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ORIGINAL PAPER

Identification and quantification of phenolic acid compounds of twenty-six mushrooms by HPLC–DAD

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Abstract

Phenolic acids are found in different foods in the human diet, for example mushrooms. Determination of phenolic acids is important because of their relationship to their role in disease prevention due to their bioactive properties. In this study, the phenolic acid profile of 26 mushroom species was analyzed by using high-performance liquid chromatography method coupled with photodiode array detector (HPLC–DAD) and 16 phenolic acid compounds were identified. The chromatographic separation was performed using Intertsil ODS-3 reverse phase C_{18} column (5 µm, 250 mm × 4.6 mm i.d), gradient solvent system with 1.5 mL/min flow rate and detected at 280 nm. The coefficient of determination (R^2) was in the range of 0.9965–0.9999. Limit of detection and quantification ranged from 0.001–0.970 to 0.001–2.940 µg/L, respectively. The phenolic compounds were characterized according to their retention times and UV data were compared with commercial standards. *S. granulatus* (71.79 µg/g) and *L. nuda* (68.38 µg/g) revealed the highest concentration of total phenolic compounds among the studied mushrooms. Gallic acid was found as the major phenolic compound in *R. aurora* (2.96 ± 0.56 µg/g) while 6,7-dihydroxy coumarin was identified as major phenolic compounds in *A. tabescens* (2.07 ± 0.25 µg/g) and *L. leucothites* (9.02 ± 0.87 µg/g). Fumaric acid was found as the most abundant compounds in 16 out of 26 mushrooms. Catechin hydrate was identified as major phenolic compounds in the rest of mushrooms. This method provided a beneficial standardization procedure of phenolic acid compounds in mushroom samples.

Keywords HPLC–DAD · Phenolic compounds · Mushroom species · Fumaric acid · Catechin hydrate

Introduction

Mushrooms have been used for centuries both as food and medicine all over the world. Mushrooms are valuable healthy foods since they are poor in calories, fat and essential fatty acids, and rich in proteins, vitamin, and minerals [1, 2]. In previous studies, it was reported that mushrooms have anti-inflammatory, antioxidant, antitumor, antiviral and antimicrobial effects also, they have hypoglycemic, antiatherogenic and hematological properties [3–8]. The medicinal properties of mushrooms are caused by the bioactive compounds such as phenolic compounds, terpenoids, lectins, polysaccharides they contain [9, 10]. Among these biologically active substances present in mushrooms, phenolics have attracted much attention due to their antioxidant, anti-inflammatory, and antitumor effects [11].

Phenolic compounds are aromatic hydroxylated compounds, possessing one or more aromatic rings with one or more hydroxyl groups. They can be divided into two classes: simple phenols and phenolic acids such as gallic acid, benzoic acid, syringic acid, chlorogenic acid, and other associates and; polyphenols, which are classified into many groups such as flavonoids, tannins, stilbenes, and so on. Natural phenolic compounds are formed as end product in shikimate and acetate pathways and can range from relatively simple molecules (phenolic acids, phenylpropanoids, flavonoids) to highly polymerized compounds (lignins, melanins, tannins) [12, 13].

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Phenolic acids are found as the main phenolic compounds in mushrooms. Phenolic acids can be divided into two major groups, hydroxybenzoic acids and hydroxycinnamic acids which are consist of benzoic and cinnamic acid, respectively. Hydroxybenzoic acid derivatives generally are present in the attached form and their structure is similar to lignins and hydrolyzable tannins. Hydroxycinnamic acid derivatives mostly occur in the bound form, linked to cell wall structural components, such as cellulose, lignin, and proteins [13, 14].

Some findings suggest that biological properties of phenolic compounds are associated with to their antioxidant activity [15]. Antioxidant properties of phenolic compounds have a vital role in the stability of food products, as well as in the antioxidative defence mechanisms of biological systems [16]. The antioxidative effect of phenolic compounds in functional foods is caused from a direct free radical scavenging activity, reducing activity and chelating of prooxidant metal ions [17–19]. Phenolic hydrogen is responsible for free radical scavenging activity of phenolic compounds and the presence of hydroxyl group at ortho and para positions increases antioxidant activity [13]. For chelating metal ions, the presence of ortho-dihydroxylation on the phenyl ring in phenolic acids and flavonoids or the presence of a 3- or 5-hydroxyl group in flavonoids is required [20]. Phenolic compounds have been reported to prevent of various degeneration of human diseases, such as Alzheimer's diseases [21, 22]. Flavonoids are considered to protect against cancer and heart diseases [23].

