Comparative evaluation of antioxidant and antimicrobial properties of some comercially available edible and medicinal mushrooms

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Abstract

Four commercially available edible and medicinal mushrooms from Croatia including shiitake (*Lentinus edodes*), black poplar (*Agrocybe aegerita*), oyster (*Pleurotus ostreatus*) and golden oyster (*Pleurotus citrinopileatus*) were assayed *in vitro* for their antioxidant and antimicrobial activities using water, ethanol and ethyl acetate as extractive solvents. Ascorbic acid, β-carotene, lycopene, α-tocopherol, total phenol, and flavonoids content were determined. Extracts obtained from dried mushrooms were evaluated for antioxidant activity by the reducing power, DPPH free radical scavenging activity and ferrous ion chelating ability. Water extracts exhibited higher antioxidant activities than ethanol and ethyl acetate extracts. Concerning EC_{50} values of scavenging abilities, the effectiveness was in descending order: ethyl acetate > ethanol > water. Scavenging activity of all the extracts has been significant compared to controls. Positive correlations were found between total phenolic content in the mushrooms extracts and their antioxidant activities. *A. aegerita* found to have the highest phenolic and flavonoid content. The four studied mushrooms showed narrow antibacterial activities against Gram-positive and Gram-negative bacteria, and strongly inhibited the growth of yeast *Candida albicans*.

Objectives

The primary objectives of this study were:

(i) to determine the total phenol, flavonoid, ascorbic acid, α-tocopherol, β-carotene and lycopene contents,

(ii) to evaluate antioxidant activity of the mushrooms extracts, and

(iii) to determine antimicrobial effect of the mushrooms extracts of four edible and medicinal mushroom species (*L. edodes*, *A. aegerita*, *P. ostreatus* and *P. citrinopileatus*).

Materials and methods

The samples of fresh fruting bodies of four commercially cultivated mushroom species were cleaned and subsequently air-dried in an oven at 50 ˚C for 12h before analysis and ground to obtain fine powder. 5 g of powders were overnight extracted with 50 ml of hot water (80 ˚C), ethanol and ethyl acetate (room temperature).

The amount of total phenols (TP) was determined by using Folin-Ciocaleau method. Flavonoids contents in the extracts were determined by colorimetric method (1). Ascorbic acid content of the extracts was assayed with 1% meta-phosphoric acid. β-carotene and lycopene content were determined with acetone:hexane mixture (4:6, v/v). α-tocopherol was determined by anhydrous ethanol and xylene extraction (2). The antioxidant activity was determined by the conjugated diene method. The reducing power assay were carried out spectrophotometrically at 700 nm. DPPH free radical scavenging activity was assayed with methanolic solution of DPPH radicals. Chelating ability of ferrous ions was assayed with ethanol and 2 mM ferrozine (3).

Results and Discussion

Mushrooms are a natural source of potent bioactive antioxidant metabolites (4). Indeed, phenolic compounds are heavily investigated for their high potential benefit on human health. Some species among higher fungi show higher antioxidant properties when compared with others. Such mushroom species include in particular shiitake (*L. edodes*) and oyster mushrooms (*P. ostreatus* and *P. citrinopileatus*). These two fungus seems to concentrate a complete “arsenal” against oxidative stresses (5). It includes primary antioxidant compounds (ascorbic acid/vitamin C, α-tocopherol/vitamin E, and β-carotene/vitamin A), the high content of phenolic compounds with extensive reducing and scavenging properties (Table 1, Fig. 1). Other mushrooms with strong antioxidant abilities include *A. aegerita* whose antioxidant effects and free radical scavenging abilities are correlated with its total phenolic content (1).

Table 1. Antioxidant components content of the extracts from *L. edodes*, *A. aegerita*, *P. ostreatus* and *P. citrinopileatus*

