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ASSESSMENT OF ANTIOXIDANT ACTIVITIES OF SOME MEDICINAL FUNGAL EXTRACTS

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The antioxidant activity (AOA) and phenolic content of *Hypholoma fasciculare*, *Agaricus bisporus*, *Pleurotus ostreatus*, *Trichaptum abietinum*, *Polyporus squamosus*, *Schizophyllum commune* fungi were investigated. The change in ORP value of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ mediatory system was used to detect AOA. Results concluded that *Agaricus bisporus* and *Pleurotus ostreatus* show the highest AOA, while *Hypholoma fasciculare* has the highest phenolic content. The phenolic content did not show any correlation with AOA, leading us to conclude that there are other antioxidant compounds present in the extracts.

***Keywords*:** ORP, antioxidants, vitamin C, phenols, medicinal fungi.

Introduction. It is well known that many biochemical reactions in organisms occur with the participation of free radicals. Reactive forms of oxygen and other free radicals can initiate uncontrollable free radical chain processes in lipoid structures of cells, leading to various dangerous diseases, such as cancer, rheumatoid arthritis, ischemia, as well as various damages to the central nervous system. Alternatively, they can complicate the course of many diseases, thus accelerating the aging process of the body [1–3].

A number of antioxidant systems exist to protect cells from the effects of free radicals. Vitamin E (α-tocopherol), vitamin A (retinol), vitamin C (ascorbic acid), carotenes, as well as antioxidant enzymes such as catalase, glutathione peroxidase, superoxide dismutase and other compounds play key roles in those systems [1, 4, 5].

In recent years special attention is paid to the study of the content of antioxidants in various fungal raw materials for further applications in medicine and other industries [4, 5].

In presented work we have investigated antioxidant properties of extracts of the following macroscopic fungi: *Hypholoma fasciculare* (Huds.) P. Kumm. (harvested in Ijevan, Armenia), *Agaricus bisporus* (J.E. Lange) Imbach (was grown in an artificial environment), *Pleurotus ostreatus* (Jacq.) P. Kumm. (harvested in Dilijan, Armenia), *Trichaptum abietinum* (Pers. ex J.F. Gmel.) Ryvarden (harvested in

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Dilijan, Armenia), *Polyporus squamosus* (Huds.) Fr. (harvested in Zikatar, Noyemberyan, Armenia), *Schizophyllum commune* Fr. (harvested in Dilijan, Armenia).

Materials and Methods. For our investigation we used following chemicals: $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]\cdot 3H_2O$, $NaCH_3COO\cdot 3H_2O$, acetic acid, ethyl alcohol, ascorbic acid, Folin-Ciocalteu's reagent, gallic acid, sodium carbonate, which were purchased from "Sigma-Aldrich GmbH" (Sternheim, Germany).

Extract Preparation. Fresh fungi samples were dried in room conditions for 14 days. For extraction purposes, dried samples (5.0 *g*) were placed on magnetic stirrer with ethanol (70%, 50 *mL*) for 24 *h*. After the extraction, extracts were filtered using a filter with pore sizes of 0.65 μm. The extracts were kept in –20º*C* and used within 30 days.

Determination of Antioxidant Activity. The antioxidant activity (AOA) was determined by potentiometric measurement of a change in Oxidation Reduction Potential (ORP) of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ mediatory system caused by antioxidants in extracts [1, 6]. Briefly, an aliquot of the extract sample (1.0 *mL*) was transferred to tubes containing $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ mediatory system (3.0 *mL*). Then the mixture was allowed to stand at 45º*C* for 30 *min*. The platinum electrode and reference electrode were used for measurements. The AOA was expressed as vitamin C equivalent in *mg*/*L* extract. The AOA of samples was derived from a standard curve of vitamin C (Pearson's correlation coefficient r^2=0.9992).

Determination of the Total Phenolic Content. The content of total phenols was determined spectrophotometrically with some modifications, using gallic acid as standard [5]. Briefly, an aliquot of the extract sample (1.0 *mL*) was transferred to tubes containing a Folin-Ciocalteu's reagent (0.5 *mL*, 2.0 N) and water (7.0 *mL*). After 3 *min* a sodium carbonate solution (0.5 *mL*, 7.5% w/v) was added. The tubes were then allowed to stand at 45º*C* for 60 *min* before absorbance at 750 *nm* was measured against water. The content of total phenols was expressed as gallic acid equivalents in *g*/100 *g* extract. The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 10 to 50 $\mu g/mL$ (Pearson's correlation coefficient $r^2 = 0.999$).

Results and Discussion. Our method of measuring AOA can detect antioxidant activity of –SH and –OH groups in compounds. Tab. 1 shows the extract's reduction equivalent of vitamin C. We can claim that extracts of *Agaricus bisporus* and *Pleurotus ostreatus* possess the highest antioxidant activities. Extract of *Trichaptum abietinum* showed no antioxidant activity at all. Negative value of that extract means that it can oxidize that amount of vitamin C.

Table 1

Total AOA of fungal extracts (negative value shows oxidative properties)

Species of fungi	Vitamin C equivalent, 10^{-4} *g*/*L*
Hypholoma fasciculare	16.5
Polyporus squamosus	13.5
Pleurotus ostreatus	88.0
Trichaptum abietinum	–32.5
Agaricus bisporus	68.0
Schizophyllum commune	13.5

Fungi contain high amounts of total phenolic compounds. Many phenolic compounds have high AOA [5]. Tab. 2 shows phenolic compounds present in each extract. The lack of correlation between AOA and total phenolic compounds can be explained by the presence of non-phenolic antioxidants in extracts.

Table 2

Total phenolic compounds of fungal extracts

Species of fungi	Gallic acid equivalent, *g/L*
Hypholoma fasciculare	0.665
Polyporus squamosus	0.104
Pleurotus ostreatus	0.172
Trichaptum abietinum	0.095
Agaricus bisporus	0.181
Schizophyllum commune	0.213

Conclusion. All analyzed extracts except *Trichaptum abietinum* showed high antioxidant activity. Thus, the phenolic content had shown no correlation to the antioxidant activity, leading us to believe that there are other antioxidant compounds present in the extracts.

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