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Health impact of commercially cultivated mushroom
Agaricus bisporus and wild-growing mushroom
Ganoderma resinaceum - a comparative overview

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Abstract: Health promoting effects of hot water extracts obtained from fruiting bodies of commercially cultivated mushroom Agaricus bisporus (AbHW) and wild-growing mushroom Ganoderma resinaceum (GrHW) originated from Northern Serbia are presented in this research. These abilities were compared in vitro by the prevention of lipid peroxidation (LPx) in the linoleic acid model system, inhibition of angiotensin converting enzyme (ACE) which can help in the maintenance of a normal blood pressure level and strengthening the ability of the central cholinergic neuron by inhibiting the activity of acetylcholinesterase (AChE). Cytotoxic activities were observed towards selected human malignant (HeLa and K562) cell lines and normal-human peripheral blood mononuclear cells (PBMC). GrHW contained higher phenolics [5.9 g (100 g)⁻¹], inhibition of LPx (EC₅₀=1.07 mg mL⁻¹), ACE (IC₅₀=0.54 mg mL⁻¹) and AChE (IC₅₀=0.37 mg mL⁻¹), and exhibited a significant selectivity in the antitumor action against HeLa (IC₅₀=0.14 mg mL⁻¹) and K562 (IC₅₀=0.11 mg mL⁻¹) cells. AbHW contained higher total protein [6.4 g (100 g)⁻¹], carbohydrate [75.4 g (100 g)⁻¹] and β-glucan [55.1 g (100 g)⁻¹] content and induced significant proliferation of healthy PBMC from 152–116 % at the concentration range of 0.047–0.187 mg mL⁻¹. The difference in the biological activity of extracts provides guidance on their use as a functional food.

Keywords: Hot water extracts; cytotoxicity; enzyme inhibition; lipid peroxidation.

INTRODUCTION

Genetic predisposition is not the only cause of acquiring chronic diseases. They can be a consequence of harmful exposures received during life, which is known as "exposome". Cardiovascular diseases, diabetes, arthritis, cancer, inflammation and neurodegenerative disorders (e.g. Alzheimer’s disease) are often linked to lifestyle choices. Although common, many chronic diseases can
be prevented. One of the most significant exposome factors which have impact on the well-being of human health is functional food.\textsuperscript{2} Functional components of food may act at physiological target sites with the potential to provide benefits and well-being including reducing the risk of chronic diseases via antihypertensive effect, lowering of blood cholesterol, neutralization of reactive species, anti-carcinogenic effect and low-glycaemic response.\textsuperscript{3}

Over the past decade supplementation with mushrooms gained much of interest in the food nutrition area.\textsuperscript{4-7} Mushrooms have been consumed by mankind for many centuries due to the attractive sensory characteristics, optimal nutritional compositions and manageable cultivate conditions.\textsuperscript{4,6,8} They have been appreciated for their vital role in prevention and alleviation of various health problems, such as immunodeficiency, cancer, inflammation, hypertension, hyperlipidemia, hypercholesterolemia and obesity.\textsuperscript{4,6,9,10} According to different purposes, mushroom species are divided into edible and medicinal mushrooms.\textsuperscript{5} Although the interest for medicinal species is in rise, edible cultivated species still make the biggest part of the market. However, some species like *Ganoderma lucidum* are also cultivated, especially in China.\textsuperscript{5}

Consequently, this study aimed to present a comparative overview of health promoting effects of hot water extracts obtained from the fruiting bodies of commercially cultivated edible mushroom *Agaricus bisporus* and wild-growing mushroom *Ganoderma resinaceum*. Health promoting effects were studied *in vitro* by the prevention of lipid peroxidation (LPx), inhibition of angiotensin converting I enzyme (ACE) which can be beneficial in maintaining a normal blood pressure level and strengthening the ability of the central cholinergic neuron by inhibiting the activity of acetylcholinesterase (AChE). Cytotoxic activities were observed towards the selected human malignant cell lines and normal-human peripheral blood mononuclear cells (PBMCs) which are included in the antitumor immune response. To the best of our knowledge, there are very few studies that describe the biological properties of *G. resinaceum* extracts. In commercially available products *G. resinaceum* is usually mixed with *G. lucidum* based items making it difficult to distinguish.\textsuperscript{11}