	L. edodes	*A. aegerita*	*P. ostreatus*	*P. citrinopileatus*
Water				
Ascorbic acid (mg/g)	1.73±0.19B[a]	1.89±0.05A	1.26±0.01C	1.23±0.05C
β-Carotene (μg/g)	nd[b]	nd	nd	nd
Lycopene (μg/g)	0.81±0.42A	0.63±0.21C	0.89±0.09A	0.72±0.11B
α-Tocopherol (μg/g)	nd	nd	nd	nd
Flavonoids (mg/g)	3.92±0.07A	1.50±0.36D	1.92±0.11C	2.04±0.05B
Total phenols (mg/g)	12.88±0.09B	13.95±0.17A	12.21±0.61D	12.56±0.01C
Ethanol				
Ascorbic acid (mg/g)	0.39±0.34B	0.56±0.28A	0.35±0.06B	0.29±0.43C
β-Carotene (μg/g)	0.04±0.11B	0.07±0.01A	0.02±0.42C	0.04±0.12B
Lycopene (μg/g)	4.91±0.14B	5.23±0.11A	4.93±0.21B	4.31±0.29B
α-Tocopherol (μg/g)	2.01±0.09A	2.09±0.11A	1.24±0.26C	1.61±0.09B
Flavonoids (mg/g)	1.75±0.17C	3.21±0.22B	4.51±0.11A	3.49±0.09B
Total phenols (mg/g)	12.61±0.01B	14.98±0.12A	9.55±0.21C	8.89±0.13D
Ethyl acetate				
Ascorbic acid (mg/g)	0.19±0.05B	0.24±0.19A	0.16±0.11D	0.17±0.04C
β-Carotene (μg/g)	0.01±0.12B	0.02±0.09A	nd	nd
Lycopene (μg/g)	3.89±0.02A	3.91±0.13AA	3.39±0.07B	3.31±0.13B
α-Tocopherol (μg/g)	1.26±0.08B	1.38±0.03A	1.19±0.27C	1.19±0.11C
Flavonoids (mg/g)	1.20±0.15C	2.21±0.01B	3.42±0.22A	2.29±0.05B
Total phenols (mg/g)	12.25±0.06B	13.01±0.07A	12.01±0.01B	12.28±0.08B

[a] Within the same row, mean followed by different letters are significantly different at $p \leq 0.05$.
[b]nd = not detected

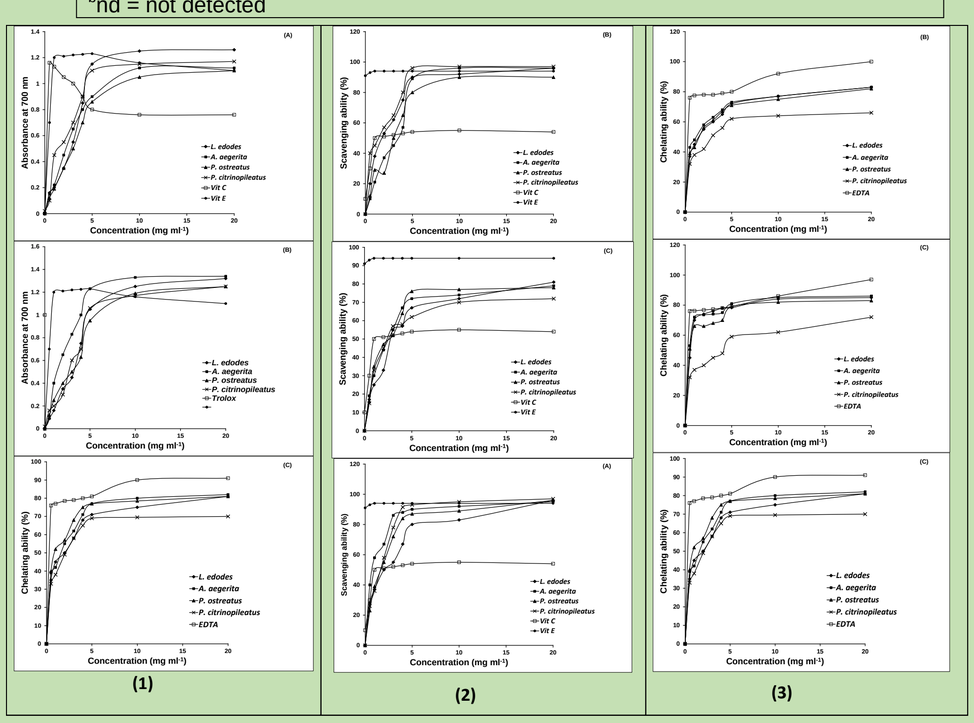

Fig. 1 . Reducing power (A), DPPH scavenging ability (B), and c helating ability (C) of hot water (1), ethanolic (2) and ethyl acetate (3) extracts

Reducing power of a compound may be serving as a significant indication of its potential antioxidant activity. The presence of reducers (i.e., antioxidants) causes the reduction of Fe^{3+}/ferrocyanide complex to ferrous form (3). The EC_{50} value for hot water extract was about 1.6 mg/ml, which was higher than ethanolic and ethyl acetate extracts. One of the most common methods for determination of antioxidant capacity is the DPPH free radical scavenging activity assay. The best scavenging effect was obtained in ethanolic extracts (*A. aegerita*, 0.78 mg/ml). The best ability for chelating of ferrous ions was obtained in hot water extracts (*P. ostreatus*, 2.28 mg/mL). Hot water extracts showed the best antioxidant activity in most investigated assays for antioxidant activity determination (Table 2).

