Health impact of the commercially cultivated mushroom
*Agaricus bisporus* and the wild-growing mushroom
*Ganoderma resinaceum* – A comparative overview

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Abstract: The health promoting effects of hot water extracts obtained from 
fruiting bodies of the commercially cultivated mushroom *Agaricus bisporus* 
(AbHW) and the wild-growing mushroom *Ganoderma resinaceum* (GrHW) 
originating from northern Serbia are presented in this research. These abilities 
were compared *in vitro* by the prevention of lipid peroxidation (LPx) in a linoleic 
acid model system, inhibition of the angiotension converting 1 enzyme (ACE) 
that could 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 contains higher 
phenolics (5.9 g (100 g)` -1), inhibition of LPx (*EC*50 = 1.07 mg mL` -1`), ACE 
(*IC*50 = 0.54 mg mL` -1`), and AChE (*IC*50 = 0.37 mg mL` -1`), and exhibited a significant selectivity in the antitumour action against HeLa (*IC*50 = 0.14 mg mL` -1`) 
and K562 (*IC*50 = 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`) contents and induced significant proliferation of healthy PBMC from 152–116 
% in the concentration range of 0.047–0.187 mg mL` -1`. The difference in the 
biological activity of the extracts provides guidance on their use as functional 
food.

Keywords: hot water extracts; cytotoxicity; enzyme inhibition; lipid peroxy-
dation.

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Genetic predisposition is not the only cause of acquiring chronic diseases. They could 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 could be prevented. One of the most significant exposome factors that have impact on the well being of human health is functional food. 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 the antihypertensive effect, lowering of blood cholesterol, neutralization of reactive species, anti-carcinogenic effect and low-glycaemic response.

Over the past decade, supplementation with mushrooms has gained much interest in the food nutrition area. Mushrooms have been consumed by mankind for many centuries due to the attractive sensory characteristics, optimal nutritional compositions and manageable cultivate conditions. 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. According to different purposes, mushroom species are divided into edible and medicinal mushrooms. Although the interest for medicinal species is in rise, edible cultivated species still make the biggest part of the market. However, some species, such as *Ganoderma lucidum*, are also cultivated, especially in China.

Consequently, this study was aimed at presenting a comparative overview of the health promoting effects of hot water extracts obtained from the fruiting bodies of the commercially cultivated edible mushroom *Agaricus bisporus* and wild-growing mushroom *Ganoderma resinaceum*. The health promoting effects were studied in vitro by the prevention of lipid peroxidation (LPx), inhibition of angiotensin converting 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 antitumour immune response. To the best of our knowledge, very few studies 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.

**EXPERIMENTAL**

Fungal materials

Freshly harvested *A. bisporus* mushrooms at the closed cap stage were obtained from the local producer EkoFungi, Belgrade, Serbia. Fresh wild-growing fruiting bodies of the auto-
Cultivated vs. Wild Mushrooms: Health Impact

Chthonous mushroom species *G. resinaceum* were collected from Fruska 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). Carpohores were identified according to the methods of classical herbarium taxonomy to confirm the correct species. 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 mycelia cultures. For further analysis, the mushroom samples were lyophilized (Telstar LyoAlfa 15-85, Terrassa, Spain) and powdered. A 100 g sample of each mushroom was extracted with 1.0 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 the dietary fibres. The samples were left overnight in a refrigerator at 4 °C. After centrifugation for 10 min 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 the extracts**

