ANTIOXIDANT ACTIVITY OF ROMANIAN AGARICUS BLAZEI MURRILL. AND AGARICUS BISPORUS J. E. LANGE MUSHROOMS

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Abstract

Mushrooms are widely used in therapeutics, due to their various biological effects. The aim of our paper was the assessment of the chemical composition and the assessment of the antioxidant activity of Agaricus bisporus (wild-growing and cultivated) and Agaricus blazei (cultivated) mushrooms. Phytochemical screening was determined by means of spectrophotometric, spectrometric, and X-ray diffraction methods. Antioxidant activity was assessed by scavenger activity towards DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS⁺ (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) free radicals, ferric reducing power and ferrous ions chelating properties. Wild-growing Agaricus bisporus and Agaricus blazei have a similar total phenolic content. The highest calcium (1783.14 mg/kg), potassium (99580.08 mg/kg) and magnesium (1070.79 mg/kg) concentrations were found for wild-growing Agaricus bisporus. Cultivated Agaricus bisporus had a weak antioxidant capacity. All analysed mushrooms are a source of bioactive compounds with antioxidant activity.

Rezumat

Ciupercile sunt mult întrebuințate în terapeutică datorită numeroaselor proprietăți biologice. Obiectivul lucrării a constat în determinarea compoziției chimice și evaluarea activității antioxidante a ciupercelor Agaricus bisporus (spontană și de cultură) și Agaricus blazei (de cultură). Pentru screening-ul fitochimic s-au utilizat metode spectrotometrice, spectrometrice și difrația de raze X. Activitatea antioxidantă a urmărit capacitatea de scavenger a radicalilor liberi 2,2-difenil-1-picirilhidrazil (DPPH), acidului 2,2-azino-bis-(3-etil-benzotiazolin-6-sulfonic) (ABTS⁺) și capacitatea de reducere, respectiv chelatare a fierului. Agaricus bisporus spontană și Agaricus blazei au un conținut asemănător de polifenoli totali. Cea mai mare cantitate de calciu (1783,14 mg/kg), potasiu (99580,08 mg/kg) și magneziu (1070,79 mg/kg) s-a obținut pentru Agaricus bisporus spontană. Forma din comerț a manifestat o activitate antioxidantă redusă. Ciupercile analizate prezintă o sursă de constituenți bioactivi cu rol antioxidant.

Keywords: Agaricus bisporus, Agaricus blazei, total phenolic content, polysaccharides, minerals, antioxidant capacity

Introduction

Agaricus genus has a wide distribution throughout the world, comprising over 300 species [12]. Agaricus bisporus J. E. Lange (it has different names depending on the stage of development and cap’s colour - white button mushroom, champignon, brown cap mushroom, portobello) and Agaricus blazei Murrill. (Agaricus brasiliensis - cogumelo do sol, himematsutake, God’s mushroom, Royal sun mushroom) are well-known, due to their chemical composition and numerous therapeutic effects.

Both mushrooms are a source of polysaccharides (glucans) [13, 22]; aminoacids [5, 24, 31, 40]; organic acids [24, 31]; sterols (ergosterol) [14]; polyols [31, 40]; fatty acids [3, 39]; vitamins [2, 3, 11]; lovastatin [5]; minerals [16, 21] and phenolic compounds (gallic acid, protocatechuic acid, caffeic acid, ferulic acid, syringic acid, pyrogallol) [3, 4, 25]. According to scientific literature, Agaricus bisporus also contains lectins [19], indolic and volatile compounds [28, 32]. In addition Royal sun mushroom contains agaritine (a hydrazine-containing compound), that exhibits anti-tumour activity upon U937 leukemic cells [1]. Regarding their biological effects, both Agaricus species show cytotoxic [1, 7, 43, 46], immunomodulatory [22], antioxidant [22, 25, 28], hepatoprotective [17], antibacterial [39], antifungal [39], antiviral [45], anti-parasitic [41], hypoglycaemic [44] and anti-inflammatory [15] properties. According to Lau et al. research, some peptides isolated from
*Agaricus bisporus* showed angiotensin I converting enzyme inhibitory effects, with anti-hypertensive properties [23]. According to Dong et al. an aqueous extract obtained from *Agaricus blazei* has oestrogenic activity, so it might be useful for preventing atherosclerosis [8].

On the strength of scientific data, the aim of our research was the determination of chemical composition and antioxidant capacity of cultivated *Agaricus blazei* Murrill. and cultivated/wild-growing *Agaricus bisporus* J. E. Lange.