**EXPERIMENTAL**

*Fungal materials*

Freshly harvested *A. bisporus* mushrooms at the closed cap stage were obtained from a local producer EkoFungi, Belgrade, Serbia. Fresh wild-growing fruiting bodies of autochthonous mushroom species *G. resinaceum* were collected from Fruška Gora (geographical coordinates: 45° 9′ 0″ N, 19° 43′ 0″ E), a large forest area and National park in Vojvodina, Northern Serbia (Republic of Serbia). Carpophores were identified according to the methods of classical herbarium taxonomy to confirm the correct species.\textsuperscript{11,12} The representative voucher specimens were deposited in the herbarium of the Department for Industrial Microbiology at the Faculty of Agriculture, University of Belgrade (No. GRF-1) together with their mycelial cultures. For further analysis, mushroom samples were lyophilized (Telstar
LyOAlfa 15-85, Terrasa, Spain) and powdered. A 100 g sample of each mushroom was extracted with 1 L of Milli-Q (MQ) water by autoclaving 1 h at 121 °C. The liquid part was concentrated to 10 % of its initial volume and semi-purified with two volumes of 75 % ethanol to precipitate the soluble fraction of dietary fibres; samples were left overnight in a refrigerator at 4 °C. After centrifugation for 10 minutes at 9000 g, washing with ethanol was repeated. The pellets were collected, dried at 40 °C and powdered. Hot water extracts of A. bisporus (AbHW) and G. resinaceum (GrHW) were dissolved in 5 % dimethyl sulphoxide (DMSO) prior to further analysis.

Chemical composition of extracts

To compare selected two species, total polysaccharide content, total α- and β-glucan content, total protein and phenol content were measured. The total polysaccharide content of the extracts was determined using a phenol–sulfuric acid method; results were expressed as g of glucose equivalents (GlcE) per 100 g of dry weight (DW) of the extract. Contents of total, α- and β-glucans were determined using the Mushroom and Yeast β-glucan Assay Procedure (Megazyme Int.); all values of glucan contents were expressed as g of GlcE per 100 g of DW of extract. Protein content was determined using a Bradford method; the total protein content was expressed as g of bovine serum albumin equivalents (BSAE) per 100 g of DW of extract. The Folin–Ciocăluţe reagent was adapted for a 96-well microplate reader (ELx808, BioTek Instruments, Inc., USA) was used to determine the total phenolic contents; results were expressed as g of gallic acid equivalent (GAE) per 100 g of DW of extract.

Analysis of monosaccharide composition

Liquid chromatography-mass spectrometry (LC-MS) was used to determine the monosaccharide profile of extracts. Each extract (15 mg) was hydrolyzed separately in 2 M trifluoroacetic acid (TFA, 15 mL) at 121 °C for 1 h, in amplified glass ampoules. The resulting hydrolysates were evaporated (IKA® Werke RV06-ML, Germany) to dryness at 45 °C, at reduced pressure. The residual TFA was removed by washing with isopropanol, 2 times, and the resulting hydrolysates were dissolved in 0.5 mL MQ water. LC-MS analyses were carried out on the high performance liquid chromatography (HPLC) device (Agilent 1200 Series, Agilent Technologies) with electrospray ion source (ESI). As a mobile phase, a mixture of solvent A (5 mM sodium formate in MilliQ water) and B (acetonitrile) in isocratic mode of elution was used: 0–20 min 25 % A at a flow rate of 1.40 mL/min. The injection volume was 10 μL for standards and 100 μL for hydrolyzed extracts, and the column temperature was 35 °C. MS data were obtained by applying the following parameters: ionization, negative ESI mode, capillary voltage 4000 V, gas temperature 350 °C, drying gas (nitrogen) 12 L/min, nebulizer pressure 45 psig (310.26 Pa), fragmentor voltage 140 V, mass range 100–2000 m/z. MassHunter Workstation software (Agilent Technologies) was used for data acquisition and processing. Monosaccharides identification was made according to their retention times (tR) and by chromatography with authentic sugar standards: D-glucuronic acid (D-GlcA), D-galacturonic acid (D-GalA), D-glucose (D-Glc), D-galactose (D-Gal), D-mannose (D-Man), D-xylose (D-Xyl), D-arabinose (D-Ara) and L-rhamnose (L-Rha), D-glucosamine (D-GlcN), N-acetyl-D-glucosamine (GlcNAc), D-fructose (D-Fru), lactose (Gal[β1-4]Glc), maltoose (Glc(α1-4)Glc) and raffinose (Gal(α1-6)Glc(α1-2)β). Inhibition of LPx