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

**Analysis of the monosaccharide composition**

Liquid chromatography–mass spectrometry (LC–MS) was used to determine the monosaccharide profile of the 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 2-propanol, 2 times, and the resulting hydrolysates were dissolved in 0.5 mL MQ water. LC–MS analyses were performed on a high performance liquid chromatography (HPLC) device (Agilent 1200 Series, Agilent Technologies) with a degasser, autosampler and thermostated column compartment with Zorbax Carbohydrate (150 mm x 4.6 mm i.d.; 5 μm) coupled with a 6210 time-of-flight LC–MS system (Agilent Technologies) with an electrospray ion source (ESI). As the mobile phase, a mixture of solvent A (5 mM solution of ammonium formate in MilliQ water) and B (acetonitrile) in the isocratic mode of elution was used: 0–20 min 25 % A at a flow rate of 1.40 mL min⁻¹. The injection volume was 10 μL for the standards and 100 μL for hydrolyzed extracts, and the column temperature was 35 °C. The 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⁻¹, nebulizer pressure 45 psig (310.26 Pa), fragmentor voltage 140 V, *m/z* range 100–2000. MassHunter workstation software (Agilent Technologies) was used for data acquisition and processing. The monosaccharides were identified...
according to their retention times ($t_R$) 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-xyllose (D-Xyl), D-arabinose (D-Fru), lactose (Gal($\beta$1–4)Glc), maltose (Glc($\alpha$1–4)Glc) and raffinose (Gal($\alpha$1–6)Glc($\alpha$1–2$\beta$)Fru).

**Inhibition of LPx**

The antioxidant activity was determined by inhibition of LPx with the conjugated diene method. Each extract solution (100 µL, 0.031–4.0 mg mL$^{-1}$) was mixed with linoleic acid (Merck) emulsion (2 mL, 10 mM). After 15 h of incubation, the absorbance of the supernatant was measured at 234 nm using a UV/Vis spectrophotometer (Shimadzu UV-1650 PC, Japan). α-Tocopherol acetate (Merck, Darmstadt, Germany) was used as a positive control. The results are expressed as the EC$_{50}$ value (concentration of the extract that prevented oxidation of 50% of linoleic acid). The correlation coefficients ($r$) between inhibition of LPx and the components of AbHW and GrHW were also determined.

**ACE inhibitory activity**

The ACE activity was analyzed using the method described in a previous investigation. The inhibition percentage of AbHW and GrHW were determined by replacing 15 µL of water with the same volume of the sample to be studied in the concentration range 0.031–4.0 mg mL$^{-1}$ in the reaction solution of 26 mU mL$^{-1}$ ACE (EC 3.4.15.1, Merck). Enalapril maleate (EM, Merck) was used as the positive control.

**AChE inhibitory activity**

AChE inhibitory potential of the tested samples (0.031–4.0 mg mL$^{-1}$) was evaluated using a colorimetric microplate assay in the reaction solution of 0.09 U mL$^{-1}$ AChE (EC 3.1.1.7, Merck). After the final incubation with 10 mM 5,5'-dithiobis(2-nitrobenzoic acid) and 14 mM acetylthiocholine iodide, the absorbance of the coloured end product was measured at 405 nm using a microplate reader (ELx808, BioTek Instruments, USA) controlled by Gen5TM Software. Galantamine hydrobromide (Ghb, Merck) was used as the positive control.

The 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% in vitro inhibition.

**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 1-glutamine, 100 µg mL$^{-1}$ streptomycin, 100 IU mL$^{-1}$ penicillin, 10% foetal bovine serum (FBS, Merck) 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 cell adherence, different concentrations, from 0.047 to 3.0 mg mL$^{-1}$, of the extracts were added to the wells. The investigated extracts were also added to a suspension of leukaemia K562 cells (5000 c/w) and PBMC (150,000 c/w) stimulated with 5 µg mL$^{-1}$ phytohaemagglutinin (PHA, Merck) 2 h after cell seeding. Only nutrient medium was added to the cells in the control wells. The corresponding concentrations of extracts in nutrient medium but without cells were used as blanks. Cisplatin was used as the positive control for all cell lines. Cell survival was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) testing after 24 h after the investigated extracts were added. The data are expressed as 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/antitumour action**

In order to evaluate further the anticancer potential of the extracts, selectivity in cytotoxic/antitumour action against specific malignant cell line, in comparison to healthy PBMC, was also determined. The selectivity coefficient ($SC$) was calculated by the following equation: $SC = IC_{50PBMC}/IC_{50}$ cancer cells.

