AGARICUS SILVATICUS - PROMISING FUNCTIONAL FOOD

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Agaricus silvaticus Schaeffer is often found in groups in mixed woodland and under trees in parks. Highly efficient biological properties of this common, edible mushroom. Important source of nutrients and nutraceuticals. Exhibits high antioxidant power.
OBJECTIVES

To determine their cytotoxic effect on malignant human breast cancer MDA-MB-453, cervical adenocarcinoma HeLa and myelogenous leukemia K562 cells.

To evaluate their antioxidant ability by measuring DPPH free radical scavenging activity, inhibition of lipid peroxidation, reducing power and chelating ability.

To determine the antibacterial ability of crude hot water extract (SV) and hot alkali extract (SNa) against selected foodborne Gram-positive and Gram-negative pathogenic bacteria by microdilution assay.
hot water extraction

- alcohol precipitation (supernatant)
  - hot water extract (SV)

- hot alkali extraction (filter cake)
  - alcohol precipitation (supernatant)
  - hot alkali extract (SNa)
Determination of the MIC by broth microdilution method

- Quantitative determination of MIC, based on the color change caused by the enzymatic activity of viable microorganisms

- Concentrations of mushroom extracts ranged from 20.0 to 0.0097 mg/mL.

- Working concentrations of approximately $10^5$-$10^6$ cfu/mL were used for antibacterial activity assay.

- To indicate cellular respiration, 2,3,5-triphenyl tetrazolium chloride TTC (0.05%) was added to the culture medium.

- The MIC was defined as the lowest sample concentration that exhibited complete inhibition of bacterial growth.
- hot water (crude) extract (SV)
- hot alkali extract (SNa)

**G+ bacterial strains**

- *Staphylococcus aureus* ATCC 25923
- *Enterococcus faecalis* ATCC 29212
- *Bacillus cereus* ATCC 10876
- *Listeria monocytogenes* ATCC 19115

**G- bacterial strains**

- *Escherichia coli* ATCC 25922
- *Salmonella enteritidis* ATCC 13076
- *Shigella sonnei* ATCC 29930
- *Yersinia enterocolitica* ATCC 27729
- *Escherichia coli* (0157:H7) ATCC 12900
Minimal inhibitory concentrations of tested mushroom extracts

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Source</th>
<th>MIC (mg/mL) SV</th>
<th>MIC (mg/mL) SNa</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>ATCC 25923</td>
<td>5.0 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>ATCC 29212</td>
<td>5.0 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.3129 ± 0.0000&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>ATCC 10876</td>
<td>5.0 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.25 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>ATCC 19115</td>
<td>5.0 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>ATCC 25922</td>
<td>5.0 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Salmonella enteritidis</em></td>
<td>ATCC 13076</td>
<td>10.0 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Shigella sonnei</em></td>
<td>ATCC 29930</td>
<td>5.0 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>ATCC 27729</td>
<td>10.0 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Escherichia coli</em> (0157:H7)</td>
<td>ATCC 12900</td>
<td>5.0 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.0 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± standard deviation (n=3)

1Within the same row, means followed by different letters are significantly different at α=0.05 (ANOVA, Tukey’s HSD Test)

- Both extracts, SV and SNa, inhibited the growth of all tested Gram-positive and Gram-negative bacteria;
- In most cases SNa possessed higher activity than SV (MIC - 0.3129 ± 0.0000 - 5.0 ± 0.0 mg/mL and 5.0 ± 0.0 - 10.0 ± 0.0 mg/mL, respectively);
- The highest antibacterial potential of SNa was achieved against *E. faecalis* (MIC - 0.3129 ± 0.0000 mg/mL)
Evaluation of the antioxidant activity

- The inhibition of lipid peroxidation was determined by the conjugated diene method

  At particularly low concentration of 0.1 mg/mL SV and SNa inhibited peroxidation of 62.7 ± 3.1 and even 81.8 ± 1.9% of lipids.

- DPPH free radical scavenging activity assay

  At 10 mg/mL scavenging ability of SV toward DPPH radicals increased to 76.8 ± 1.2%, while 74.8 ± 2.3% was achieved in the presence of four times lower concentration (2.5 mg/mL) of SNa.

- Ferric-reducing antioxidant power assay

  The reducing power of SV and SNa was 1.4 ± 0.8 and 2.2 ± 1.6 at 5 mg/mL.

- Chelating ability on ferrous ions

  At 5 mg/mL SV and SNa chelated 87.7 ± 2.7% and 81.8 ± 1.4% of ferrous ions.
An increase in antioxidant activity with increasing concentration of the extracts was confirmed in all applied assays.

Extraction in hot sodium hydroxide solution contributed to the better antioxidant activity.

SNa was better antioxidant shown by the lower EC$_{50}$ values of inhibition of lipid peroxidation, ferric-reducing antioxidant power, DPPH scavenging ability, and ferrous ion-chelating ability.
Many wild mushroom could be a valuable natural source of antioxidant compounds, suggesting their potential role to be used in functional foods.
In vitro cytotoxic activity

- human cervix adenocarcinoma HeLa
- human myelogenous leukemia K562
- human breast carcinoma MDA-MB-453 cells

Both investigated extracts exerted selective dose-dependent cytotoxic actions on malignant cells.

The decrease in survival of target cancer cells induced by the extracts is shown in graphs.

Cell survival was determined by MTT test.
With IC$_{50}$ values (concentration of extract that is required for 50% inhibition in vitro) ranging from 0.7 to 1.7 mg/mL, following continuous incubation, both examined extracts possess moderate cytotoxicity.

The highest cytotoxicity was found in a SNa treated HeLa cells (IC$_{50}$=0.7 ± 0.1 mg/mL).
Crude hot water extract and hot alkali extract obtained from *A. silvaticus* possess higher antibacterial potential against tested Gram-positive than Gram-negative bacterial strains.

The highest activity exhibited hot alkali extract towards *E. faecalis*.

As infections caused by this bacterium are very difficult to treat due to its frequent resistance to multiple antibiotics, the use of *A. silvaticus* extracts as supplements to certain types of food might lead to the destruction of bacteria in food and thus to contribute to the reduction of poisoning with this type of bacteria.

At a time of increasing resistance of microorganisms to antibiotics, naturally-derived antimicrobial substances are very desirable.
Conclusion

✓ The results from different in vitro assay systems, including the inhibition of lipid peroxidation, the scavenging effects on DPPH radical, the reducing power and the ferrous ions chelating effect, demonstrated that these polysaccharide extracts have effective antioxidant activities.

✓ These findings could be important in terms of development of natural, easily accessible sources of antioxidant agents which are able to protect the human body from free radicals and to slow down the progress of many chronic diseases.

✓ The results of this study confirm a high biological potential of mushroom A. silvaticus.

Due to its very pleasant taste and nutritional value, antibacterial potential as well as a high content of antioxidant components it could be considered as functional food and might be able to contribute to the reduction of cancer risks.
ACKNOWLEDGEMENTS

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