Antioxidant activity was determined by inhibition of LPx with a conjugated diene method. Each extract solution (100 μL, 0.031–4 mg mL⁻¹) was mixed with linoleic acid
KOZARSKI et al. (Merck KGaA) emulsion (2 mL, 10 mM). After 15 h of incubation, absorbance of the supernatant was measured at 234 nm using a UV/Vis spectrophotometer (Shimadzu UV-1650 PC, Japan). α-Tocopherol acetate (Merck KGaA Darmstadt, Germany) was used as a positive control. The results were expressed as EC_{50}; concentration of the extract that prevented oxidation of 50% of linoleic acid. The correlation coefficients (r) between inhibition of LPx and components of AbHW and GrHW were also determined.

**ACE inhibitory activity**

The ACE activity was analyzed using the method described in our previous investigation.20 The inhibition percentage of AbHW and GrHW were determined by replacing the 15 μL of water with the same volume of the sample to be studied at concentration range of 0.031–4 mg mL⁻¹ in the reaction solution of 0.09 U mL⁻¹ ACE (EC 3.4.15.1, Merck KGaA). Enalapril maleate (EM, Merck KGaA) was used as a positive control.

**AChE inhibitory activity**

AChE inhibitory potential of tested samples (0.031–4 mg mL⁻¹) was evaluated using colorimetric microplate assay21 in the reaction solution of 0.09 U mL⁻¹ AChE (EC 3.1.1.7, Merck KGaA). After final incubation with 10 mM 5,5'-dithiobis(2-nitrobenzoic acid) and 14 mM acetylthiocholine iodide, absorbance of the colored end-product was measured at 405 nm using microplate reader (ELx808, BioTek Instruments, Inc., USA) controlled by Gen5TM Software. Galantamine hydrobromide (Ghb, Merck KGaA) was used as a positive control. Enzyme inhibitory activities in both assays were expressed as inhibition percentage and IC_{50} values, which were calculated using linear regression analyses, as the concentration of extract required for 50% inhibition *in vitro*.

**In vitro cytotoxic activity**

PBMC, HeLa and K56 cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The complete nutrient medium was RPMI 1640 supplemented with 3 mM L-glutamine, 100 μg mL⁻¹ streptomycin, 100 IU mL⁻¹ penicillin, 10 % fetal bovine serum (FBS, Merck KGaA) and 25 mM Hepes adjusted to pH 7.2 with a bicarbonate solution. Extracts were heated before application at 95 °C for 20 min.

HeLa (2000 c/w) cells were seeded into a 96-well plate and 20 h later, after the cell adherence, different concentrations, from 0.047 to 3 mg mL⁻¹, of the extracts were added to the wells. The investigated extracts were also added to a suspension of leukemia K562 cells (5000 c/w) and PBMC (150,000 c/w) stimulated with 5 μg mL⁻¹ phytohaemagglutinin (PHA, Merck KGaA) 2 h after cell seeding. Only nutrient medium was added to the cells in the control wells. Corresponding concentrations of extracts in nutrient medium but without cells were used as blanks. Cisplatin was used as a positive control for all cell lines. Cell survival was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) testing.22 24 h after the investigated extracts were added. The data were expressed as of cells viability, % and IC_{50} values, concentration of the extract required to decrease cell viability by 50% compared to the control.