**Statistical analysis**

All experiments were performed in triplicate and expressed as the mean±standard deviation (SD). Statistical analyses were performed with the Statistica 12.0 software package (Statistica, Tusla, OK, USA), using one-way analysis of variance (ANOVA) for all data collected. Differences between the means for each treatment were determined using Duncan 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 comparison to AbHW. This could be explained by the less efficient extraction process caused by highly lignified and hard fruiting bodies of *G. resinaceum* that are characteristic of the species belonging to the genus *Ganoderma* versus the soft fruiting bodies of champignons.

The results of the quantitative analysis of the components of AbHW and GrHW are given 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. The water-soluble glucan fraction of AbHW comprised 83% of the total carbohydrate content and consisted of almost 90% $\beta$-glucans. The glucan fraction of GrHW comprised about 56% of total carbohydrate content; approximately 86% were $\beta$-glucans. The $\beta$-glucans present in the mushrooms showed different water affinities. This particular behaviour is probably due to different molecular structures, different polymerization degrees and different molecular weights.

**TABLE I. Chemical composition of hot water extracts derived from *A. bisporus* cultivated strain (AbHW) and *G. resinaceum* wild-growing strain (GrHW)**

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total content, g (100 g)$^{-1}$</th>
<th>Glucan content, g (100 g)$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carbohydrates$^a$</td>
<td>Proteins$^b$</td>
</tr>
<tr>
<td>AbHW</td>
<td>75.4±4.1A$^d$</td>
<td>6.4±0.5A</td>
</tr>
<tr>
<td>GrHW</td>
<td>52.1±3.2B</td>
<td>4.7±0.2B</td>
</tr>
</tbody>
</table>

$^a$Glucose equivalents (GlcE); $^b$bovine serum albumin equivalents (BSAE); $^c$gallic acid equivalents (GAE); all values are expressed per dry weight (DW) of extract; $^d$data are the means±SD ($n = 3$); within the same column, means followed by different letters are significantly different at $P < 0.05$.
is important to note that, so far, several methods have been used for the extraction and quantification of the soluble and insoluble β-glucans, and the results may vary according to the method used.23,24

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 a total phenolic content of 36.39±1.20 mg GAEs g–1 in G. resinaceum hot water extract,25 which was almost 40 % lower than in G. resinaceum hot water extract analyzed in the present study. The observed difference could be explained by different extraction procedures and different origin of G. resinaceum. In the study of Zengin et al.,25 the extraction was realized by boiling deionised water during 15 min versus water extraction at 121 °C in an autoclave, for 1 h, applied in this study. In addition, different growth conditions of strains from diverse areas, e.g., different substrates, could significantly influence the composition of their biological components.11 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 BSAE (100 g)–1 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 the dominant component of both extracts and the monosaccharide composition was analyzed. Based on the LC–MS analysis (Table II), the polysaccharides in AbHW and GrHW were found to contain mainly Glc (tR at 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>M_r / g mol⁻¹</th>
<th>Molecular ions (m/z)</th>
<th>tR / min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rha</td>
<td>C₆H₁₂O₅</td>
<td>164.06847</td>
<td>[M–H]⁻ 163.06363; [M+Cl]⁺ 199.04155</td>
<td>2.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[M+HCOO]⁻ 209.07177; [M+CH₃COO]⁻ 223.07989</td>
<td></td>
</tr>
<tr>
<td>Xyl</td>
<td>C₅H₁₀O₅</td>
<td>150.05282</td>
<td>[M–H]⁻ 149.04796; [M]¹ 150.05231</td>
<td>3.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[M+Cl]⁻ 185.02767; [M+HCOO]⁻ 195.05615</td>
<td></td>
</tr>
<tr>
<td>Ara</td>
<td>C₅H₁₀O₅</td>
<td>180.06339</td>
<td>[M–H]⁻ 149.03933; [M]¹ 150.04244</td>
<td>3.42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[M+Cl]⁻ 185.01767; [M+HCOO]⁻ 195.04736</td>
<td></td>
</tr>
</tbody>
</table>
Inhibition of LPx

Linoleic acid (LA) is the most abundant polyunsaturated fatty acid (PUFA) in vivo, and accordingly, prevention of LPx in the linoleic acid model system was investigated in this study. The results regarding the inhibition of LPx activity in hot water extracts are presented in Table III. The higher the LPx inhibition capacity, the lower was the value of \( EC_{50} \).