### Materials and Methods

**Material.** Whole fruiting bodies of *Agaricus bisporus* and *Agaricus blazei* have been used as samples. Brown wild-growing *Agaricus bisporus* (ABS) was collected in October 2015 from Morărești, Argeș district (44°58’ N, 24°34’ E), Romania. Cultivated *Agaricus bisporus* (ABC) was purchased from a supermarket in Bucharest, Romania. Cultivated *Agaricus blazei* (ABL) was obtained from a specific Romanian manufacturer. All mushrooms were freeze-dried, using a Christ Alpha 1-2/B Braun, Biotech International lyophilisator.

**Reagents, solvents and apparatus.** All chemicals were purchased from Roth (Germany), unless otherwise stated. 2,2-diphenyl-1-picrylhydrazyl, diammonium salt of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) and ferrozine were from Sigma-Aldrich (Germany). For all spectrophotometric determinations a Jasco V-530 spectrophotometer (Jasco, Japan) was used.

For phytochemical screening we have used spectrophotometric, spectrometric and X-ray diffraction methods.

**Preparation of samples.** For the spectrophotometric and the antioxidant assays, 5 g of each mushroom were heated on a reflux condenser with 100 mL water for 15 min. After cooling, the solutions were brought to 100 mL volumetric flasks. The extractive solutions were encoded ABS (for wild-growing *Agaricus bisporus*), ABC (for cultivated *Agaricus bisporus*) and ABL (for cultivated *Agaricus blazei*).

**Spectrophotometric determination of total phenolic content.** Total polyphenols were determined with Folin-Ciocalteu reagent according to Singleton et al., method [37]. Results were expressed as g gallic acid/100 g dried mushroom (d.w.), based on a calibration curve (1.22 - 7.22 mg/mL, \( R^2 = 0.9989, n = 6 \)).

**Determination of mineral elements** was carried on by means of semi-quantitative (X-ray fluorescence = XRF) and quantitative (inductively coupled plasma atomic emission spectroscopy = ICP - AES) methods [10].

**X-ray diffraction (XRD)** was used for characterizing the crystalline phases of different active substances from analysed mushrooms. Determinations were performed using a Rigaku SmartLab equipment, operating conditions: 45 kV, 200 mA, Cu Ka radiation (1.54059 Å), parallel beam configuration (20/0 scan mode), from 3 to 90 2°; the components were identified using the Rigaku Data Analysis Software PDXL 2, database provided by ICDD.

The **antioxidant capacity** was determined by means of well-known methods: scavenger capacity upon 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•−) free radicals, ferric reducing power (FRAP method) and chelating activity upon ferrous ions. These methods have also been used by other authors for evaluation of antioxidant activity of several medicinal mushrooms [26].

**DPPH, ABTS•− assays.** Determinations were carried on according to our previously described methods (Popescu et al., 2016) [33]. The concentration range was 3.03 - 9.09 mg/mL (for DPPH method) and 0.5 - 2.5 mg/mL (for ABTS•− assay) respectively.

**Ferric reducing power (FRAP method)** was determined according to Wong et al. (2006) [42]. The concentrations range was 2 - 9 mg/mL.

**Metal chelating activity.** The chelation of ferrous ions was estimated by the method of Dinis et al. [6] with slight modifications. Briefly, 200 µL of ABS, ABC, ABL solutions (6 - 22 mg/mL) were treated with 50 µL 2 mM FeCl₂ and 2.5 mL distilled water. Solutions were incubated for 5 min. at room temperature. Then, 200 µL 5 mM ferrozine were added and samples were incubated for 10 min. The absorbance of the mixture was measured at 562 nm against water, used as blank. The ability of mushrooms extracts to chelate ferrous ions was determined using the following formula:

\[
\text{chelating effect (\%)} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

Antioxidant activity was assessed by means of IC₅₀ (mg/mL) and ascorbic acid equivalents (for DPPH, ABTS•− and FRAP methods), EDTA-Na equivalents for ferrous ions chelating activity. IC₅₀ values were determined graphically from the linear regression curve plotted between percent (\%) of inhibition/absorbance values and extractive solutions concentration (mg/mL). Ascorbic acid and EDTA-Na equivalents were calculated based on calibration curves: 0.005 - 0.04 mg/mL, \( R^2 = 0.9975, n = 5 \) (DPPH method); 0.01 - 0.07 mg/mL, \( R^2 = 0.9973, n = 6 \) (ABTS•− method); 0.008 - 0.04 mg/mL, \( R^2 = 0.9969, n = 5 \) (FRAP method) and for metal chelating activity 0.04 - 0.16 mg/mL, \( R^2 = 0.9963, n = 6 \).

**Statistical analysis.** For each one of the selected mushrooms, three samples were analysed and spectrophotometric/antioxidant assays were carried out in triplicate (\( n = 3 \)). The results are expressed as mean ±
standard deviation and were processed by Microsoft Office Excel, 2007.