In recent years, the increase in mushroom consumption in relation to the beneficial effects of bioactive compounds such as phenolic compounds on human health has further increased the studies on mushroom species. Therefore, investigations about the quantification of bioactive compounds present in the mushrooms gained importance. One of the main analytical techniques used to obtain the chemical profile of mushroom is high performance liquid chromatography (HPLC) [24]. In this context, the objective of the present study was to determine of phenolic compounds of 26 mushroom species; namely, *Agaricus bisporus*, *Amanita vaginata*, *Armillaria tabescens*, *Clitocybe odora*, *Collybia confluens*, *Collybia dryophila*, *Coprinus atramentarius*, *Chroogomphus rutilus*, *Lactarius deliciosus*, *Lactarius salmonicolor*, *Laetiporus sulphureus*, *Lepista nuda*, *Lepista personata*, *Leucoagaricus leucothites*, *Leucopaxillus tricolor*, *Marasmius oreades*, *Morchella elata*, *Morchella esculenta*, *Pleurotus ostreatus*, *Ramaria flava*, *Russula aurora*, *Russula azurea*, *Russula delica*, *Russula vinosa*, *Suillus granulatus* and *Tapinella panuoides* were collected from Anatolia by using high performance liquid chromatography coupled to photodiode array detector (HPLC–DAD). This described method benefits the analysis of phenolic acids in mushroom samples with sensitivity, trusty, rapidity, and selectivity.

Materials and methods

Chemicals and reagents

HPLC grade solvents were obtained from E. Merck (Darmstadt, Germany). Gallic acid (≥ 99%), fumaric acid (≥ 99%), protocatechuic acid (97%), catechin hydrate (≥ 98%), *p*-hydroxy benzoic acid (99%), 6,7-dihydroxy coumarin (98%), caffeic acid (≥ 98%), vanillin (99%), 2,4-dihydroxy benzoic acid (98%), *p*-coumaric acid (≥ 98%), ferulic acid (99%), coumarin (≥ 99%), *trans*-2-hydroxy cinnamic acid (99%), ellagic acid (≥ 98%), rosmarinic acid (≥ 98%) and *trans*-cinnamic acid (99%) were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany).

Instrument

The phenolic acid analysis was carried out using a Shimadzu 20 AT series high performance liquid chromatograph (HPLC–DAD, Shimadzu Cooperation, Japan).

Mushroom samples

The species names, collection localities and dates, family and edibility of 26 mushroom species are listed in Table 1. Voucher specimens were deposited at the fungarium of Natural Products Laboratory of Muğla Sıtkı Koçman University.

Extraction

The phenolic acids were determined according to the method of Barros et al. [25] with slight modification [26]. The mushroom sample (3 g) was extracted with acetone: water (80:20 v/v; 30 mL) at − 18 °C for 24 h. After ultrasonic bath for 15 min, the mushroom extract was centrifuged at 4000 rpm for 10 min and filtered through Whatman no. 4 paper. The residue was then re-extracted by two additional 30 mL of the acetone:water. The combined extracts were evaporated at 40 °C under reduced pressure to remove acetone. The obtained extract solved in water:methanol (80:20) and filtered through a 0.20 µm disposable LC filter disk for HPLC–DAD.

HPLC–DAD conditions

Separation was achieved on an Intertsil ODS-3 reverse phase C_{18} column (5 µm, 250 mm × 4.6 mm i.d) thermostatted at 40 °C. The solvent flow rate was 1.5 mL/min. The sample volume injection was 20 µL. The mobile phases used were: (A) 0.5% acetic acid in water, (B) 0.5% acetic acid in methanol. The elution gradient was as follows: 0–20%

Table 1 Collection localities and dates, family and edibility of the studied mushroom species

