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PHENOLIC AND FLAVONOID CONTENT IN *HERICIUM ERINACEUS*, *GANODERMA LUCIDUM*, AND *AGROCYBE AEGERITA* UNDER SELENIUM ADDITION

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The phenolic and flavonoid contents and composition and the antioxidant ability in *Hericium erinaceus*, *Ganoderma lucidum*, and *Agrocybe aegerita* under selenium (Se) addition to growth medium were studied. The contents of total Se in fruiting bodies of controls (0 mM of Se) were 4.58 (*A. aegerita*), 8.53 (*G. lucidum*), and 14.29 (*H. erinaceus*) mg kg⁻¹ dry weight (DW), and was significantly increased by Se enrichment of substrate. The total phenolics in fruiting bodies of controls of *H. erinaceus*, *G. lucidum*, and *A. aegerita* were significantly lower (17.10, 28.11, and 16.05 mg of gallic acid equivalent (GAE)/g of extract, respectively) than for Se-rich mushrooms (26.29, 40.29, and 20.07 mg GAE/g of extract, respectively). Total flavonoid content for *H. erinaceus*, *G. lucidum*, and *A. aegerita* increased after Se supplementation from 368.6 to 445.6, 469.9 to 627.7, and 318.1 to 393.9 µg g⁻¹ of extract, respectively. The results show that the mushrooms have superior antioxidant properties after Se addition, because the scavenging ability on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was improved.

Keywords: mushroom, scavenging ability, antioxidant components

Edible mushrooms are sources of many substances beneficial for human health (CHIU et al., 2000; SULLIVAN et al., 2006). Because of the presence of bioactive compounds, some mushrooms are known as medicinal mushrooms. They have been used in traditional Asian medicine for several thousand years as life-prolonging mushrooms, remedies for asthma, hepatitis, bronchitis, insomnia, and sources of nutrients, and are also becoming popular in Western countries (AJITH & JANARDHANAN, 2007; VAZ et al., 2011; DONG et al., 2012).

Hericium erinaceus, *Ganoderma lucidum*, and *Agrocybe aegerita* are appreciated for antibacterial, antiviral, antitumor, antidiabetic, hypolipidemic, and antioxidant properties, and their extracts function as a dietary supplements (CHIU et al., 2000; ZHANG et al., 2003; SULLIVAN et al., 2006).

In many studies the bioactive compounds from mushroom have been identified and their antioxidant activity has been analysed. However, there is still little information on the effect of mushroom enrichment with micronutrients on antioxidants. The aim of this work was to assess the possibility to enhance the phenolic and flavonoid content and antioxidant activity

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of *H. erinaceus*, *G. lucidum*, and *A. aegerita* under selenium (Se) addition to substrates. This work is a part of experiments with Se-enrichment of mushrooms, which focus on estimating the impact of the micronutrient on antioxidant properties of edible mushrooms.

1. Materials and methods

1.1. Experimental design

The experiment was designed as previously described by NIEDZIELSKI and co-workers (2014).

1.2. Chemical analyses

Se determination, extraction, total phenolic content (TPC), total flavonoid content (TFC), and the radical scavenging activity on DPPH radicals were performed as previously described by GAŚECKA and co-workers (2015). EC_{50} – the concentration of a sample having 50% of the DPPH radical scavenging effect.

1.3. Chromatographic analysis

Chromatographic analysis was performed as described by BOROWIAK and co-workers (2015). The preferred wavelength for protocatechuic, *p*-hydroxybenzoic, vanillic, syringic, and *t*-cinnamic acids was $\lambda=280$ nm, while the preferred wavelength for chlorogenic, caffeic, *p*-coumaric, ferulic, sinapic acids, quercetin, rutin, and kaempferol was $\lambda=320$ nm.

1.4. Statistical analysis

Statistical analysis was done using STATISTICA 10 with ANOVA followed by post-hoc Tukey's test ($\alpha=0.05$). The Pearson correlation coefficients for selected pairs of parameters were estimated.

