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Chemical composition and biological activities of the *Agaricus mushrooms*

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Abstract: Two species of Agaricus mushroom grown in Mongolia were analyzed for their element content. Biological activity and chemical components study of *Agaricus*, grown in the Mongolian flora has been investigated for the first time. The ethanol extracts of dried Agaricus sp. mushrooms were analyzed for antioxidant activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and interferon-like activity. The ethanol extracts from *Agaricus arvensis* showed the most potent radical scavenging activity. The IC_{50} of *A. silvaticus* and *A. arvensis* were 216 and 17.75 μg/ml respectively. Among the twenty three mushroom extracts, the extracts from *A. silvatisus* and *A. arvensis* have shown the interferon-like activity.

Keywords: Agaricus silvaticus, *Agaricus arvensis*, Free radical-scavenging activity, DPPH, IFN-like activity, luciferase

INTRODUCTION

Scientific evidence has attested the presence of bioactive substances in *Agaricaceae* fungi with essential nutritional and metabolic properties including: glucans, proteoglucans, ergosterol, lectins and arginine. *Agaricus* is a large and important genus of mushroom containing both edible and poisonous species, with possibly over 300 members worldwide. This genus belongs to Phylum Basidiomycota, Class: Hymenomycetes (newly described Class Agaricomycetes), Order: Agaricales, Family: *Agaricaceae* [1].

The *Agaricales* order is one of the major classes of fungi and contains a great number of important species that are used as nutritional supplements and therapeutic resources. *Agaricus silvaticus* and *Agaricus arvensis* are common, edible mushrooms belonging to *Agaricaceae* family which are known for their therapeutic properties. There are several studies that report the effects of *A. silvaticus* (Sun mushroom) on various diseases and these properties may also be associated to the presence of bioactive compounds with medicinal value, such as phenolic compounds, polyketides, terpenes and steroids recognized as excellent antioxidants. There are several studies that report the effects of *A. silvaticus* on various diseases and these properties may also be associated to the presence of bioactive compounds with medicinal value, such as phenolic compounds, polyketides, terpenes and steroids recognized as excellent antioxidants [2]. According to Elmastas et al. [3], phenolic compounds seem to antioxidant activity be the main component responsible for the in mushroom extracts. According to Tsai et al. [4], the antioxidant properties of *Agaricus blazei* may be associated with its high concentration of tocopherols. Antioxidant activity has been investigated for this and some other *Agaricus* species, and in China it is claimed to have anticancer properties and has been used to cure lower back pain and pain in tendons and veins [5]. The *A. arvensis* (Horse mushroom) is regarded as one of the most delicious edible fungi, although the fruiting bodies of this and other yellow-staining Agaricus species often have a build-up of heavy metals, such as cadmium and copper [6].

Clinical and experimental studies demonstrate that dietary supplementation with Agaricales mushrooms and other medicinal fungi exert positive nutritional, medicinal and pharmacological effects and can be used as an adjuvant in cancer therapy. The mechanisms of action of bioactive compounds found in mushrooms are yet to be fully elucidated in the literature, but scientific evidence suggests that these substances are able to modulate carcinogenesis not only at early stages, but at more advanced phases of disease progression as well, providing benefits to individuals with various types of cancer, mainly by stimulating the immune system [7].

The aim of this study were to evaluate the chemical composition of two dehydrated species of the *Agaricus* fungus with respect to protein, lipids, minerals as well as determine the antioxidant and interferon-like activity of alcoholic extracts from those two species of *Agaricus*.

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EXPERIMENTAL

General: Chemical analyses of two dehydrated species of Agaricus mushroom are studied by common method: moisture (kiln at 105°C), ash (muffle furnace at 550°C), proteins (Kjeldahl) and lipids (Soxhlet). Analyses of minerals were performed by atomic absorption spectrometry.

***Mushroom material*:** The whole mushrooms of *A. silvaticus* and *A. arvensis* were collected from Bogd mountain, Shajin hurahiin am, Ulaanbaatar and Bayantes soum, Zavkhan province, Mongolia, during 2010-2011. Identification was done by B.Burenbaatar at the Institute of Botany, MAS, by comparing their morphological, anatomical and physiological characteristics and monographs with descriptions given in the manual and also through the electronic data on identification keys of mushrooms. The specimens were deposited at the herbarium of Institute of Botany and Institute of Chemistry and Chemistry Technology, MAS.