Code	Mushroom name	Collection localities and dates	Family	Edibilty
AB	*Agaricus bisporus* (J.E. Lange) Imbach	Uşak-Banaz, December 2013	Agaricaceae	Edible
AV	*Amanita vaginata* (Bull.) Lam	Uşak-Eşme, June 2014	Pluteaceae	Poisonous
AT	*Armillaria tabescens* (Scop.) Emel	Uşak-Sivaslı, November 2013	Physalacriaceae	Inedible
CO	*Clitocybe odora* (Bull.) P. Kumm	Denizli-Buldan, November 2013	Tricholomataceae	Poisonous
CC	*Collybia confluens* (Pers.) P. Kumm	Uşak-Eşme, November 2013	Tricholomataceae	Inedible
CD	*Collybia dryophila* (Bull.) P. Kumm	Uşak-Eşme, November 2013	Tricholomataceae	Poisonous
CA	*Coprinus atramentarius* (Bull.) Fr	Denizli- Honaz, November 2013	Agaricaceae	Poisonous
CR	*Chroogomphus rutilus* (Schaeff.) O.K. Mill	Uşak-Banaz, May 2014	Gomphidiaceae	Edible
LD	*Lactarius deliciosus* (L.) Gray	Fethiye-Babadağ, October 2013	Russulaceae	Edible
LS1	*Lactarius salmonicolor* R. Heim & Leclair	Bolu, April 2013	Russulaceae	Edible
LS2	*Laetiporus sulphureus* (Bull.) Murrill	Uşak-Subaşı, December 2014	Fomitopsidaceae	Edible
LN	*Lepista nuda* (Bull.) Cooke	Denizli-Babadağ, December 2014	Tricholomataceae	Edible
LP	*Lepista personata* (Fr.) Cooke	Uşak, December 2013	Tricholomataceae	Edible
LL	*Leucoagaricus leucothites* (Vittad.) Wasser	Uşak-Banaz, September 2013	Agaricaceae	Edible
LT	*Leucopaxillus tricolor* (Peck) Kühner	Denizli-İncirpınar, December, 2014	Tricholomataceae	Inedible
MO	*Marasmius oreades* (Bolton) Fr	Uşak-Eşme, November 2014	Marasmiaceae	Edible
ME1	*Morchella elata* Fr	Denizli-Babadag, April 2013	Morchellaceae	Edible
ME2	*Morchella esculenta* (L.) Pers	Muğla-Fethiye, April 2013	Morchellaceae	Edible
PO	*Pleurotus ostreatus* (Jacq.) P. Kumm	Uşak-Eşme, December 2014	Pleurotaceae	Edible
RF	*Ramaria flava* (Schaeff.) Quél	Muğla, December 2014	Gomphaceae	Edible
RA1	*Russula aurora* Krombh	Denizli-Honaz, November 2013	Russulaceae	Edible
RA2	*Russula azurea* Bres	Denizli-Honaz, November 2013	Russulaceae	Edible
RD	*Russula delica* Fr	Muğla, December 2014	Russulaceae	Edible
RV	*Russula vinosa* Lindblad	Denizli-Honaz, November 2014	Russulaceae	Edible
SG	*Suillus granulatus* (L.) Roussel	Uşak-Banaz, November 2014	Suillaceae	Edible
TP	*Tapinella panuoides* (Fr.) E.-J. Gilbert	Uşak-Eşme, November 2013	Tapinellaceae	Inedible

B (0–0.01 min); 20–60% B (0.01–2 min); 60–80% B (2–15 min); 100% B (15–30 min); 100–10% B (30–35 min); 10–0% B (35–40 min). Detection was carried out photodiode array detector (PDA) using 280 nm as the preferred wavelength.

Method validation

The proposed chromatographic method was validated in terms of linearity, LOD, LOQ, and repeatability. The phenolic compounds were characterized according to their retention times, and UV data were compared with commercial standards. Three parallel analyses were performed. For the quantitative analysis of phenolic compounds, calibration curves were obtained via the injection of known concentrations (0.0, 0.00782, 0.01563, 0.03125, 0.0625, 0.125, 0.25, 0.5 and 1.0 ppm) of different standards compounds i.e. gallic acid, fumaric acid, protocatechuic acid, catechin hydrate, *p*-hydroxy benzoic acid, 6,7-dihydroxy coumarin, caffeic acid, vanillin, 2,4-dihydroxy benzoic acid, *p*-coumaric acid, ferulic acid, coumarin, *trans*-2-hydroxy cinnamic acid, ellagic acid, rosmarinic acid, *trans*-cinnamic acid. Linear concentration range was studied using mixed standard solutions ranging from 0.01 to 1 mg/L. The linearity was examined using coefficient of determination (R^2) values. Determination of signal-to-noise ratio was calculated under the proposed chromatographic condition. LOD was considered as 3:1 and LOQ as 10:1. The analytical parameters and numbers of phenolic compounds are described in Table 2.

Statistical analysis

All the experiments were carried out at least in triplicate with constant results. Data were recorded as mean ± S.E.M. Significant differences between means were determined by Student's test, p values < 0.05 were regarded as significant.

Results

Studies on mushrooms for the preparation of alternative pharmaceutical components in the management of various diseases are increasing. Among the bioactive compounds present in mushrooms, phenolics are one of the most