**Selectivity coefficient in the cytotoxic/antitumor action**

In order to further evaluate the anticancer potential of the extracts, selectivity in cytotoxic/antitumor action against specific malignant cell line, in comparison to healthy PBMC, was determined as well. Selectivity coefficient (SC) was calculated by the following equation: \( SC = \frac{IC_{50 \text{ PBMC}}}{IC_{50 \text{ cancer cells}}} \)
Statistical analysis

All experiments were carried out in triplicate and expressed as the mean±standard deviation (SD). Statistical analyses were performed with the Statistica 12.0 software package (Statistica, Tulsa, OK, SAD), using one-way analysis of variance (ANOVA) for all data collected. Differences between the means for each treatment were determined using Duncan’s multiple range tests (P<0.05).

RESULTS AND DISCUSSION

Extraction yields and chemical composition

Following the water extraction at 121 °C and ethanol precipitation, the yields of AbHW and GrHW were 5.9±0.7 and 2.6±0.4 g (100 g)⁻¹ of mushrooms DW, respectively. A significant reduction of about 56 % of the GrHW yield was observed in the comparison of AbHW. It could be explained by the less efficient extraction process caused by G. resinaceum highly lignified and hard fruiting bodies that are characteristic of the species belonging to the genus Ganoderma versus soft fruiting bodies of champignons.

Results of components quantitative analysis of the AbHW and GrHW are shown in Table I. According to the results, carbohydrates were the most abundant constituents in the analyzed hot water extracts, and AbHW yielded the highest content of total carbohydrates, 75.2±5.1 g (100 g)⁻¹ of extracts DW. Water soluble glucan fraction of AbHW comprised 83 % of the total carbohydrate content and consisted of almost 90 % β-glucans. The glucan fraction of GrHW comprised about 56 % of total carbohydrate content; approximately 86 % were β-glucans. β-Glucans present in mushrooms show different water affinities. This particular behavior is probably due to different molecular structures, different polymerization degrees and different molecular weights. It is important to note that, so far, several methods have been used for extraction and quantification of soluble and insoluble β-glucans, and the results may vary according to the methods used.

Phenolic compounds were present in both extracts (Table I). GrHW yielded the highest content of total phenolics, 5.9±0.4 g GAE (100 g)⁻¹ of extract DW. Zengin et al. reported the total phenolic content of 36.39±1.20 mg GAEs g⁻¹ in G. resinaceum hot water extract, and it was almost 40 % lower than in G. resinaceum hot water extract analyzed in our study. Observed difference could be
explained by different extraction procedures and different origin of *G. resinaceum*. In a study of Zengin *et al.* extraction was done by boiling deionized water during 15 min versus water extraction at 121 °C in an autoclave, for 1 h, applied in this study. Also, different growth conditions of strains from diverse areas, e.g. different substrates, could significantly influence the composition of their biological components. A considerable amount of proteins were presented in all extracts even upon thermal treatment (Table I). The highest content was detected in AbHW, 6.4±0.4 g of BSA (100 g)−1 of DW of extract. The protein content in GrHW was statistically different and approximately 44% lower than that in AbHW.