Determination of free radical-scavenging capacity: The antioxidant activity was measured by a modification of the DPPH radical-scavenging method first described by Brand-Wiliams et al. [8]. In its radical form, $DPPH^{\cdot}$ has an absorption band at 517 nm which disappears upon reduction by an antiradical [5]. Different concentrations (200, 100, 50, 25 μg/ml in methanol) of extract were added $6*10^{-5}$M methanol DPPH solution. The decrease in absorbance was determined at 517 nm for 30 minute until the reaction reached a plateau. After 30 minute the absorbance values were measured at 517nm and covered into the percentage antioxidant activity (ARA) using the following formula:

$$ARA,\% = 100 - \left(\frac{Ae-Ab}{Ac}\right) * 100 \quad (1)$$

Where:

Ae = A517 in the presence of crude extract;

Ac = A517 of negative control solution and Ab is the absorbance of DPPH solution before adding the antioxidant;

Percentages of radical consumption for different antioxidant concentrations were measured. IC_{50} value corresponds to the concentration that scavenged 50% of the radicals, expressed as the antioxidant/DPPH mole ratio. Other parameters such as antiradical power (ARP), defined as the inverse of IC_{50} and the stoichiometric factor (n), corresponding to the number of radical moles consumed per mole of antioxidant added were calculated.

Interferon-like activity: The IFN-like activity was determined by Luciferase Reporter Assay System according to the manufacturer's instruction (Promega), using Thermo Scientific Varioskan® Flash. The extracts library were dissolved in DMSO to a final concentration of 100 mg/mL and stored at -20°C until use. Working solution was prepared in RPMI 1640 medium at a final concentration of 100 μg/ml in the plate *[9]*.

Cell line: The human hepatocellular carcinoma HepG2 cell line was obtained from ATCC, and was maintained in RPMI-1640 (GIBCO, Thermo) medium supplemented with 10% (v/v) calf serum and antibiotics (100 U/ml penicillin and 0.1 g/L streptomycin) at 37°C in the presence of 5% CO_2. The HepG2-ISRE-Luc2 firefly Luciferase reporter cells was generated by transfecting the HepG2 cells with pISRE-Luc plasmid.

Luciferase Assay for screening: Screen was performed at 96 well format, for each plate, 16 wells were used for negative and positive control, the remaining 80 wells contain test samples which were diluted to 100μg/ml. HepG2-ISRE-Luc2 cells were plated at 5Ч 10^4 cells/well in 96-well plates. After incubation for 24 h at 37 °C in 5% CO_2, cells were stimulated with test samples and 200IU IFN-α as positive control for 24 h. Cells were lysed in Reporter Lysis buffer and luciferase activity was measured by Luciferase Reporter Assay System according to the manufacturer's instruction (Promega), using Thermo Scientific Varioskan® Flash.

RESULTS AND DISCUSSION

The mushrooms were crashed and subjected to several analyses the results of which are given in tables 1 and 2. It is evident from table 1 that the main components of the two species of *Agaricus* are the carbohydrates and proteins. *A. arvensis* grown in Mongolia was low in protein content and high in mineral contents in comparison to *A. arvensis* grown in Brazil, while carbohydrate contents of these fungi were comparable. Ash content of mushrooms is usually 5-12% dry matter and its variability seems to be lower than that of crude protein and carbohydrates. For *A. silvaticus* from Mongolia, the fat content was approximately 3.7 times lower, and the mineral content was 2.1 times higher than same fungi grown in Brazil. These differences might be due to growth conditions, genetic factors, geographical variations, soil contents and analytical procedures.

Table 1. The chemical composition of *Agaricus* mushroom

	Components, %	*A. silvaticus*	*A. silvaticus* **[1]**	*A. arvensis*	*A. arvensis* **[8]**
1	Moisture	5.06±0.25	6.31	5.16±0.33	-
2	Ash	15.63±0.12	7.38	16.63±0.15	3.53
3	Fat	1.78±0.07	6.60	2.13±0.31	2.75
4	Protein	46.1±0.14	41.16	44.4±0.21	56.27
5	Water soluble carbohydrate	40.3±0.84	36.31	37.5±0.25	37.45

Forty five minerals were scanned; however, since detectable levels of Be, Sb, Te and Ti were not found in the samples, these metals were excluded from the tables.