Table 2 Analytical parameters for HPLC–DAD analysis

No	Compounds	RT	R^2	Linearity range (mg/L)	LOD (µg/L)	LOQ (µg/L)
1	Gallic acid	4.37	0.9986	0.06–1	0.114	0.345
2	Fumaric acid	5.59	0.9999	0.06–3	0.970	2.940
3	Protocatechuic acid	6.87	0.9997	0.01–1	0.021	0.063
4	Catechin hydrate	8.46	0.9965	0.06–1	0.094	0.285
5	*p*-Hydroxybenzoic acid	10.64	0.9971	0.01–1	0.112	0.342
6	6,7-Dihydroxy coumarin	11.62	0.9998	0.01–1	0.019	0.060
7	Caffeic acid	13.13	0.9999	0.02–1	0.010	0.031
8	Vanillin	14.89	0.9995	0.01–0.5	0.020	0.061
9	2,4-Dihydroxy benzoic acid	15.54	0.9999	0.01–0.5	0.006	0.021
10	*p*-Coumaric acid	18.74	0.9994	0.01–0.5	0.022	0.067
11	Ferulic acid	19.76	0.9997	0.01–1	0.157	0.475
12	Coumarin	20.96	0.9999	0.01–0.5	0.001	0.001
13	*trans*-2-Hydroxy cinnamic acid	21.98	0.9998	0.03–0.5	0.018	0.055
14	Ellagic acid	22.54	0.9982	0.02–1	0.079	0.239
15	Rosmarinic acid	23.61	0.9999	0.03–2	0.060	0.181
16	*trans*-Cinnamic acid	24.52	0.9975	0.01–1	0.199	0.603

RT retention time (min), R^2 coefficient of determination, *LOD* limit of detection, *LOQ* limit of quantification

important and probably the main candidates responsible for the most health beneficial properties of the mushrooms.

The analytical parameters and numbers of phenolic compounds are described in Table 2. Phenolic and organic acid compositions of the mushroom species were given in the Table 3. The results were expressed as µg per g of dry weight (dw). Totally 16 phenolic and organic acid compounds, namely; gallic acid, fumaric acid, protocatechuic acid, catechin hydrate, *p*-hydroxy benzoic acid, 6,7-dihydroxy coumarin, caffeic acid, vanillin, 2,4-dihydroxybenzoic acid, *p*-coumaric acid, ferulic acid, coumarin, *trans*-2-hydroxy cinnamic acid, ellagic acid, rosmarinic acid, and *trans*-cinnamic acid were identified in the mushroms. Figure 1 shows the HPLC–DAD chromatogram of standard phenolic compounds.

According to obtained results, the highest level of total phenolic compounds was determined in *S. granulatus* (71.79 µg/g), *L. nuda* (68.38 µg/g), *R. vinosa* (58.41 µg/g), *R. azurea* (45.05 µg/g), *L. personata* (43.44 µg/g), *C. rutilus* (40.33 µg/g), and *A. vaginata* (39.60 µg/g), respectively.

Gallic acid (3,4,5-trihydroxy benzoic acid) is a trihydroxy benzoic acid, a type of phenolic acid. Earlier studies have shown that gallic acid indicated various bioactivities including anticancer, anti-HIV, anti-inflammatory, antimicrobial, and antifungal. Furthermore, gallic acid is used in skin and leather industry as a chelating agent and preservative in food and beverages [27]. When *R. aurora* (2.96 ± 0.56 µg/g) has the higher level of gallic acid, *R. delica* (0.07 ± 0.01 µg/g) has the lower level of the gallic acid (Table 3).

Fumaric acid ((*2E*)-but-2-enedioic acid) is one of the important organic acids due to its antioxidant, antimicrobial and acidifying properties [28]. The highest fumaric acid content was found in *L. nuda* (53.70 ± 3.66 µg/g), whereas the lowest fumaric acid content was found in *L. leucothites* (4.42 ± 0.64 µg/g) (Table 3).

Protocatechuic acid (3,4-dihydroxy benzoic acid) is known to have a range of bioactivities including anti-inflammatory, antioxidant, antimicrobial, free radical-scavenging activities, peroxidation inhibition and estrogenic/antiestrogenic activity [29]. The protocatechuic acid content of the mushroom species ranged from 0.75 ± 0.06 to 4.89 ± 0.32 µg/g (Table 3).

Catechin ((*2R,3S*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol) is a natural secondary metabolite. In previous studies, catechin was reported to show antioxidant, antimicrobial, anti-allergy and anticancer effects. Also, consuming catechin rich teas lowers the blood glucose levels and prevents from type-2 diabetes [30]. The lowest and highest catechin amounts in mushroom species were found to be in range of 1.33 ± 0.31–20.50 ± 1.26 µg/g. These values were determined in *L. personata* and *A. vaginata*, respectively (Table 3).

p-Hydroxy benzoic acid is an organic compound can be obtained naturally or synthesized chemically. Various pharmacological activities of *p*-hydroxy benzoic acid include antimicrobial, antialgal, antimutagenic, antiestrogenic, hypoglycemic, anti-inflammatory, anti-platelet aggregating, nematicidal, antiviral and antioxidant [31]. *p*-Hydroxy benzoic acid contents of the mushrooms ranged