**Monosaccharide composition**

Polysaccharides were observed as a dominant component of both extracts, and monosaccharide composition was analyzed. On the basis of LC-MS analysis (Table II) polysaccharides in AbHW and GrHW were found to contain mainly Glc (tR of 3.17 min). In the AbHW sample a minor proportion of Gal and traces of Man, Xyl and Ara were found. In GrHW the presence of Gal, Man, Xyl, Ara, Rha and Fru in small quantities was determined.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Formula</th>
<th><em>M</em>&lt;sub&gt;r&lt;/sub&gt;/g mol&lt;sup&gt;−1&lt;/sup&gt;</th>
<th>Molecular ions (m/z)</th>
<th>tR/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xyl</td>
<td>C&lt;sub&gt;5&lt;/sub&gt;H&lt;sub&gt;10&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt;</td>
<td>150.05282</td>
<td>[M-H] 149.04796; [M] 150.05231</td>
<td>3.00</td>
</tr>
<tr>
<td>Ara</td>
<td>C&lt;sub&gt;5&lt;/sub&gt;H&lt;sub&gt;10&lt;/sub&gt;O&lt;sub&gt;5&lt;/sub&gt;</td>
<td>150.05282</td>
<td>[M+Cl] 185.02767; [M+HCOO] 195.05615</td>
<td>3.42</td>
</tr>
<tr>
<td>Man</td>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;12&lt;/sub&gt;O&lt;sub&gt;6&lt;/sub&gt;</td>
<td>180.06339</td>
<td>[M+Cl] 179.05573; [M] 180.05902</td>
<td>3.80</td>
</tr>
</tbody>
</table>

**Inhibition of LPx**

Linoleic acid (LA) is the most abundant polyunsaturated fatty acid (PUFA) *in vivo*, and accordingly, prevention of the LPx in the linoleic acid model system was investigated in this study. The results regarding the LPx inhibition activity of hot water extracts are presented in Table III. The higher the LPx inhibition capacity, the lower was the value of EC<sub>50</sub>.
TABLE III. EC\textsubscript{50} and IC\textsubscript{50} values of AbHW and GrHW in the inhibition of LPx, enzyme inhibition and cytotoxicity against human malignant cells and normal-human cells

<table>
<thead>
<tr>
<th>Properties</th>
<th>AbHW EC\textsubscript{50}, mg mL\textsuperscript{-1}</th>
<th>GrHW EC\textsubscript{50}, mg mL\textsuperscript{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of LPx</td>
<td>2.71±0.25\textsuperscript{A}</td>
<td>1.07±0.08\textsuperscript{B}</td>
</tr>
<tr>
<td>Enzyme inhibition IC\textsubscript{50}, mg mL\textsuperscript{-1}</td>
<td>&gt;4.00</td>
<td>0.54±0.09</td>
</tr>
<tr>
<td>ACE-inhibitory activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AChE inhibitory activity</td>
<td>1.74±0.31\textsuperscript{A}</td>
<td>0.37±0.07\textsuperscript{B}</td>
</tr>
<tr>
<td>Cytotoxicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HeLa</td>
<td>0.97±0.09\textsuperscript{A}</td>
<td>0.14±0.03\textsuperscript{B}</td>
</tr>
<tr>
<td>K562</td>
<td>0.72±0.07\textsuperscript{A}</td>
<td>0.11±0.04\textsuperscript{B}</td>
</tr>
<tr>
<td>PBMC</td>
<td>1.57±0.44\textsuperscript{A}</td>
<td>0.34±0.05\textsuperscript{B}</td>
</tr>
</tbody>
</table>

\textsuperscript{A} Each value is expressed as mg extract mL\textsuperscript{-1}; data are the the mean±SD (n=3); means with different letters within a row are significantly different (P<0.05).

The mean values of EC\textsubscript{50} indicated that AbHW and GrHW were potent antioxidants. A significant difference was found at P<0.05 for EC\textsubscript{50} values between both extracts. GrHW showed an almost three-fold higher potential for LPx inhibition compared to AbHW. Regression analysis revealed a highly significant positive correlation between the EC\textsubscript{50} and total carbohydrates, glucans, β-glucans and proteins. In contrast, a decrease in EC\textsubscript{50} value correlated with higher phenol contents. There was a significant negative correlation of very high strength (r=-0.961, P<0.05). α-Tocopherol acetate, one of the most potent antioxidant widely used in the industry, showed an EC\textsubscript{50} of 0.036±0.004 mg mL\textsuperscript{-1}.