Results and Discussion

Brown wild-growing *Agaricus bisporus* and cultivated *Agaricus blazei* have a similar total phenolic content (Table I). Owing to different extraction techniques, we could not compare our spectrophotometric results with those reported in other studies. So that, Stojković *et al.*, found a total phenolic content of 4.17 g gallic acid/100 g d.w. for *Agaricus bisporus* and 1.49 g gallic acid/100 g d.w. for *Agaricus blazei* [39], whilst Elmastas *et al.*, found 1.31 g gallic acid/100 g dried wild-growing button mushroom from Turkey Black Sea region [9].

Table I

<table>
<thead>
<tr>
<th>Active substance</th>
<th>MUSHROOM</th>
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<tbody>
<tr>
<td></td>
<td>ABS</td>
</tr>
<tr>
<td><strong>Total phenolic content</strong> (g gallic acid/100 g d.w.)</td>
<td></td>
</tr>
<tr>
<td>Al</td>
<td>914.82</td>
</tr>
<tr>
<td>Ca</td>
<td>1783.14</td>
</tr>
<tr>
<td>Fe</td>
<td>89.98</td>
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<tr>
<td>Mg</td>
<td>1070.79</td>
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<td>Na</td>
<td>13960.71</td>
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<td>P</td>
<td>6793.64</td>
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<tr>
<td>Cu</td>
<td>37.49</td>
</tr>
<tr>
<td>K</td>
<td>99580.08</td>
</tr>
</tbody>
</table>

"d.w." – dry weight

XRF and ICP-AES assays (Figure 1, Table I) pointed out that all mushrooms contain macro- and microelements, that were also reported by other authors [16, 21, 29, 36]. Although, mushrooms are well known as important metal accumulators, cadmium, lead, nickel and mercury were not identified, so we concluded that all samples proceed from unpolluted areas [20]. Other heavy metals, such as copper and iron are within the accepted range of concentrations (20 - 100 mg/kg d.w. and 50 - 150 mg/kg d.w.), for mushrooms native of unpolluted areas [20]. Copper and iron concentrations for cultivated *Agaricus bisporus* are lower compared to other studies (72.1 - 533.73 mg/kg copper and 78 mg/kg iron) [21, 27, 29]. As far as brown cap wild-growing mushroom is concerned, copper and iron concentrations are higher than those found by Sheikh *et al.*, (21.2 mg/kg copper and 61.37 mg/kg iron) [36].

Aluminium content is higher compared to other medicinal mushrooms (*Armillaria mellea* – 4.8 mg/kg; *Boletus edulis* – 370 mg/kg; *Lycoperdon perlatum* – 42.2 mg/kg) [35]. Phosphorus content is within the concentration range that is frequently found in mushrooms (5000 - 10000 mg/kg) [20]. Calcium, potassium and magnesium concentrations are higher compared to other authors’ results [16, 29, 36]. Despite other studies [16, 21, 29, 36], micro-elements such as zinc, manganese, chromium, vanadium and selenium (well known for their antioxidant properties) were not identified. From our point of view, these differences are the consequence of mushrooms source and pedoclimatic conditions. XRD analysis (Figure 2.) has revealed that mannitol and galactose are the main crystalline active compounds. Our results are similar to scientific literature, since *Agaricus bisporus* and *Agaricus blazei* polysaccharides contain galactose [22, 43], while mannitol is the main polyol, found in these mushrooms [39].
In vitro antioxidant capacity was determined by several methods, as natural compounds act as radical scavengers, reducing agents or transition metals (copper, iron) chelators [30]. Our results (Tables II and III) showed that wild-growing Agaricus bisporus has the best scavenger activity upon ABTS·⁺ free radical and ferric reducing power. As shown in Figure 3B, the scavenger activity upon ABTS·⁺ free radical, at 2.5 mg/mL was 99.15% for ABS, 72.63% for ABC and 85.57% for ABL. Concerning ferric reducing power (FRAP method), at 9.09 mg/mL the absorbance was 1.1894 for ABL, 1.7028 for ABS and only 0.6614 for ABC (Figure 4A). As shown in Figure 3A, mushrooms scavenged DPPH free radical in a dose-dependent manner. The mushrooms aqueous extracts displayed scavenging activity with values ranging from 86.27% for ABL to 74.28% for ABS and only 57.08% for ABC (Figure 3A). The best DPPH scavenger activity was observed for royal sun mushroom (Tables II and III).

Oxidative stress is a key factor in the aetiology of cardiovascular, metabolic neurodegenerative diseases, cancer etc. [18, 30]. Chelating activity upon ferrous ions is an important aspect of natural compounds antioxidant activity, since high levels of ferrous ions are responsible for lipid peroxidation through Fenton reaction [30].