Table 3 Composition (μg/g) of phenolic and organic acids

Compounds																	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	Total
AB	0.83 ± 0.02	10.10 ± 0.98	nd	2.28 ± 0.57	nd	9.28 ± 0.76	nd	nd	0.40 ± 0.01	nd	nd	0.04 ± 0.01	nd	nd	0.05 ± 0.01	0.45 ± 0.02	23.43
AV	0.82 ± 0.12	17.67 ± 1.17	nd	20.50 ± 1.26	0.08 ± 0.02	0.38 ± 0.04	0.08 ± 0.01	nd	nd	nd	nd	nd	nd	nd	nd	0.07 ± 0.01	39.60
AT	2.26 ± 0.43	nd	1.37 ± 0.18	nd	nd	2.07 ± 0.25	nd	nd	nd	nd	nd	0.06 ± 0.02	nd	nd	nd	0.09 ± 0.01	5.85
CO	0.13 ± 0.01	nd	0.76 ± 0.09	2.14 ± 0.21	0.56 ± 0.12	nd	nd	nd	nd	nd	nd	nd	nd	0.79 ± 0.48	nd	nd	4.38
CC	0.13 ± 0.03	5.59 ± 0.47	0.75 ± 0.06	2.14 ± 0.21	0.55 ± 0.12	0.37 ± 0.14	nd	0.09 ± 0.01	nd	0.06 ± 0.01	nd	nd	nd	nd	0.16 ± 0.04	0.02 ± 0.01	9.86
CD	1.86 ± 0.14	29.95 ± 2.15	nd	3.72 ± 0.18	0.52 ± 0.07	nd	nd	0.08 ± 0.02	0.09 ± 0.01	0.08 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	nd	nd	nd	0.01 ± 0.01	36.35
CA	1.44 ± 0.32	8.93 ± 0.87	2.01 ± 0.39	7.04 ± 0.85	2.02 ± 0.16	3.44 ± 0.23	0.25 ± 0.12	0.23 ± 0.10	0.14 ± 0.07	0.03 ± 0.01	0.03 ± 0.01	nd	0.18 ± 0.04	nd	nd	0.08 ± 0.02	25.82
CR	1.20 ± 0.10	27.82 ± 2.08	1.18 ± 0.22	7.81 ± 0.78	0.27 ± 0.06	nd	nd	nd	1.33 ± 0.65	0.05 ± 0.01	nd	0.36 ± 0.08	nd	nd	0.31 ± 0.05	nd	40.33
LD	0.69 ± 0.11	6.95 ± 0.89	0.85 ± 0.11	15.82 ± 1.25	1.14 ± 0.12	0.73 ± 0.17	nd	0.10 ± 0.03	0.61 ± 0.09	0.02 ± 0.01	nd	0.09 ± 0.01	nd	nd	0.09 ± 0.01	0.16 ± 0.04	27.25
LS1	0.43 ± 0.04	11.01 ± 0.85	0.87 ± 0.08	1.44 ± 0.21	0.44 ± 0.07	nd	nd	nd	0.28 ± 0.06	nd	nd	0.03 ± 0.01	nd	nd	0.18 ± 0.04	0.04 ± 0.01	14.72
LS2	0.24 ± 0.02	5.72 ± 0.58	nd	4.01 ± 0.35	0.14 ± 0.02	0.28 ± 0.03	0.16 ± 0.01	nd	nd	nd	nd	0.01 ± 0.01	nd	0.20 ± 0.02	nd	nd	10.76
LN	1.90 ± 0.35	53.70 ± 3.66	1.90 ± 0.35	2.76 ± 0.41	5.44 ± 0.67	1.11 ± 0.12	nd	nd	0.99 ± 0.10	0.05 ± 0.01	nd	nd	0.30 ± 0.13	nd	0.07 ± 0.01	0.16 ± 0.02	68.38
LP	1.71 ± 0.16	34.27 ± 2.54	4.03 ± 0.68	1.33 ± 0.31	0.30 ± 0.08	0.29 ± 0.07	nd	0.22 ± 0.06	nd	0.04 ± 0.01	0.32 ± 0.06	nd	nd	nd	0.85 ± 0.15	0.08 ± 0.01	43.44
LL	0.21 ± 0.02	4.42 ± 0.64	0.91 ± 0.14	nd	0.47 ± 0.15	9.02 ± 0.87	nd	nd	0.13 ± 0.02	nd	nd	nd	nd	0.34 ± 0.08	nd	0.38 ± 0.09	15.88
LT	1.18 ± 0.12	nd	1.95 ± 0.19	2.11 ± 0.27	0.41 ± 0.09	nd	nd	nd	0.29 ± 0.06	nd	nd	nd	nd	0.25 ± 0.05	nd	nd	6.19
MO	nd	25.85 ± 1.14	2.83 ± 0.32	nd	nd	nd	nd	nd	nd	nd	0.05 ± 0.01	0.01 ± 0.01	0.10 ± 0.02	nd	0.20 ± 0.02	0.01 ± 0.01	29.05
ME1	1.17 ± 0.15	nd	3.85 ± 0.26	5.04 ± 0.41	0.17 ± 0.02	nd	0.18 ± 0.02	nd	nd	0.08 ± 0.02	0.01 ± 0.01	nd	nd	nd	nd	0.02 ± 0.01	10.52
ME2	1.32 ± 0.32	nd	1.98 ± 0.22	10.24 ± 0.78	nd	nd	nd	nd	nd	0.11 ± 0.02	nd	nd	nd	0.39 ± 0.09	0.04 ± 0.01	nd	14.08
PO	1.38 ± 0.06	4.86 ± 0.37	2.07 ± 0.24	1.79 ± 0.20	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.27 ± 0.02	nd	0.04 ± 0.01	10.41
RF	0.29 ± 0.02	4.72 ± 0.31	0.89 ± 0.10	5.77 ± 0.41	nd	nd	nd	nd	nd	0.01 ± 0.01	nd	0.09 ± 0.02	nd	nd	nd	0.05 ± 0.01	11.82
RA1	2.96 ± 0.56	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.45 ± 0.14	0.59 ± 0.16	0.39 ± 0.12	4.39
RA2	1.45 ± 0.06	41.76 ± 3.02	nd	nd	nd	0.49 ± 0.04	nd	nd	nd	0.07 ± 0.01	0.11 ± 0.02	nd	nd	0.73 ± 0.41	0.09 ± 0.02	0.35 ± 0.08	45.05
RD	0.07 ± 0.01	15.59 ± 1.21	4.89 ± 0.32	2.27 ± 0.20	nd	nd	nd	nd	nd	nd	0.35 ± 0.07	nd	nd	nd	nd	0.05 ± 0.01	23.22
RV	2.50 ± 0.41	52.08 ± 3.21	nd	3.65 ± 0.21	nd	nd	nd	nd	nd	0.01 ± 0.01	nd	nd	nd	nd	nd	0.17 ± 0.02	58.41
SG	nd	48.38 ± 1.28	2.11 ± 0.18	16.59 ± 0.71	2.55 ± 0.23	nd	nd	nd	0.91 ± 0.18	nd	nd	nd	nd	0.84 ± 0.10	0.29 ± 0.07	0.12 ± 0.02	71.79
TP	0.18 ± 0.01	nd	0.39 ± 0.05	3.32 ± 0.49	nd	nd	nd	nd	nd	nd	nd	nd	0.27 ± 0.14	nd	2.76 ± 0.27	0.15 ± 0.04	7.07