Zengin et al. revealed a very high total antioxidant activity of methanol and water extracts from G. resinaceum, measured by phosphomolybdenum method.\textsuperscript{25} The strongest antioxidant capacity was observed in G. resinaceum water extract, which had the highest concentration of phenolics with apigenin, benzoic acid, and catechin as the major phenolic compounds.\textsuperscript{25} According to literature, the antioxidant properties of phenolic compounds are due to the presence of structural elements like catechol moieties and hydroxyl groups, which are directly involved in antiradical activity.\textsuperscript{27,28} Likewise, Socrier et al. highlighted a relation between the structure of phenolic glycosides and the antioxidant efficiency; aglycone compounds were significantly more efficient than glucoside compounds in the prevention of induced oxidation of liposomes.\textsuperscript{28} Mushroom extracts possess high levels of phenolic compounds, composed of one or more aromatic rings bearing one or more hydroxyl groups, which can exhibit free radical-scavenging activities as hydrogen or electron donating agents and metal ion-chelators.\textsuperscript{29} The presence of radical chain-breaking phenolic antioxidants provides a means of intercepting the lipid peroxidation process by reducing the alkoxy or peroxy radicals.\textsuperscript{29}

ACE inhibitory activity

Among processes related to hypertension, ACE plays an important physiological role in the regulation of blood pressure by converting angiotensin I
to angiotensin II, a potent vasoconstrictor.\textsuperscript{30} There are various types of ACE inhibitors that are extensively used to treat hypertension, but they are also reported to have adverse side effects.\textsuperscript{31} An alternative therapy such as natural origin drugs is preferred because natural products are considered to have fewer side effects.\textsuperscript{32} The inhibitory activities for ACE exhibited by AbHW and GrHW are presented in Fig 1A. At concentrations of 0.031 to 4 mg mL\textsuperscript{-1}, the inhibition of ACE of AbHW was between 3.2–36.4 %. ACE inhibition of the GrHW increased as the concentration increased from 0.031 to 1 mg mL\textsuperscript{-1} and reached a plateau of 71.6–72.9 % at 1–4 mg mL\textsuperscript{-1}. IC\textsubscript{50} values in ACE inhibition are presented in Table III. GrHW displayed the strongest inhibitory activity and exhibited a moderate potential when compared with a standard inhibitor EM, IC\textsubscript{50} < 0.031 mg mL\textsuperscript{-1}, Fig 1A.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Inhibitory activities of A) ACE and B) AChE, exhibited by AbHW and GrHW; positive control-enalapril maleate (EM) and galantamine hydrobromide (Ghb); each value is expressed as mean±SD (n = 3).}
\end{figure}

To the best of our knowledge, \textit{G. resinaceum} has not been examined before for antihypertensive activity. GrHW yielded the highest content of total phenolics, Table I. Many investigations indicate that polyphenol-rich food is effective in the protection and treatment of hypertension namely via ACE inhibition.\textsuperscript{33} Polyphenols can form noncovalent complexes with globular proteins, and such interactions may result in complexation, and protein unfolding.\textsuperscript{34} The strength of the interactions depends on the size of the polyphenols, the polyphenol structure, and the amino acid sequence of the proteins.\textsuperscript{34} IC\textsubscript{50} value in ACE inhibition of AbHW was not determined for the tested range of concentrations, Table III.