Our results regarding God’s mushroom weak ferrous ions chelating activity were unexpected (Tables II and III, Figure 4B), since other studies found good antioxidant properties [4, 38].

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**Figure 2.**
XRD analysis
Legend: DL, D = DL/D-mannitol; G = D-galactose

**Figure 3.**
Evaluation of the antioxidant activity: A – DPPH method; B – ABTS·⁺ method

**Figure 4.**
Evaluation of the antioxidant activity: A – FRAP method, B – ferrous chelating activity
assume that these differences might be the result of distinct chemical composition. According to Carvajal et al. (2012) studies, the ferrous ions chelating activity was higher for methanolic mycelial extracts compared to fruiting body extracts, due to a higher citric acid content [4].

### Table II

<table>
<thead>
<tr>
<th>MUSHROOM</th>
<th>DPPH</th>
<th>ABTS</th>
<th>FRAP</th>
<th>CHELATING ACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABS</td>
<td>1.694 ± 0.4973</td>
<td>0.5947 ± 0.0195</td>
<td>2.5478 ± 0.2792</td>
<td>13.7650 ± 0.10163</td>
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<tr>
<td>ABC</td>
<td>7.6243 ± 0.1516</td>
<td>1.3248 ± 0.0801</td>
<td>7.1437 ± 0.7159</td>
<td>18.883 ± 0.0805</td>
</tr>
<tr>
<td>ABL</td>
<td>1.3727 ± 0.2641</td>
<td>0.9829 ± 0.0123</td>
<td>3.6536 ± 0.1546</td>
<td>-</td>
</tr>
</tbody>
</table>

*" not determined

### Table III

<table>
<thead>
<tr>
<th>MUSHROOM</th>
<th>DPPH</th>
<th>ABTS</th>
<th>FRAP</th>
<th>CHELATING ACTIVITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABS</td>
<td>5.7698 ± 1.4588</td>
<td>73.96 ± 17.6352</td>
<td>7.3530 ± 0.1551</td>
<td>6.4525 ± 0.5976</td>
</tr>
<tr>
<td>ABC</td>
<td>2.6200 ± 0.0572</td>
<td>42.6925 ± 11.8245</td>
<td>2.6775 ± 0.0943</td>
<td>4.7057 ± 0.1687</td>
</tr>
<tr>
<td>ABL</td>
<td>8.4119 ± 1.3577</td>
<td>54.3133 ± 14.2781</td>
<td>5.1071 ± 0.2354</td>
<td>-</td>
</tr>
</tbody>
</table>

*" not determined, "d.w." – dry weight

Studies regarding the antioxidant activity have been undertaken by other authors as well [3, 4, 9, 22, 25, 27, 36, 38, 39], but a direct comparison with their results can’t be made, since the mushroom’s source and extraction methods were different. We assume that magnesium and mannitol are responsible for the antioxidant activity. Magnesium inhibits NADPH-oxidase and maintains a constant level of reduced glutathione [34]. Other active substances (polysaccharides, organic acids, phenolic compounds) might be involved in the overall antioxidant capacity [3, 4, 22, 24, 25].

### Conclusions

The analysed mushrooms are a source of phenolic compounds and polysaccharides. Wild-growing *Agaricus bisporus* and cultivated *Agaricus blazei* have a similar total phenolic content (1 g gallic acid/100 g dried mushroom), while cultivated *Agaricus bisporus* has the lowest content (0.51 g gallic acid/100 g dried mushroom). In addition, analysed mushrooms proved to be an important source of functional minerals (calcium, magnesium and potassium). The highest content was found for wild-growing *Agaricus bisporus* (magnesium – 1070.79 mg/kg, calcium – 1783.14 mg/kg and potassium – 99580.08 mg/kg). The content of heavy metals (iron – 32.7 - 89.9 mg/kg; copper – 21.4 - 85.9 mg/kg) was within the accepted range of concentrations, thus suggesting that the sampling area was not polluted. The overall antioxidant activity is strongly correlated with the total phenolic content. Our results pointed out that wild-growing *Agaricus bisporus* has the best scavenger activity towards ABTS⁻ free radical (IC₅₀ = 0.59 mg/mL), ferric reducing power (IC₅₀ = 2.54 mg/mL) and ferrous ions chelating properties (IC₅₀ = 13.76 mg/mL). Taking into consideration the role of oxidative stress in different diseases pathology (cardiovascular, neurologic, autoimmune, cancer etc.), further pharmacological research is needed in order to determine the mushrooms therapeutic profile.

### References

19. Permeable lectin