2. Results and discussion

2.1. Accumulation of Se

The highest content of Se in the controls was confirmed for *H. erinaceus*, then for *G. lucidum* and *A. aegerita* (Tables 1–3). Mushrooms usually contain small amounts of Se, but some species have the capacity to accumulate the element (COSTA-SILVA et al., 2011; KALAC, 2013). The addition of Se to the substrate resulted in an increase of its content in the fruiting bodies (Tables 1–3). The fruiting bodies of *H. erinaceus* and *A. aegerita* were not formed at 0.8 mM of Se addition. The stage of maturity, the amount of Se in soil and in the substrates (including Se supplementation of substrate) used in cultivation influenced the Se concentration in fruiting bodies (WERNER & BEELMAN, 2002; ESTRADA et al., 2009; BHATIA et al., 2013, 2014; NIEDZIELSKI et al., 2014). The daily requirement of Se is up to 150–200 μg (NAVARRO-ALARCON & CABRERA-VIQUE, 2008). Thus, the tested Se-rich mushrooms can be used as a possible supplement of Se-rich human diet.

Table 1. Se concentration, phenolics composition, and radical scavenging activity of *H. erinaceus* at different concentrations of Se in substrate

		Concentration of Se (mM)				
		0	0.1	0.2	0.4	0.6
Se	mg kg ⁻¹ DW	14.29 ^c	16.88 ^c	23.43 ^{bc}	29.89 ^b	40.08 ^a
Protocatechuic acid		21.49 ^d	27.06 ^d	82.87 ^c	90.29 ^{ab}	102.50 ^a
4-Hydroxybenzoic acid		0.40 ^d	0.56 ^d	1.01 ^c	1.49 ^b	6.98 ^a
Chlorogenic acid		0.35 ^e	0.76 ^d	1.87 ^c	3.03 ^b	3.88 ^a
Vanillic acid		0.19 ^d	2.66 ^c	3.26 ^c	13.38 ^b	28.13 ^a
Caffeic acid		55.81 ^d	60.22 ^d	89.04 ^c	122.69 ^b	157.44 ^a
Syringic acid		17.76 ^d	46.98 ^c	63.72 ^b	69.83 ^{ab}	71.06 ^a
<i>p</i> -Coumaric acid	µg g ⁻¹ DW	13.70 ^c	22.92 ^c	40.17 ^b	34.87 ^b	100.15 ^a
Ferulic acid		21.03 ^e	126.21 ^d	159.43 ^c	184.89 ^b	221.65 ^a
Sinapic acid		48.79 ^d	43.47 ^d	95.36 ^c	119.27 ^b	136.76 ^a
<i>t</i> -Cinnamic acid		22.35 ^d	35.42 ^c	44.65 ^b	52.25 ^b	75.61 ^a
Rutin		17.37 ^d	22.69 ^c	26.49 ^{bc}	27.89 ^b	33.07 ^a
Quercetin		17.43 ^d	19.49 ^d	30.48 ^c	43.02 ^b	68.95 ^a
Kaempferol		22.86 ^c	23.23 ^c	27.82 ^b	28.33 ^b	46.01 ^a
DPPH	% (in 5 mg ml ⁻¹)	68.99 ^e	70.14 ^d	74.26 ^c	79.69 ^b	84.63 ^a
EC ₅₀	mg ml ⁻¹	0.81 ^a	0.77 ^b	0.72 ^c	0.69 ^d	0.63 ^e

Mean values (n=3); identical superscripts denote no significant ($\alpha < 0.05$) differences in lines

Table 2. Se concentration, phenolics composition, and radical scavenging activity of *G. lucidum* at different concentrations of Se in substrate