According to these results (table 2) mineral contents of two species of *Agaricus* fungi were higher than other previous study [10]. Mineral contents varied within two species of *Agaricus*.

The values of minerals of *A. arvensis* which was collected from Bogd Khaan mountain, near Ulaanbaatar, were higher than *A. silvaticus* collected from province. The main components of *Agaricus* spp. were K and P. The Calcium content of these mushrooms ranged from 797.1 to 2644.2 mg/kg.

Significant amounts of iron were found (2328 mg/kg) in the *A. arvensis*, which makes the mushroom a rich source of this mineral.

A. sylvaticus has presented an important source of zinc (755.7mg/kg). Zinc has an important physiological role, acting as an antioxidant, as well as preventing lipid peroxidation. Concentrations of the elements in fruiting bodies are generally species-dependent. Substrate composition is an important factor, but great differences exist in uptake of individual metals.

From the above results, it is apparent that *A. silvaticus* and *A. arvensis* should be regarded as important sources of many macro elements (K, Mg and Ca) and trace metals such as Mg, Zn and Cu.

Table 2. Mineral contents (mg/kg, dry weight) of *Agaricus spp.*

Elements	*A. silvaticus*	*A. arvensis*	*A. arvensis* **[10]**	*A. silvaticus* **[11]**
Al	2922.8	5504.5	237 ± 6	
As	5.5	4.9	3.69 ± 0.18	1.18 ± 0.17
Ca	797.1	2644.2	550 ± 34	
Cd	25.0	4.9	10.6 ± 0.25	51.9 ± 4.1
Ce	12.7	22.6	-	
Co	3.1	4.2	2.85 ± 0.06	
Cr	15.6	16.6	1.69 ± 0.21	
Cu	164. 9	210.4	70.6 ± 1.10	181.5 ± 28.6
Fe	1312.9	2328.2	232 ± 87	
Ga	3.4	5.6	-	
K	23445	19922.7	33400 ± 1530	
La	6.3	12.5	n.d.	
Li	7.8	45.7	0.09 ± 0.03	
Mg	1703. 7	2195.2	1210 ± 42	
Mn	276.6	364.2	52.9 ± 7.10	
Mo	30.5	138.9	0.19 ± a	
Na	1172.3	2444.6	527 ± 130	
Nd	4.2	7.6	-	
Ni	7.0	9.1	0.98 ± 0.04	0.13 ± 0.05
P	12597.8	11840.6	10700 ± 230	
Pb	14.8	14.1	n.d	9.3 ± 0.7
S	2235.0	1446.8	-	
Sr	55.5	148.8	1.87 ± 0.35	
Th	1.56	2.5	-	
V	15.6	26.6	0.47 ± 0.01	
W	15.6	23.4		
Y	3.9	8.3	n.d.	
Zn	755.7	429.9	92.8 ± 1.84	
Zr	3.1	6.6	0.40 ± a	

The levels of some heavy metals such as As, Ni, Pb and Sr of fruiting bodies of *Agaricus* spp. grown in Mongolia was higher than those reported earlier [10, 11]. It is known that high metal levels (Cr, Co, Pb Ni) have been observed in mushrooms growing in heavily contaminated areas, such as those in the close vicinity of highways with heavy traffics, emission areas of metal smelters, domestic heating and long-range transport. Some authors report higher metal concentrations in younger fruiting bodies. This is explained by the transport of a metal from mycelium to the fruiting body during the start of fructification. With further increase of the fruiting body mass, the metal concentration decreases. The proportion of metal concentrations from atmospheric depositions seems to be of less important due to the short lifetime of a fruiting body, which is usually 10 to 14 days [12].

Thus, the differences in the mineral contents of mushrooms reported in various studies can be attributed to the ecosystems in which they were grown and by the environmental factors such as climate, growing conditions and soil content. All these factors cause very wide variability in the trace element concentrations within a species, commonly to one order of magnitude.

Determination of free radical-scavenging capacity: Free radical scavenging is one of the mechanisms in inhibiting lipid oxidation commonly used to estimate antioxidant activity. The radical scavenging activity of mushroom extracts was tested against the DPPH.