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

\( EC_{50} \) values for AbHW and GrHW in the inhibition of LPx, enzyme inhibition and cytotoxicity against human malignant cells and normal-human cells.

<table>
<thead>
<tr>
<th>Property</th>
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<th>GrHW</th>
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<td>0.34±0.05</td>
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<tr>
<td>PBMC</td>
<td></td>
<td></td>
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</tbody>
</table>

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

The mean values of \( EC_{50} \) indicated that AbHW and GrHW were potent antioxidants. A significant difference was found at \( P < 0.05 \) for the \( EC_{50} \) 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 \( EC_{50} \) value and the total carbohydrates, glucans, \( \beta \)-glucans and proteins. In contrast, a decrease in the \( EC_{50} \) value correlated with higher phenol contents. There was a significant negative correlation of very high strength (\( r = -0.961, P < 0.05 \)). \( \alpha \)-Tocopherol acetate, one of the most potent antioxidant widely used in the industry, showed an \( EC_{50} \) value of 0.036±0.004 mg mL\(^{-1} \).

Zengin et al. revealed a very high total antioxidant activity of the methanol and water extracts from G. resinaceum, measured by the phosphomolybdenum method. 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. According to the literature, the antioxidant properties of phenolic compounds are due to the presence of structural elements, such as catechol moieties and hydroxyl groups, which are directly involved in antiradical activity. 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 peroxyl radicals.\textsuperscript{29}

\textit{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 GrHW increased as the concentration increased from 0.031 to 1.0 mg mL\textsuperscript{-1} and reached a plateau of 71.6–72.9 % at 1–4 mg mL\textsuperscript{-1}. The \( IC_{50} \) values in ACE inhibition are presented in Table III. GrHW displayed the strongest inhibitory activity and exhibited a moderate potential when compared with the standard inhibitor EM, \( IC_{50} < 0.031 \) mg mL\textsuperscript{-1}, Fig. 1A.

![Fig. 1. 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 (\( n = 3 \)).](image)

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 \textit{via} ACE inhibition.\textsuperscript{33}

Available on line at www.shd.org.rs/JSCS/  
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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} The $IC_{50}$ value in ACE inhibition of AbHW was not determined for the tested range of concentrations, Table III.

**AChE inhibitory activity**

In the cholinergic hypothesis, strengthening the ability of the central cholinergic neuron by inhibiting the activity of AChE, the enzyme involved in the breakdown of acetylcholine (Ach), is one of the important options in the treatment of AD.\textsuperscript{35} Several commercially accessible synthetic AChE inhibitors are used to alleviate the symptoms related to AD.\textsuperscript{36} Nevertheless, some of them have side effects, including gastrointestinal disturbance and hepatotoxicity.\textsuperscript{36} 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 $0.37\pm0.07$ mg mL$^{-1}$. At a concentration of 1 mg mL$^{-1}$, GrHW achieved 81.6$\pm6.5$ % of enzyme inhibition. AbHW expressed an almost 5 times weaker inhibition potential, Table III. The $IC_{50}$ 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 Zengin et al., the inhibitory effect of a water extract of *G. resinaceum* for AChE was observed with a value of 0.62 mg galanthamine equivalents per g of extract.\textsuperscript{25} The inhibitory ability of the extract was explained by the presence of a higher amount of apigenin, catechin and epicatechin, which were already confirmed as enzyme inhibitors.\textsuperscript{25}

**Cytotoxic activity**

The decrease in the survival of target cells induced by AbHW and GrHW is presented in Fig. 2 and Table III.