		Concentration of Se (mM)					
		0	0.1	0.2	0.4	0.6	0.8
Se	mg kg ⁻¹ DW	8.53 ^d	28.70 ^c	37.28 ^c	47.64 ^b	49.37 ^b	71.38 ^a
Protocatechuic acid		nd	17.63 ^d	22.60 ^{cd}	23.74 ^c	33.36 ^b	42.83 ^a
4-Hydroxybenzoic acid		0.41 ^c	0.48 ^{bc}	0.51 ^{bc}	0.57 ^b	0.61 ^b	1.94 ^a
Chlorogenic acid		1.99 ^c	3.69 ^b	3.90 ^b	4.21 ^b	6.88 ^a	7.64 ^a
Vanillic acid		2.28 ^e	5.62 ^d	7.43 ^c	9.48 ^b	11.34 ^a	11.71 ^a
Caffeic acid		10.83 ^d	13.00 ^{cd}	15.97 ^{bc}	17.74 ^{bc}	19.84 ^b	34.31 ^a
Syringic acid		nd	20.86 ^c	21.47 ^c	26.92 ^b	29.49 ^b	35.19 ^a
<i>p</i> -Coumaric acid	µg g ⁻¹ DW	nd	nd	nd	10.36 ^b	12.59 ^{ab}	14.87 ^a
Ferulic acid		nd	nd	nd	nd	32.53 ^a	33.61 ^a
Sinapic acid		16.94 ^d	18.03 ^{cd}	21.39 ^{bcd}	22.30 ^{bc}	23.74 ^{ab}	28.19 ^a
<i>t</i> -Cinnamic acid		11.33 ^c	14.79 ^{bc}	14.91 ^{bc}	15.13 ^{bc}	18.09 ^b	34.10 ^a
Rutin		3.31 ^d	4.06 ^e	11.78 ^b	13.91 ^b	14.04 ^b	23.02 ^a
Quercetin		8.34 ^d	10.47 ^d	13.44 ^c	16.45 ^b	17.62 ^b	27.04 ^a
Kaempferol		16.28 ^e	22.53 ^d	24.09 ^d	26.18 ^c	28.52 ^b	36.54 ^a
DPPH	% (in 5 mg ml ⁻¹)	60.93 ^f	66.14 ^e	71.44 ^d	76.67 ^c	82.30 ^b	86.99 ^a
EC ₅₀	mg ml ⁻¹	0.57 ^a	0.52 ^b	0.50 ^{bc}	0.47 ^{cd}	0.45 ^d	0.38 ^e

Mean values (n=3); identical superscripts denote no significant ($\alpha < 0.05$) differences in lines, nd: not detected

Table 3. Se concentration, phenolics composition, and radical scavenging activity of *A. aegerita* at different concentrations of Se in substrate

		Concentration of Se (mM)				
		0	0.1	0.2	0.4	0.6
Se	mg kg ⁻¹ DW	4.58 ^d	14.97 ^c	10.61 ^c	23.03 ^b	33.42 ^a
Protocatechuic acid		22.87 ^c	27.04 ^c	37.03 ^c	92.34 ^b	125.01 ^a
4-Hydroxybenzoic acid		1.28 ^b	1.45 ^b	2.06 ^{ab}	2.80 ^b	3.96 ^a
Chlorogenic acid		2.38 ^b	2.82 ^b	2.94 ^b	3.26 ^b	6.93 ^a
Caffeic acid		81.96 ^b	105.11 ^b	74.32 ^b	82.70 ^b	135.60 ^a
Vanillic acid		12.97 ^a	13.12 ^a	14.19 ^a	12.76 ^a	15.50 ^a
<i>p</i> -Coumaric acid		22.22 ^b	24.29 ^b	22.22 ^b	44.55 ^a	47.84 ^a
Ferulic acid	µg g ⁻¹ DW	nd	nd	43.49 ^c	86.07 ^b	94.09 ^a
Sinapic acid		26.59 ^c	26.38 ^c	34.37 ^b	36.91 ^b	48.84 ^a
<i>t</i> -Cinnamic acid		27.85 ^d	49.32 ^b	43.63 ^c	42.87 ^c	75.81 ^a
Rutin		11.93 ^c	10.47 ^c	12.72 ^c	80.49 ^b	118.93 ^a
Quercetin		17.32 ^d	23.69 ^c	37.40 ^b	40.70 ^b	66.51 ^a
Kaempferol		71.91 ^d	116.68 ^b	127.55 ^c	168.73 ^a	184.03 ^c
DPPH	(in 5 mg ml ⁻¹)	74.16 ^d	79.02 ^c	84.99 ^b	91.21 ^a	93.56 ^a
EC ₅₀	mg ml ⁻¹	0.82 ^a	0.79 ^{ab}	0.75 ^b	0.70 ^c	0.62 ^d

Mean values (n=3); identical superscripts denote no significant ($\alpha < 0.05$) differences in lines, nd - not detected

2.2. Phenolic and flavonoid content

We confirmed the highest TPC and TFC for *G. lucidum*, while *A. aegerita* had the lowest amount of the metabolites (Figs 1 and 2). The lowest TPC and TFC were confirmed for the controls of each mushroom (0 mM of Se). Se addition significantly increased their contents.