Table 2. EC_{50} values of various extracts from *L. edodes*, *A. aegerita*, *P. ostreatus* and *P. citrinopileatus*

Compound	EC_{50} value[a] (mg/ml)			
	L. edodes	*A. aegerita*	*P. ostreatus*	*P. citrinopileatus*
Water				
Antioxidant activity	2.12±0.11B[b]	2.43±0.22A	2.01±0.13C	2.26±0.23B
Reducing power	1.42±0.05C	1.78±0.15A	1.65±0.06B	1.38±0.07C
Scavenging ability on DPPH radicals	1.82±0.03B	3.56±0.08A	3.01±0.02A	1.61±0.15B
Chelating ability of ferrous ions	2.48±0.19B	2.69±0.09A	2.28±0.13C	2.56±0.01B
Ethanol				
Antioxidant activity	3.34±0.09B	3.67±0.05A	2.77±0.02C	2.98±0.13C
Reducing power	2.11±0.12B	2.41±0.13A	1.99±0.04C	2.32±0.09B
Scavenging abiliy on DPPH radicals	2.05±0.07A	0.78±0.17C	1.85±0.09B	1.69±0.01B
Chelating ability of ferrous ions	6.23±0.11B	6.86±0.09A	5.76±0.23C	6.72±0.12A
Ethyl acetate				
Antioxidant activity	2.75±0.04A	2.78±0.09A	2.12±0.13C	2.34±0.17B
Reducing power	0.98±0.18B	1.21±0.08A	0.88±0.09C	1.18±0.01A
Scavenging ability on DPPH radicals	2.87±0.06A	2.84±0.21A	2.81±0.02A	2.93±0.14A
Chelating ability of ferrous ions	4.87±0.12B	5.23±0.14A	3.99±0.05C	5.09±0.11A

[a]EC_{50} value, the effective concentration at which the antioxidant activity was 50%; the absorbance was 0.5 for reducing power; 1,1-diphenyl-2-picrylhydrazil (DPPH) was scavenged by 50%; and ferrous ions were chelated by 50%, respectively. EC_{50} value was obtained by interpolation from linear regression analysis.
[b]Each vaue is expressed as mean±SE (n = 3). Means with the same letter within the same row are significantlly different ($p < 0.05$).
[c]Obtained by extrapolation from linear regression analysis.

The antimicrobial activities of *L. edodes*, *A. aegerita*, *P. ostreatus* and *P. citrinopileatus* ethanol extracts and standard antibiotics was quantitatively assessed by the presence or absence of clear zones indicating strong inhibition, and hazy (partial) inhibition zones, as given in Table 3. To determine antimicrobial activities , mushrooms were tested against Gram-negative (*S. typhimurium* and *E. coli*) bacteria, Gram-positive bacteria (*B. subtilis*, *B. cereus* and *S. aureus*) and yeasts (*C. albicans* and *Sch. pombe*). Among the selected bacteria studied, extracts inhibited the growth of Gram-positive bacteria slightly better than Gram-negative bacteria. The ethanol extracts exhibited clear inhibition zones only against fungi *C. albicans* and *Sch. pombe*. Furthermore, hazy inhibition zones were obtained against all tested bacteria except *B. cereus* and *B. subtilis* in the case of *P. ostreatus* extracts.

Table 3. Antimicrobial activity of ethanol extracts of *L. edodes*, *A. aegerita*, *P. ostreatus* and *P. citrinopileatus* and standard antibiotics

Bacteria/Fungi	*L. edodes*	*A. aegerita*	*P. ostreatus*	*P. citrinopileatus*	Chloramphenicol	Nystatin
Bacillus cereus	3+*	3+*	-	+*	4+	-
Bacillus subtilis	3+*	3+*	-	2+*	3+	-
Staphylococcus aureus	3+*	3+*	2+*	3+*	4+	-
Salmonella typhimurium	3+*	3+*	2+*	2+*	4+	-
Escherichia coli ATCC 35218	3+*	2+*	+*	2+*	4+	-
Candida albicans	3+	4+	3+	+	-	3+
Schizosaccharomyces pombe	2+	2+	2+	+	-	2+

Symbols: *hazy zone; diameter of inhibition zone: + low level of activity (7-10 mm); 2+ (11 to 15 mm); 3+ (16 to 20 mm);
4+ high level of activity (21 to 25 mm); - no inhibition

Conclusions

- The aim of this research was to extend knowledge of nutritional and nutraceutical quality of commercially cultivated mushrooms consumed in Croatia
- Total phenols were the major antioxidant components found in all four mushrooms extracts, while vitamins were found in small amounts.
- EC_{50} values of extracts showed descending order: ethyl acetate > ethanol > water
- Results of this study showed that water extract has maximum antioxidant and antimicrobial properties, which are potentially useful for overall health and nutritional purposes.

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