3]. The first objective of the study was to determine the antioxidant properties of ethanolic extract from dried mushrooms sold in the supermarkets of Bucharest. The antioxidant properties were assayed in terms of antioxidant activity by their reducing power and their scavenging abilities on radicals. The amounts of potential antioxidant components in the ethanolic extract were also determined. The second objective of the study was to evaluate the antibacterial activity against Gram-negative and Gram-positive bacteria, and two *Candida* strains.

2. Experimental

Preparation of samples. Mushrooms *A. bisporus* (trays weighing 500 g, collected over one day) were purchased from the Bucharest supermarkets. Mushrooms without any damage were chosen and dried in a stream of dry air. The drying process took place at a constant temperature of 25 °C in the oven for 15 days, until a constant weight was reached.

Obtaining of extracts. The dried samples were subjected to 70% ethanol extraction; 10 g of the dried mushroom sample was extracted using 100 mL solvent (ethanol), over a period of 24 h, at 25 °C and at 150 rpm. The extract was filtered using a Whatman No. 4 filter paper [4,5]. The solvents used for extraction were removed using a rotary vacuum evaporator Buchi R215, with a vacuum controller V-850, and a heating bath for parallel evaporation

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Multivapor P-6, at 50 °C under vacuum. The resulting extracts were lyophilized in a freeze-dryer ALPHA 1-2/LD plus (Martin Christ GmbH), at –55 °C, for 48 h. Extract yield (g/g) was expressed as the weight of extract (g) per gram of dry mushroom of *A. bisporus* [6].

Antioxidant activity (DPPH free radical scavenging activity) of ethanolic mushroom extract. The antioxidant activity of the plant extracts and the standard (ascorbic acid) was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical activity. The different concentration of the extracts (2-10 mg/mL) was prepared in ethanol. 0.002% of DPPH was prepared in ethanol and 1 mL of this solution was mixed with 1 mL of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Helios λ spectrophotometer. % inhibition was calculated using the formula given below (14): $(A - B)/A \times 100$, where A - optical density of the blank and B - optical density of the sample [5].

Reducing power. Different extract concentration (2 to 10 mg/mL) in ethanol (2.5 mL) was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide, and the mixture was incubated at 50°C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 200 ×g for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm against a blank. Ascorbic acid was used as standard [7].

Nitric oxide radical scavenging assay. The procedure is based on the method [8] where sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions.

Hydrogen peroxide radical scavenging (SO) assay. A solution of hydrogen peroxide (2 mmol L^{-1}) was prepared in a phosphate buffer (pH 7.4). The hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm with molar absorbtivity of 81 M^{-1} cm^{-1}. Each extract (100 μg mL^{-1}) was added to the hydrogen peroxide solution (0.6 mL). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank containing phosphate buffer without hydrogen peroxide [9].

Superoxide radical scavenging (SO) assay. The reaction mixture contained Nitroblue tetrazolium (0.1 mM) and Nicotinamide adenine dinucleotide (0.1 mM) with or without sample to be assayed in a total volume of 1 mL of Tris-HCl buffer (0.02 M, pH 8.3). The reaction was started by adding Phenazine methosulphate (10 μM) to the mixture and change in the absorbance was recorded at 560 nm. The percent inhibition was calculated against a control without test sample [10].

The total phenolic and flavonoid content of ethanolic extract, and several organic fractions, were determined using Folin-Ciocalteu reagent and aluminium chloride colorimetric method, respectively [10].

β-carotene and lycopene determination. The dried ethanolic extract (100 mg) was vigorously shaken with 10 mL of acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 645 and 663 nm. Contents of β-carotene and lycopene were calculated according to the following equations: lycopene (mg/100 mL) = $-0.0458\ A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$; β-carotene (mg/100 mL) = $0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$ [11].

Ascorbic acid determination. Ascorbic acid contents of the extract were determined spectrophotometrically by metaphosphoric acid extraction of 2,6-dichlorophenol indophenol dye using a Helios spectrophotometer in a wavelength of 500 nm with a 1 cm quartz cell. The ascorbic acid content was reported as mg/100 mL [12].