The results demonstrated that 95% alcoholic extract of *Agaricus* sp. reacted with DPPH radicals at different concentration inhibited the reduced the radical cations effectively, and their % of inhibition against these radicals increased when their concentration increased. The results were normalized and expressed as IC_{50} values for *A. silvaticus* and *A. arvensis* found to be 216 μg/ml and 17.75 μg/ml against DPPH radicals respectively.

In previous report made by Barros et al., [6] *A.silvaticus* was the most efficient species (lower IC_{50} values) concerning antioxidant activity, while *A.arvensis* presented lower antioxidant properties (higher IC_{50} values) which are compatible to its lower phenols content. Some authors have already reported a direct correlation between mushrooms antioxidant activity and total phenolic content, although the antioxidant action is raised by other substances such as tocopherols and β-carotene [13].

The values presented in this study obtained from Huang et al. [14] also found that the methanol extract from *Agaricus blazei* showed a high scavenging ability of 97.1% at 2.5 mg/ml.

Interferon-like activity: Interferon (INF) is a protein produced by cells in response to a viral infection. The biological activity of interferon is to convert cells of the same species into a viral refractory state. A great interest has recently been roused by natural inducers of interferon. According to Smolarz et al. the ethanol extracts from the herb of *Poligonum amphibium* L. and rhizome and fruit of *Polygonum bistorta* L. induced a substance showing an interferon-like activity. The protective titre (the highest dilution which protected cells by 50% against virus infection) on the interferon-like materials was 1:10 – 1:15 [15].

Alkaline extracts of 23 mushrooms grown in Mongolia were examined for antiviral activity. However, only two extracts from *Agaricus* species have shown an interferon-like activity compared with a-INF which was a control. The interferon-like activity of *A. silvaticus* was 56% while the activity of *A. arvensis* was 35%.

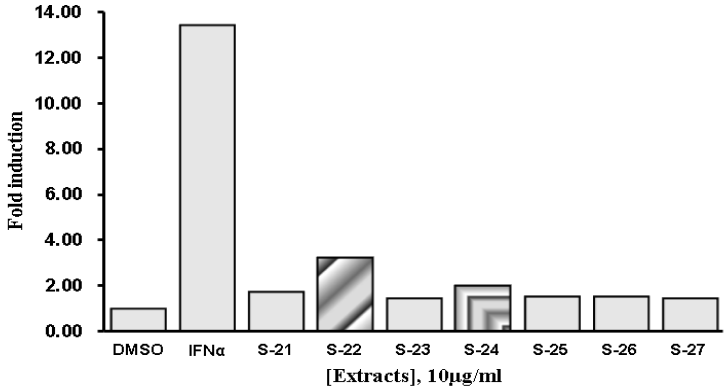

Fig. 1. IFN-like activity extracts of some mushrooms grown in Mongolia

Some species of *Agaricus* possess medicinal properties. The antiviral activity of extracts and compounds isolated from *Agaricus* species mushrooms has been described by various authors. Sorimachi et al. [16] demonstrated that the AqE obtained from *A.brasiliensis* was capable of blocking the cytopathic effect (CPE) of Western Equine Encephalitis virus (WEE). The aqueous extract of *Agaricus blazei* Murill ss. Heinem, was assessed to its antiviral action against herpes simplex type 1 (HSV-1) and bovine herpes type 1 (BoHV-1) in HEp-2 cell culture [17].

CONCLUSIONS

In this study we were able to observe the rich chemical composition of *Agaricus* spp. highlighting the variety and quantity of minerals and the biological activity of these mushrooms. The highest mineral concentrations of analyzed mushrooms were K, Na, and P.

We observed that the ethanol extracts from *Agaricus arvensis* was more effective in inhibiting free radicals. The IC_{50} of *A. silvaticus* and *A. arvensis* were 216 and 17.75 μg/ml respectively. Through this study we were able to observe the rich chemical composition of *A. silvaticus* and *A. arvensis* , highlighting the variety and quantity of minerals and the high protein content of these mush rooms. It was found that the chemical composition of the mushroom showed differences when compared to the composition of the same mushroom in other studies and other mushrooms of the Agaricales genus.