The increase of TPC was also confirmed in *Pleurotus* and *Volvariella* cultivated on Se-rich substrate (BHATIA et al., 2014). Se probably indirectly affected other metabolites including phenolics. It enhanced accumulation of some sugars being important substrates in many metabolic pathways (LEI et al., 2014). BHATIA and co-workers (2014) suggested inhibition of enzymatic polyphenol oxidation by antioxidants containing Se.

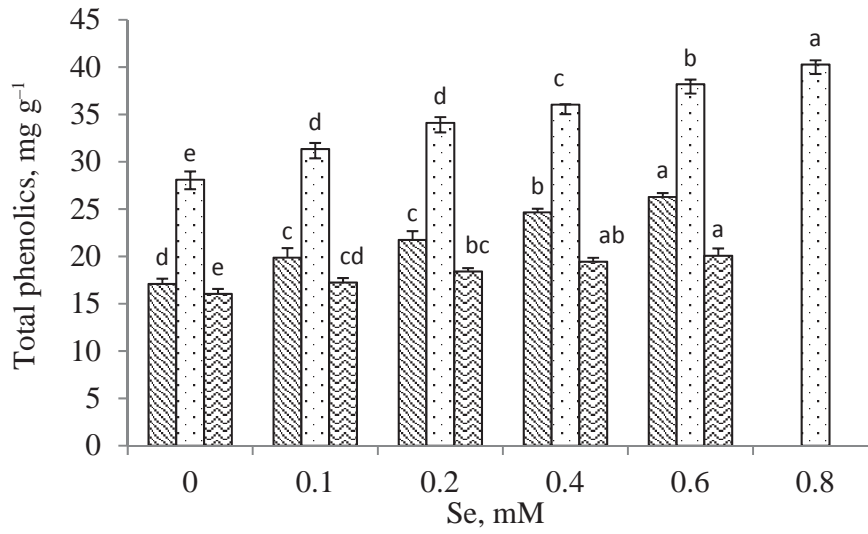


Fig. 1. Total phenolic content in fruiting bodies. Each value is expressed as mean \pm standard deviation SD (n=3), identical superscripts denote no significant ($\alpha < 0.05$) differences for each mushroom
 ■ *H. erinaceus*; ■ *G. lucidum*; ■ *A. aegerita*

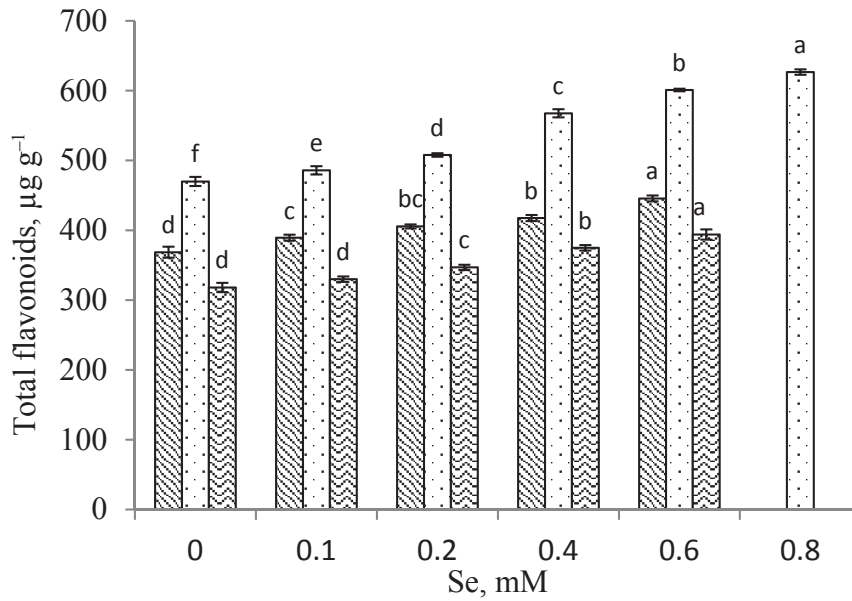


Fig. 2. Total flavonoid content in fruiting bodies. Each value is expressed as mean \pm standard deviation SD (n=3), identical superscripts denote no significant ($\alpha < 0.05$) differences for each mushroom
 ■ *H. erinaceus*; ■ *G. lucidum*; ■ *A. aegerita*

2.3. Phenolic profile

Among 22 analysed phenolic compounds we identified phenolic acids and flavonoids in fruiting bodies. Protocatechuic, 4-hydroxybenzoic, chlorogenic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, sinapic, and *trans*-cinnamic acids, rutin, quercetin, and kaempferol were detected in *H. erinaceus* (Table 1, Fig. 3). Li and co-workers (2012) detected 4-hydroxybenzoic, *p*-coumaric, ferulic, syringic, and α -resorcylic acids in *H. erinaceus*.