Determining the antimicrobial capacity (Minimum Inhibitory Concentration - MIC). For tests, *Escherichia coli* CBAB 2, *Bacillus cereus* CMGB 215, *Listeria innocua* CMGB 218, *Staphylococcus aureus* ATCC 6588, *Pseudomonas aeruginosa* ATCC 15442, *Candida albicans* ATCC 20231, *Candida sp*. ICCF 15 strains were used. Each strain was inoculated separately (10^6 UFC/mL) in a Petri dish on which LB/YPG agarised medium was poured. The extract was incorporated into nutrient agar at concentrations from 1 mg/mL to 25 mg/mL. 20 μL of extract were added and the dish was kept 30 minutes to absorb the extract. Then it was

inserted in the thermostat, at 28 – 30^{0}C, for 24 hours. The minimum inhibitory concentrations (MICs) of the extract for each test microorganism were regarded as the agar plate with the lowest concentrations without growth. [13].

Statistical analysis. All the essays were assessed in triplicate, and the results were expressed as mean ±SD values of three observations ($p < 0.05$). The mean values and standard deviation were calculated with the EXCEL program from Microsoft Office 2010 package.

3. Results and Discussions

Ethanol is a bipolar solvent with high penetration power, therefore, being mainly used in extractions of biologically active compounds from natural products. Using a mixture of ethanol:distilled water of 70:30 (v/v), an extraction yield of 4.73 ± 0.32% was obtained. The extraction yield of phenolic compounds in *A. bisporus* was 0.19 ± 0.12%.

In vitro antimicrobial activity

The results of the *in vitro* antimicrobial activity of ethanolic extract are detailed in **Table 1.** All extracts from *A. bisporus* have antimicrobial activity against the potentially pathogenic strains tested. This is due to the fact that the ethanolic extract contains various components with antimicrobial activity. The freeze-dried extract displayed maximum inhibition (MIC 5 mg/mL) towards *Escherichia coli* CBAB 2, and also against *S. aureus* ATCC 6588 (Gram-negative). In exchange, *Pseudomonas aeruginosa* ATCC 15442 proved to be the most resistant strain, with a minimum inhibitory concentration value of 15 mg/mL. The antimicrobial activity on the tested strains confirms the use of such raw extracts, and justifies the researches on new sources of bioactive compounds.

2,2-diphenyl-1-picrylhydrazyl scavenging activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical is a very stable one when it accepts an electron. The action of antioxidants determines the reduction of absorbance to 517 nm. The reduction of absorbance caused by the antioxidants determines a change in color from violet to yellow [14].

Thus, DPPH is normally used as a sublayer to assess the antioxidant activity of an extract [15]. **Figure 1** presents (p <0.01) the reduction of the DPPH radicals' concentration due to the ethanolic extract in *A. bisporus* and the standard (ascorbic acid). At a concentration of 10 mg/mL, the scavenging ability of the DPPH radical was approximately 43% lower than that of the standard, and by 31% as compared with the ethanolic extract from *A. blazei*. The study confirms the moderate antioxidant effect of the ethanolic extract from *A. blazei*. Similar values were obtained for strains of *Pleurotus*. They were lower than those of the extract from *Coprinus comatus*, approximately 65%, for similar concentrations of extract [16]. This result is compliant with prior researches.

Reducing power

Figure 2 presents the reducing power of the ethanolic extract as compared with the ascorbic acid. It is well known that the reducing power is associated with the antioxidant activity and the total quantity of phenols. The yellow color of the testing solution changes to various tones of green-blue, depending on the reducing power of each extract or on the concentration thereof. The presence of antioxidants in an extract determines the reduction of Fe^{3+} to Fe^{2+} [17, 18, 19]. The reducing power of the ethanolic extract increased with its concentration.

Table 1. Minimum inhibitory concentration (MIC) of *Agaricus bisporus* ethanolic extract

Tested strains	Antimicrobial activity (mg/mL)	Ampicillin (µg/mL)
Escherichia coli CBAB 2	5	22
Bacillus cereus CMGB 215	12.5	22
Listeria innocua CMGB 218	7.5	10
Staphylococcus aureus ATCC 6588	5	23
Pseudomonas aeruginosa ATCC 15442	15	28
Candida albicans ATCC 20231	7.5	25
Candida sp. ICCF 15	7.5	25

Table 2. EC_{50} values (mg/mL) obtained in the antioxidant activity of ethanolic extract from *Agaricus bisporus*