Values represent the means ± S.E.M. of three parallel measurements ($p < 0.05$)

n.d. not detected

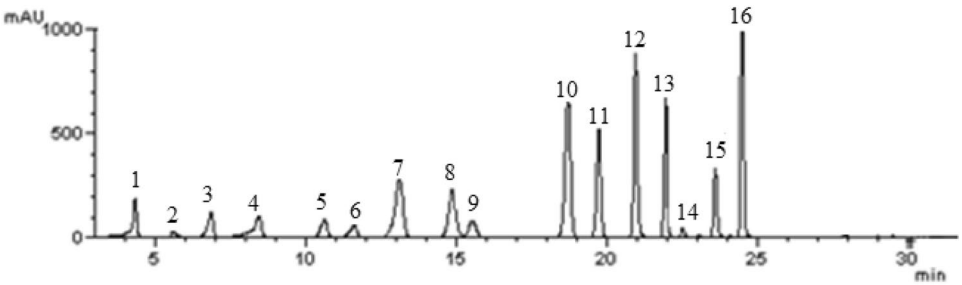

Fig. 1 HPLC–DAD chromatogram of standard phenolic compounds

from 0.08 ± 0.02 to 5.44 ± 0.67 µg/g. The lowest and highest *p*-hydroxy benzoic acid levels observed in *A. vaginata* and *L. nuda*, respectively. The minimum and maximum contents of 2,4-dihydroxy benzoic acid were 0.09 ± 0.01 µg/g in *C. dryophila* and 1.33 ± 0.65 µg/g in *C. rutilus*, respectively (Table 3).

Caffeic acid (3,4-dihydroxy cinnamic) is mostly found in plants. Caffeic acid is also found in many food sources such as coffee, blueberries, apples and cider. It is known that caffeic acid has antioxidant and antimicrobial activities in vivo, protects against atherosclerosis and cardiovascular diseases. Moreover, caffeic acid is used as photo-protective agents [32]. Caffeic acid was only found in *A. vaginata*, *C. atramentarius*, *L. sulphureus*, and *M. elata* mushrooms and caffeic acid contents were 0.08 ± 0.01, 0.25 ± 0.12, 0.16 ± 0.01 and 0.18 ± 0.02 µg/g, respectively (Table 3).