REFERENCES

1. J.V.Costa, M.R.Carvalho, G.Novaes, E.R.Asquieri. (2011) Chemical and antioxidant potential of *Agaricus sylvaticus* mushroom grown in Brazil. *J. Bioanal. Biomed.*, **3**(2), 49-54
2. Cheung L.M., Cheung P.C.K., Ooi V.E.C. (2003) Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chemistry*, **81**, 249-255.
3. Elmastas M., Isildak O., Turkekul I., Temur N. (2007) Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *Journal of Food Composition and Analysis*, **20**, 337-345.
4. Tsai S., Tsai H., Bad J. (2007) Antioxidant properties of *Agaricus blazei*, *Agrocybe cylindracea* and *Boletus edulis*. *Lebensmittel Wissenschaft und Technologie - Food Science and Technology,* **40**, 1392-1402.
5. J.Y.Zhao, J.H.Ding, Z.H.Li, Z.J.Dong, T.Feng, H.B.Zhang, J.K.Liu. (2013) Two new sesquiterpenes from culturesof the basidiomycete *Agaricus arvensis*. *Journal of Asian Natural Products Research*, **15**(3), 305-309
6. L.Barros, S.Falcro, P.Baptista, C.Ferreira, M.Vilas-Boas. (2008) Antioxidant activity of *Agaricus sp.* mushrooms by chemical, biochemical and electrochemical assays. *Food Chemistry*, **111**, 61–66
7. R.C.Fortes, M.R.Carvalho Garbi Novaes. (2011) The effects of *Agaricus sylvaticus* fungi dietary supplementation on the metabolism and blood pressure of patients with colorectal cancer during post surgical phase. *Nutr. Hosp.*, **26**(1), 176-186
8. Y.Y.Thoo, S.K.Ho, J.Y.Liang, C.W.Ho, C.P.Tan. (2010) Effects of binary solvent extraction system, extraction time and extraction temperature on phenolic antioxidants and antioxidant capacity from mengkudu (*Morinda citrifolia*). *Food Chemistry*, **120**, 290–295
9. Z.F.Tai, G.L.Zhang, F.Wang. (2012) Identification of small molecule activators of the Janus Kinase/ signal transducer and activator of transcription pathway using a cell-based screen. *Biol. Pharm. Bull.,* **35**(1), 65-71
10. F.A.Ayaz, H.Torun, A.Colak, E.Sesli, M.Millson, R.H.Glew. (2011) Macro- and microelement contents of fruiting bodies of wild-edible mushrooms growing in the east black sea region of Turkey. *Food and Nutrition Sciences*, **2**, 53-59.
11. X.H.Chen, H.B.Zhou, G.Z.Qiu. (2009) Analysis of several heavy metals in wild edible mushrooms from regions of China. *Bull. Environ. Contam. Toxicol*., **83**, 280–285
12. P.Kalac, L.Svoboda. (2000) A review of trace element concentrations in edible mushrooms. *Food Chemistry*, **69**, 273-281.
13. Cheung L.M., Cheung P.C., Ooi, V.C. (2003) Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chemistry*, **81**, 249–255.
14. Huang S.J., Huang L.C., Chen C.C., Mau J.L. (1999) Antioxidant properties of *Agaricus blazei*. In: Broderick A., Nair T. (Eds.), Proceedings of the third international conference on mushroom biology and mushroom products, P.266-274, Sydney, Australia.
15. H.D.Smolarz, T.Skwarek. (1999) The investigation into the interferon-like activity of *Polygonum* L. genus. *Acta Poloniae Pharmaceutica Drag Research*, **56**(6), 459-462.
16. Sorimachi K.. IkeharaY., Maezato G., Okubo A. Yamazaki S., Akimoto K., Niwa A. (2001) Inhibition by *Agaricus blazei* Murill fractions of cytopathic effect induced by Western equine encephalitis (WEE) virus on VERO cells in vitro. *Biosci. Biotechnol. Biochem*., **65**, 1645-1647.
17. R.Bruggemann, J.M.Orlandi, F.J.Benati, L.C.Faccin, M.S.Mantovani et al. (2006) Antiviral activity of *Agaricus blazei Murrill* ss heinem extract against human and bovine herpesviruses in cell culture. *Brazilian Journal of Microbiology*, **37**, 561-565.