	EC_{50} values
DPPH scavenging activity	3.59±0.08
Reducing power	4.71±0.15
Nitric oxide scavenging activity	4.22±0.29
Superoxide anion scavenging activity	10.00±1.37
Hydrogen peroxide scavenging activity	2.75±0.2

The results obtained (for 10 mg/mL) are similar to those for *A. arvensis* and *Sarcodon imbricatus*, at a concentration of 5 mg/mL [11]. Also, the values of the reducing power are similar to those obtained from the fructification bodies of the mushrooms *P. ferula* (0.57), *P. ostreatus* (0.56), and even higher for the extract from *Boletus edulis* (0.44) at 13.72% [17]. In exchange, as compared with the standard (ascorbic acid), the results were, on average, 62.5% lower, which is a moderate reducing power. These results are compliant with the scavenging ability of the DPPH radical.

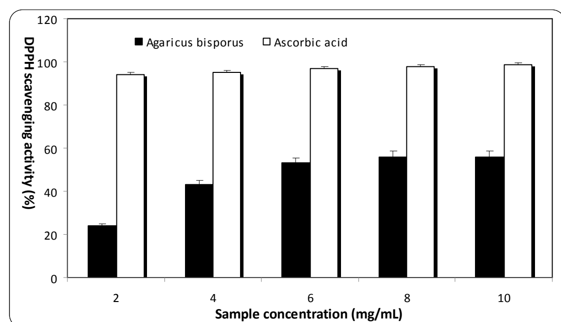

Fig. 1. DPPH radical scavenging activity of the ethanolic extract from *Agaricus bisporus*

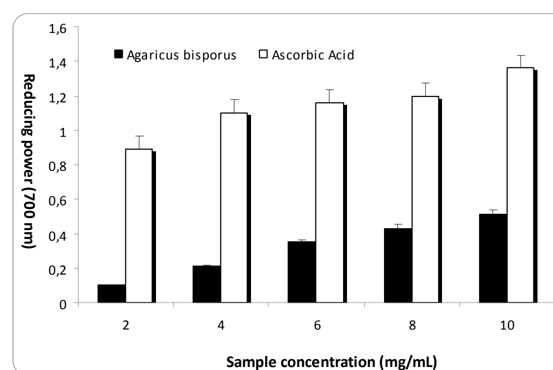

Fig. 2. Reducing power of the ethanolic extract from *Agaricus bisporus*

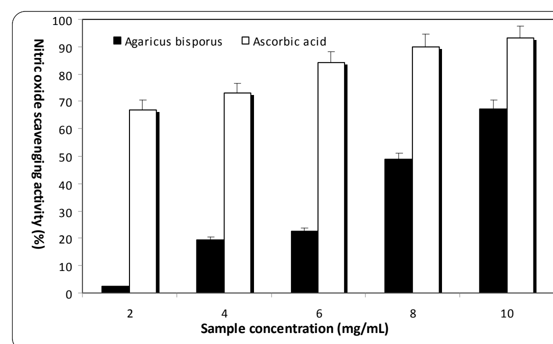

Fig. 3. Nitric oxide scavenging activity of the ethanolic extract from *Agaricus bisporus*

Nitric oxide radical scavenging activity

The nitric oxide may spontaneously occur by oxidation. It is very unstable and reacts with oxygen. It is a strong mediator for certain physiological processes, such as relaxation of the soft muscles and neuronal signaling, while the deficit of nitric oxide is associated with hypertension [20,21]. The ethanolic extract inhibits the nitric oxide depending on the concentration (**Fig. 3**). At a concentration of 10 mg/mL, the binding ability was 27.74% lower than that of the standard 67.2%. Thus, the EC_{50} value was 4.22 ± 0.29 mg/mL, and the standard 0.08 ± 0.01 mg/mL (**Table 2**).

Superoxide anion Radical Scavenging Activity

This radical is involved in the acceleration of aging processes. Superoxide anions play an important role in the formation of other reactive oxygen species, such as singlet oxygen, hydrogen peroxide and hydroxyl radical, which induce oxidative damage in DNA, lipids and proteins [22]. **Figure 4** indicates a reduced inhibiting ability of the superoxide radical. An ability of 50% was

determined for 10 mg/mL, and the ascorbic acid used as standard displayed a value of 97% for the same concentration of the sample. The determinations are confirmed by the EC_{50} value of the standard, i.e. 0.6 ± 0.02 mg/mL.