In general, both extracts exhibited selective dose-dependent cytotoxic activity against the target malignant cell lines, whereby, AbHW displayed less pronounced cytotoxicity, Fig. 2. Concerning the specific sensitivity of different cells to the cytotoxic activity of the extracts, the K562 cells were the most sensitive to the cytotoxic actions of both extracts, with GrHW reaching over 80 % inhibition at a concentration of 0.750 mg mL$^{-1}$. The HeLa cells exhibited a lower sensitivity. AbHW displayed a several times lower cytotoxicity than GrHW against HeLa cells. The $IC_{50}$ value of cisplatin, which served as a positive control, in the 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 the selected tumour cell lines, Itharat et al. indicated that
the criteria of cytotoxicity for crude extracts, as established by the American National Cancer Institute (NCI), is an $IC_{50} < 30 \mu g \text{ mL}^{-1}$ in the preliminary assay.\textsuperscript{37} In the above research, inhibition of tumour cell growth was observed after 24, 48 and 72 h of incubation and different patterns of cytotoxic action were noticed among the selected plant extracts and tumour cell lines with $IC_{50}$ values in the range >100–6.2 $\mu g \text{ mL}^{-1}$.\textsuperscript{37} In the present study, the 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 crude extracts. It should be noted that cytotoxic potential of GrHW and AbHW against tumour cells was analyzed as preliminary screening after incubation for 24 h and for the initial comparison of the antineoplastic potential between commercially cultivated mushrooms vs. wild mushroom species.

![Graphs showing 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.](Fig. 2)

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

Considering the possible effects of antitumour drugs on normal healthy immunocompetent cells, which are a normal part of the tumour microenvironment,\textsuperscript{38} the activities of the investigated extracts were evaluated against healthy
PBMC. Fig. 2. Both extracts exhibited weaker cytotoxic effects against stimulated PBMC than against the target malignant cell lines. AbHW showed a significant lower cytotoxicity against PBMC than GrHW, with an $IC_{50}$ value of 1.57±0.4 mg mL$^{-1}$.

To evaluate further the antitumour potential of the extracts, the selectivity in the antitumour 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 antitumour action compared to AbHW, especially against K562 cells.

Table IV. Selectivity in antitumour action of AbHW and GrHW

<table>
<thead>
<tr>
<th>SC$^a$ in the antitumour action</th>
<th>$IC_{50}$ (PBMCs+PHA) / $IC_{50}$ HeLa</th>
<th>$IC_{50}$ (PBMCs+PHA) / $IC_{50}$ K562</th>
</tr>
</thead>
<tbody>
<tr>
<td>AbHW</td>
<td>1.62</td>
<td>2.18</td>
</tr>
<tr>
<td>GrHW</td>
<td>2.43</td>
<td>3.10</td>
</tr>
</tbody>
</table>

$^a$Selectivity coefficient

The content of phenolic compounds could be used as an important indicator of antitumour action. Concerning cytotoxicity against U937 cells of ethanol–water extracts obtained from two *G. resinaceum* mycelia strains, it was observed that proliferation of the tumour cells could be correlated to the absence of flavonoid fractions. Morin, myrecetin and rutin were detected in *G. resinaceum* F-2 strain and residual growth of about 40 %, at 0.75 mg mL$^{-1}$ of U937, was observed. *G. resinaceum* F-1 strain was ineffective on U937 cell viability and none of the compounds mentioned above were detected. Besides phenols, many homopolysaccharides with antitumour activity have been isolated from basidiomycetes. There are two basic mechanisms of polysaccharide action against tumour cells: indirect-immunostimulation and direct-inhibition of tumour cell growth and apoptosis induction. A considerable content of $\beta$-glucans was observed in AbHW (Table I). Likewise, AbHW induced significant proliferation of PBMC from 152 to 116 % in the concentration range 0.047–0.187 mg mL$^{-1}$ ($P < 0.05$), compared to the control. A previous report confirmed an immunomodulatory effect on activated PBMC and the synthesis of interferon-gamma (IFN-$\gamma$) of polysaccharides obtained from *A. bisporus* fruiting bodies, after 48 and 72 h of stimulation. IFN-$\gamma$ plays important roles in modulating the immune system and resistance to tumour growth.

CONCLUSIONS

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

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**REFERENCES**