\textit{AChE inhibitory activity}

In the cholinergic hypothesis, strengthening the ability of the central cholinergic neuron by inhibiting the activity of AChE, enzyme involved in breakdown of acetylcholine (Ach), is one of the important option in a treatment of
AD. Several commercially accessible synthetic AChE inhibitors are used to alleviate the symptoms related to AD. Nevertheless, some of them have side effects including gastrointestinal disturbance and hepatotoxicity. The inhibitory activities for AChE exhibited by AbHW and GrHW are presented in Table III and Fig 1B; obviously, both extracts had the ability to inhibit this enzyme. GrHW displayed the strongest inhibitory activity among the investigated extracts with an IC_{50} value of 0.37±0.07 mg mL\(^{-1}\). At the concentration of 1 mg mL\(^{-1}\) GrHW achieved 81.6±6.5 % of enzyme inhibition. AbHW expressed almost 5 times weaker inhibition potential, Table III. IC_{50} value in AChE inhibition of Ghb, which is currently used for the treatment of cognitive decline in mild to moderate AD and various other memory impairments, was <0.031 mg mL\(^{-1}\). According to the Zengin et al., inhibitory effect of water extract of G. resinaceum for AChE was observed with a value of 0.62 mg galanthamine equivalents per g of extract. Inhibitory ability of extract was explained by the presence of a higher amount of apigenin, catechin and epicatechin which were already confirmed as enzyme inhibitors.

Cytotoxic activity

Decrease in survival of target cells induced by AbHW and GrHW is shown in Fig 2 and Table III. In general, both extracts exhibited selective dose-dependent cytotoxic actions against target malignant cell lines; AbHW displayed less pronounced cytotoxicity, Fig 2. Concerning to the specific sensitivity of different cells to the cytotoxic activity of the extracts, K562 cells were the most sensitive to the cytotoxic actions of both extracts; GrHW reached over 80 % inhibition at a concentration of 0.750 mg mL\(^{-1}\). HeLa cells exhibited a lower sensitivity; AbHW displayed several times lower cytotoxicity than GrHW against HeLa cells. IC_{50} value of cisplatin, which served as a positive control, in cytotoxicity testing against HeLa and K562 cells was <0.047 mg mL\(^{-1}\).

In the study of cytotoxic potential of water and ethanol extracts from Thai medicinal plants against selected tumor cell lines, Itharat et al. pointed that the criteria of cytotoxicity for the crude extracts, as established by the American National Cancer Institute (NCI) is an IC_{50} < 30 μg mL\(^{-1}\) in the preliminary assay. In the above research tumor cell growth inhibition was observed after 24, 48 and 72 h of incubation and different patterns of cytotoxic action were noticed among selected plant extracts and tumor cell lines with IC_{50} values at the range >100–6.2 μg mL\(^{-1}\). In our study K562 cells were the most sensitive to the growth suppression activity of both extracts, but the IC_{50} values (Table III) were higher than the suggested criteria of cytotoxicity for the crude extracts. It should be noted that cytotoxic potential of GrHW and AbHW against tumor cells was analyzed as preliminary screening after 24 h of incubation and for the initial comparison of antineoplastic potential between commercially cultivated mushrooms vs. wild mushroom species.
Fig. 2. Viability of PHA-stimulated PBMC, HeLa and K562 cells in the presence of increasing concentrations of AbHW and GrHW. Data are expressed as the mean±SD (n = 3). Significantly different from the control, *$P<0.05$ after 24 h.

Selectivity coefficient in the cytotoxic/antitumor action

Considering the possible effects of antitumor drugs on normal healthy immunocompetent cells, which are a normal part of the tumor microenvironment, the activities of the investigated extracts were evaluated against healthy PBMC, Fig 2. Both extracts exhibited weaker cytotoxic effects against stimulated PBMC than against target malignant cell lines. AbHW showed significant lower cytotoxicity against PBMC than GrHW, with an IC$_{50}$ value of 1.57±0.4 mg mL$^{-1}$.

To further evaluate the antitumor potential of the extracts, the selectivity in the antitumor action against specific malignant cell line in comparison to healthy PBMC was determined, Table IV. It was observed that GrHW exhibited significantly higher selectivity in antitumor action compared to AbHW, especially against K562 cells.