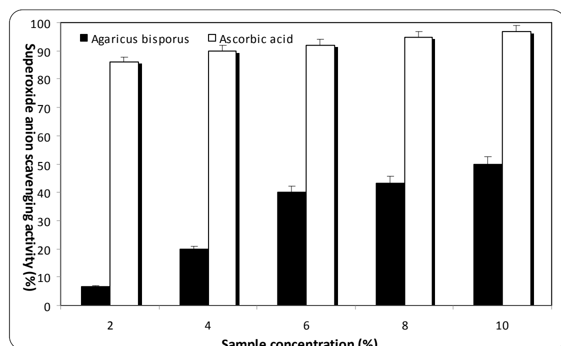

Fig. 4. Superoxide radical scavenging activity of the ethanolic extract from *Agaricus bisporus*

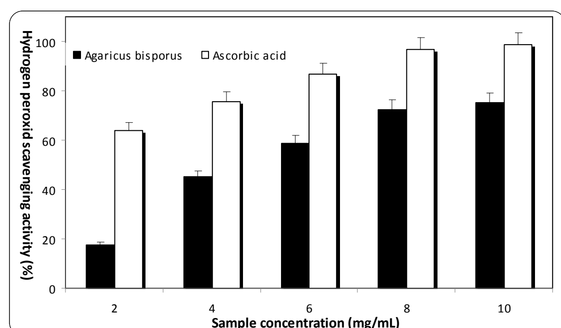

Fig. 5. Hydrogen peroxide radical scavenging activity of the ethanolic extract from *Agaricus bisporus*

Scavenging of hydrogen peroxide

Hydrogen peroxide is formed *in vivo* by enzymatic oxidation processes. It may go through the cellular membrane, having significant oxidation abilities. Although not toxic in itself, its negative role results from the ability to generate hydroxyl radicals at cellular level [23]. The biological value of the ethanolic extract from *A. bisporus* comes from the high inhibiting ability of the hydrogen peroxide. This conclusion is strengthened by the low value of EC_{50}, 2.75 ± 0.2 mg/mL (Table 2), as compared with that of the ascorbic acid, 1.08 ± 0.13 mg/mL.

The validation of the results obtained is supported by the values which correspond to recent researches in which both the antioxidant activity and the reducing power are concerned (Table 2). In this case, the values for EC_{50} were less than 10 mg/mL, with the exception of the superoxide anion scavenging activity. A very good correlation coefficient (r^2) was calculated between the quantity of phenols and the DPPH scavenging activity (0.7864), reducing power (0.9843), nitric oxide scavenging activity (0.9346), superoxide anion scavenging activity (0.935) and hydrogen peroxide scavenging activity (0.8956). In exchange, the correlation coefficient between the content of flavonoids and DPPH scavenging activity was 0.0776, reducing power (0.0027), nitric oxide scavenging activity (0.0928), superoxide anion scavenging activity (0.0087) and hydrogen peroxide scavenging activity (0.0076). These low values of the correlation coefficient (r^2) prove the different roles exercised by the flavonoids as compared with phenols on various species of free radicals [24].

In the ethanolic extract from *A. bisporus*, compounds with antioxidant effect were identified (**Table 3**). A very small quantity of carotenoid compounds, especially lycopene, was identified, as compared with other extracts from *A. bisporus* [25] and *P. ostreatus* [26].

Table 3. Antioxidant contents of ethanolic extract from *Agaricus bisporus*

Antioxidant components	Ethanolic extract
Total phenols (mg gallic acid/g extract)	19.74±0.3
Flavonoids (mg quercetina/g extract)	123±0.62
Ascorbic acid (mg/g extract)	0.01±0.01
β-carotene (µg/100 g extract)	9±0.5
Lycopene (µg/100 g extract)	0.1±0. 005

On the other hand, the total determined quantity of phenols and flavonoids was higher than in the case of certain species related to or belonging to the same species in the case of methanolic extract [27].

4. Conclusions

A. bisporus has significant antioxidant activities and free radicals binding abilities. The extract represents a source of compounds useful in

the preparation of natural products with high biological value. Such an extract may help in preventing certain diseases caused by free radicals. The results obtained by way of this study also demonstrate that the mycelium extract may act as an antimicrobial agent against bacteria responsible for various infections. The solvent used directly influences its use as a nutraceutical agent and the bioactive properties thereof.

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6. References

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