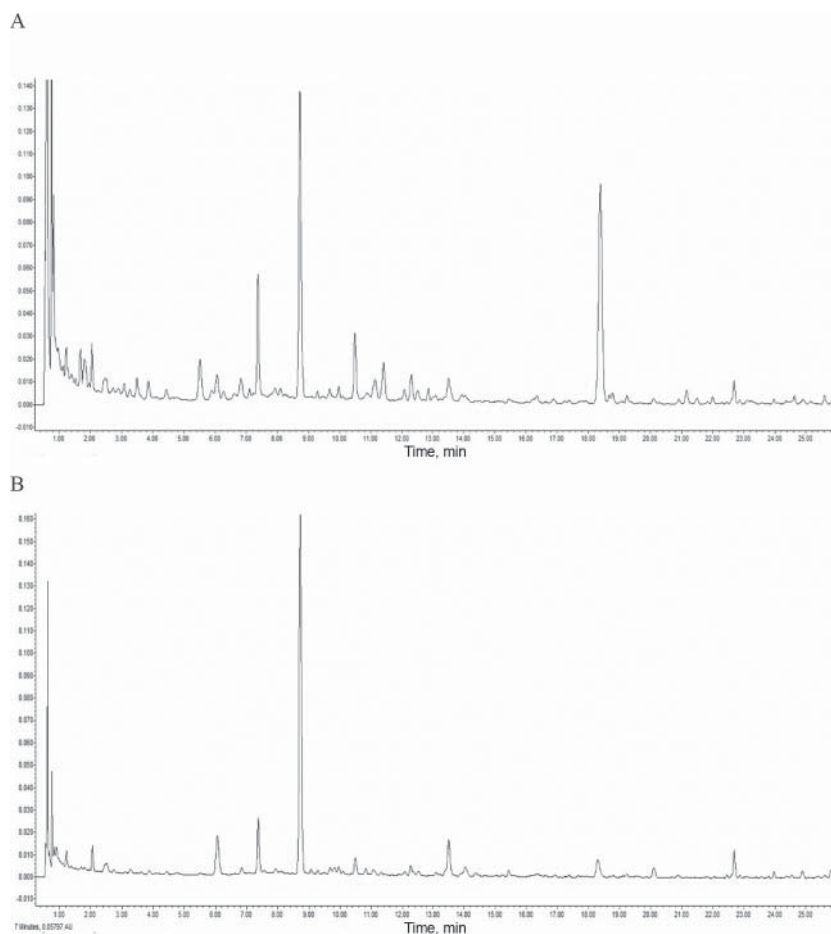


Fig. 3. HPLC chromatograms of phenolic compounds obtained for extract from *H. erinaceus* A: $\lambda=280$ nm; B: $\lambda=320$ nm. Retention times: protocatechuic acid 2.06 min, 4-hydroxybenzoic acid 3.86 min, vanillic acid 5.51 min, caffeic acid 6.059 min, syringic acid 7.37 min, *p*-coumaric acid 8.27 min, ferulic acid 10.48 min, sinapic acid 11.072 min, chlorogenic acid 12.069 min, rutin 13.51 min, *t*-cinnamic acid 18.29 min, quercetin 20.11 min, kaempferol 22.68 min

In control of *G. lucidum* 4-hydroxybenzoic, chlorogenic, vanillic, caffeic, sinapic, and *trans*-cinnamic acids, rutin, quercetin, and kaempferol were confirmed (Table 2). Additionally, protocatechuic and syringic acids were detected from 0.1 mM of Se in substrate, *p*-coumaric

acid was quantified from 0.4 mM of Se in substrate, while ferulic acid was detected from 0.6 mM of Se in substrate. Another studies confirmed gallic, 4-hydroxybenzoic, *p*-coumaric, protocatechuic, 5-sulfosalicylic, and cinnamic acids, pyrogallol, catechin, naringin, hesperidin, myricetin, quercetin, kaempferol, formononetin, biochanin, rutin, apigenin, biochanin, and luteolin in *G. lucidum* (KIM et al., 2008; HELANO et al., 2012; CELIK et al., 2013).