The content of phenolic compounds could be used as an important indicator of antitumor action. Concerning cytotoxicity against U937 cells of ethanol-water extracts obtained from two G. resinaceum mycelia strains, it was observed
that proliferation of the tumor cells could be correlated to the absence of the flavonoid fractions.\textsuperscript{40}

Table IV. Selectivity in antitumor action of AbHW and GrHW

\begin{tabular}{|c|c|c|}
\hline
 & IC\(_{50}\) (PBMCs+PHA) / IC\(_{50}\) HeLa & IC\(_{50}\) (PBMCs+PHA) / IC\(_{50}\) K562 \\
\hline
AbHW & 1.62 & 2.18 \\
GrHW & 2.43 & 3.10 \\
\hline
\end{tabular}

\textsuperscript{a}SC-selectivity coefficient

Morin, myrecetin, and rutin were detected in \textit{G. resinaceum} F-2 strain and residual growth of about 40\%, at 0.75 mg mL\(^{-1}\) of U937, was observed.\textsuperscript{40} \textit{G. resinaceum} F-1 strain was ineffective on U937 cell viability and none of the compounds mentioned above were detected.\textsuperscript{40} Besides phenols, many homopolysaccharides with antitumor activity have been isolated from the basidiomycetes.\textsuperscript{41} There are two basic mechanisms of polysaccharide action against tumor cells: indirect-immunostimulation and direct-inhibition of tumor cell growth and apoptosis induction.\textsuperscript{31} Considerable content of β-glucans was observed in AbHW (Table I). Likewise, AbHW induced significant proliferation of PBMC from 152 to 116\% at the concentration range of 0.047–0.187 mg mL\(^{-1}\) (\(P<0.05\)), compared to control. Our previous report confirmed an immune-modulatory effect on activated PBMC and synthesis of interferon-gamma (IFN-\(γ\)) of polysaccharides obtained from \textit{A. bisporus} fruiting bodies, after 48 and 72 h of stimulation.\textsuperscript{19} IFN-\(γ\) plays important roles in modulating the immune system and resistance to the tumor growth.\textsuperscript{19}

CONCLUSION

Hot water extract of wild-growing \textit{G. resinaceum} is a natural source of ACE and AChE inhibitors which can be a part of novel nutraceuticals or pharmaceuticals. GrHW exhibited significant selectivity in antitumor action against HeLa and K56 cells in comparison to healthy PBMC. Its chemical complexity can affect multiple tumor-related processes in synergistic ways when used as a treatment. Hot water extract of commercially cultivated \textit{A. bisporus} expressed milder effect in the inhibition of the linoleic acid peroxidation, enzyme inhibition, as well as lower cytotoxicity against investigated tumor cell lines. On the contrary, a significant proliferation of healthy PBMC, immunocompetent cells included in the antitumor immune response, was confirmed. Identification of \textit{G. resinaceum} secondary metabolites and their mode of action are necessary tasks for further comparison of the biological potential.

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ИЗВОД
КОМПАРАТИВНИ ПРЕГЛЕД ЗДРАСТВЕНОГ ЕФЕКАТА КОМЕРЦИЈАЛНО УЗГАЈАНЕ ГЉИВЕ Agaricus bisporus И САМОНИКЛЕЛНЕ ВРСТЕ ГЉИВЕ Ganoderma resinasceum
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У овоме раду поређен је здаствени ефекат врелих водених екстраката добијених из плодоносних тела комерцијално узгајане јестиве гљиве Agaricus bisporus (AbHW) и самониклељне врсте гљиве Ganoderma resinasceum (GrHW) из региона северне Србије. Здаствени ефекат је поређен in vitro превенцијом липисне пероксидације (LPx) у модел систему липисне киселине, инхибицијом ангиотензин конвертујућег ензима (ACE) који има улогу у одржавању нормалног нивоа крвног притиска и јачањем способности централних холинергичних нервних инхибицијом активности ацетилхолинееразе (AChE). Цитотоксична активност је праћена на хуманим ћелијама тумора грлића мате материца (HeLa) и ћелијама хроничне мијелоидне леукемије (K562), као и на здравим монојамским хеомонобетиљама (PBMC). Разлика у биолошкој активности екстраката даје смернице у њиховој примени као цитотоксици биохемијску процедуру.

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