Vanillin (4-hydroxy-3-methoxy benzaldehyde), commonly used as a flavouring agent in food industry since ancient times and it has antimicrobial, anticancer and antioxidant activities [33]. The minimum and maximum contents of vanillin were 0.08 ± 0.02 µg/g in *C. dryophila* and 0.23 ± 0.10 µg/g in *C. atramentarius*, respectively (Table 3).

p-Coumaric acid is mostly found in plants and mushrooms in free or bound form. *p*-coumaric acid is known to exhibit a range of bioactivities including antioxidant, anti-inflammatory, antimutagenic, anti-ulcer, antiplatelet and anti-cancer [34]. The highest *p*-coumaric acid content was found in *M. esculenta* (0.11 ± 0.02 µg/g), whereas the lowest *p*-coumaric acid content was found in *R. flava* and *R. vinosa* (0.01 ± 0.01 µg/g) (Table 3).

Ferulic acid (4-hydroxy-3-methoxy cinnamic acid) is naturally occurring phenolic compound, abundant in citrus fruits, banana, coffee, orange juice, eggplant, bamboo shoots, beetroot, cabbage, spinach and broccoli. Ferulic acid is also used as a food preservative in Japan. Recent studies have indicated that ferulic acid possesses anti-atherosclerotic and antioxidant activities. Besides its biological properties ferulic acid has protective properties against Alzheimer's, inflammatory disease, diabetes, and hypertension [35]. Value of ferulic acid content was ranged from 0.01 ± 0.01 to 0.35 ± 0.07 µg/g for mushroom species. The highest and lowest of ferulic acid concentrations were obtained in *R. delica* and *M. elata*, respectively (Table 3).

Coumarins are seconder metabolites of plants and fruits. It has been reported that coumarins show cyclooxygenase inhibition, antimutagenic, scavenging of reactive oxygen species, anti-inflammatory, anticoagulant, lipoxygenase, CNS stimulants, antithrombotic, vasodilatory and anticancer activity [36]. The highest and lowest concentration of coumarin were detected in *C. rutilus* (0.36 ± 0.08 µg/g) and *L. sulphureus* and *M. oreades* (0.01 ± 0.01 µg/g), respectively. *A. bisporus* indicated the highest amount of 6,7-dihydroxy coumarin with the value of 9.28 ± 0.76 µg/g, whereas *L. sulphureus* indicated the lowest amount of 6,7-dihydroxy coumarin with the value of 0.28 ± 0.03 µg/g (Table 3).

Ellagic acid (2,3,7,8-tetrahydroxy-chromeno[5,4,3-cde] chromene-5,10-dione) is a lactone derivative found in various plants and fruits. Ellagic acid is famous for its biological and pharmacological activities such as antimutagenic, anticarcinogenic, antioxidant and anti-inflammatory [37]. The minimum and maximum contents of ellagic acid were 0.20 ± 0.02 µg/g in *L. sulphureus*, and 0.79 ± 0.48 µg/g in *C. odora*, respectively (Table 3).

Rosmarinic acid ((*R*)-(+)-3-(3,4-dihydroxy phenyl) lactic) is a phenolic ester derivate, found in many plants. Well documented biological activities of rosmarinic acid are antioxidant, anti-inflammatory, antimutagenic, antitumor, antigenotoxic, cytotoxic, antimetastatic, antiangiogenic, neuroprotective, antimicrobial, immunomodulatory, melanogenic and antivenom effects [38]. Rosmarinic acid contents of mushroom samples were found between 0.04 ± 0.01–2.76 ± 0.27 µg/g. The lowest and highest rosmarinic acid values were observed in *M. esculenta* and *T. panuoides*, respectively (Table 3).

Cinnamic acid ((*2E*)-3-Phenylprop-2-enoic acid) is widely distributed in plants. The literature survey reveals cinnamic acid derivatives have various biological properties such as antibacterial, antifungal, antioxidant, anti-inflammatory and antitumor [39]. The highest amount of cinnamic acid was found in *A. bisporus* (0.45 ± 0.02 µg/g) while the lowest amount of cinnamic acid was found in *C. dryophila*, and *M. oreades* (0.01 ± 0.01 µg/g). *Trans*-2-hydroxy cinnamic acid was only observed in *C. atramentarius*, *L. nuda*, *M. oreades* and *T. panuoides* mushrooms and *trans*-2-hydroxy cinnamic acid values were 0.18 ± 0.04, 0.30 ± 0.13, 0.10 ± 0.02 and 0.27 ± 0.14 µg/g, respectively (Table 3).