In controls of *A. aegerita*, protocatechuic, 4-hydroxybenzoic, chlorogenic, caffeic, vanillic, *p*-coumaric, sinapic, and *trans*-cinnamic acids, rutin, quercetin, and kaempferol were detected (Table 3). Additionally, ferulic acid was detected from 0.2 mM of Se addition. Se-enrichment enhanced the concentrations of phenolics in comparison to the controls. It can be very important for human health, because some phenolic compounds, such as caffeic, protocatechuic, and *p*-coumaric acids, kaempferol, quercetin, and rutin, are excellent antioxidants (SUN et al., 2004; HAN et al., 2009).

2.4. Antioxidant properties

For non-enriched mushrooms we confirmed the highest scavenging activity for *A. aegerita*, then for *H. erinaceus* and *G. lucidum* (Fig. 4). Some species of mushrooms are excellent antioxidants, because the EC₅₀ values are lower than for *tert*-butylated hydroxytoluene (KARAMAN et al., 2009). The strongest scavenging ability and a drop of EC₅₀ value obtained for the highest concentration of Se (Tables 1–3) indicate the improvement of the antioxidant properties and suggest that the micronutrient acts as an antioxidant.

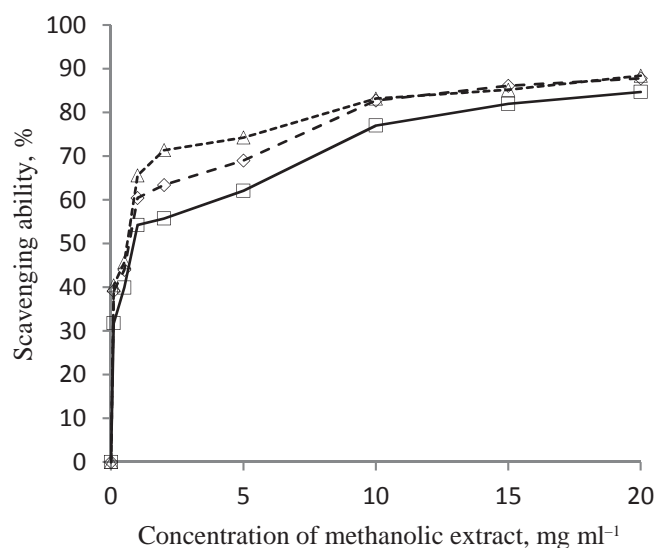


Fig. 4. Scavenging ability of non-enriched methanolic extracts on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals
 -◇-: *H. erinaceus*; -□-: *G. lucidum*; --△-: *A. aegerita*

Significant ($\alpha < 0.05$) positive correlations were confirmed between Se concentration and phenolics (besides syringic and ferulic acids for *H. erinaceus*; *p*-hydroxybenzoic and syringic

acids for *G. lucidum*; vanillic, caffeic, and sinapic acids for *A. aegerita*). Significant ($\alpha < 0.05$) negative correlations were confirmed between EC_{50} and Se concentration and phenolics (besides *p*-hydroxybenzoic acid for *H. erinaceus*; ferulic acid for *G. lucidum*; vanillic, caffeic, and *t*-cinnamic acids for *A. aegerita*). The results indicate that the addition of Se is able to improve the antioxidant properties of mushrooms. Other authors (TSAI et al., 2007; KIM et al., 2008; HELANO et al., 2012) also documented positive correlations between phenolics and DPPH radical scavenging activity.

3. Conclusions

To conclude, Se addition to substrate induced changes in the composition and concentrations of phenolics and flavonoids in fruiting bodies of *H. erinaceus*, *G. lucidum*, and *A. aegerita*. The concentrations of all components and scavenging ability of methanolic extract were higher for Se-rich mushrooms. Se is necessary for human health and is obtained only from food. It is not toxic at the appropriate doses, and therefore we suggest Se addition to growth medium of mushrooms to improve Se content and antioxidant activity of fruiting bodies.

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