Discussion

There are many studies about phenolic compounds of mushroom species. A summary of the literature studies on phenolic compounds of the studied mushroom species is shown in the Table 4. The phenolic acid compositions of *A. bisporus* collected from different countries i.e. Northeast Portugal, Spain, Korea, and Australia have been reported by Reis et al. [2], Palacios et al. [40], Kim et al. [41], Taofiq et al. [42], Barros et al. [25]. They studied phenolic compounds, namely; gallic, *p*-coumaric acid, cinnamic acid, caffeic acid, chlorogenic acid, ferulic acid, *p*-hydroxy benzoic acid, homogentisic acid, protocatechuic acid, catechin, and pyrogallol. In a study on *C. atramentarius*, *p*-hydroxy benzoic acid, *p*-coumaric acid, and cinnamic acid contents have been reported [43]. The phenolic acid compositions such as gallic acid, gentisic acid, 2,3,4-trihydroxy benzoic acid as of *C. rutilus* have been studied by Islam et al. [44]. Another studies, by Palacios et al. [40], Taofiq et al. [42] and Barros et al. [25] reported phenolic acid compositions such as caffeic acid, chlorogenic acid, ferulic acid, gallic acid, gentisic acid, *p*-hydroxy benzoic acid, homogentisic acid, protocatechuic acid, and pyrogallol in *L. deliciosus* species. *p*-Hydroxy benzoic acid and cinnamic acid compositions of *L. salmonicolor* have

Table 4 Phenolic compositions of studied mushroom species

Mushroom species	Phenolic compounds	Collected area	References
A. bisporus	Gallic acid, *p*-coumaric acid, cinnamic acid	Northeast Portugal	Reis et al. [2]
A. bisporus	Caffeic acid, catechin, chlorogenic acid, *p*-coumaric acid, ferulic acid, gallic acid, *p*-hydroxy benzoic acid, homogentisic acid, protocatechuic acid, pyrogallol	Spain	Palacios et al. [39]
A. bisporus	Gallic acid, pyrogallol, protocatechuic acid	Korea	Kim et al. [40]
A. bisporus	Cinnamic acid	Australia	Taofiq et al. [41]
A. bisporus	*p*-Hydroxy benzoic acid	Northeast Portugal	Barros et al. [24]
C. atramentarius	*p*-Hydroxy benzoic acid, *p*-coumaric acid, cinnamic acid	Northeast Portugal	Heleno et al. [42]
C. rutilus	Gallic acid, gentisic acid, 2,3,4-trihydroxy benzoic acid	China	Islam et al. [43]
L. deliciosus	Caffeic acid, chlorogenic acid, ferulic acid, gallic acid, gentisic acid, *p*-hydroxy benzoic acid, homogentisic acid, protocatechuic acid, pyrogallol	Spain	Palacios et al. [39]
L. deliciosus	*p*-Hydroxy benzoic acid, cinnamic acid	Spain	Taofiq et al. [41]
L. deliciosus	*p*-Hydroxy benzoic acid	Northeast Portugal	Barros et al. [24]
L. salmonicolor	*p*-Hydroxy benzoic acid, cinnamic acid	Northeast Portugal	Vaz et al. [44]
L. sulphureus	Caffeic acid, gallic acid, *p*-hydroxy benzoic acid	Ethiopia	Woldegiorgis et al. [45]
L. sulphureus	*p*-Hydroxy benzoic acid, *p*-coumaric acid, salicylic acid	Poland	Nowacka et al. [46]
L. sulphureus	Protocatechuic acid	Poland	Sulkowska-Ziaja et al. [47]
L. nuda	Protocatechuic acid, *p*-hydroxy benzoic acid, *p*-coumaric acid	Northeast Portugal	Barros et al. [24]
L. leucothites	Catechin, *p*-coumaric acid	Dumlupinar-Turkey	Aslim and Ozturk [48]
M. oreades	*p*-Hydroxybenzoic acid, *p*-coumaric acid, vanillic acid, salicylic acid	Poland	Nowacka et al. [46]
M. esculenta	Gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, chlorogenic acid, ferulic acid, epicatechin	Turkey	Yıldız et al. [49]
M. esculenta	Gallic acid, protocatechuic acid, *p*-hydroxy benzoic acid, vanillic acid, caffeic acid	China	Islam et al. [43]
M. esculenta	cinnamic acid	Northeast Portugal	Taofiq et al. [41]
P. ostreatus	Protocatechuic acid, *p*-hydroxy benzoic acid, *p*-coumaric acid, cinnamic acid	Northeast Portugal	Reis et al. [2]
P. ostreatus	*p*-Coumaric acid, ferulic acid, gallic acid, gentisic acid, homogentisic acid, *p*-hydroxy benzoic acid, protocatechuic acid	Spain	Palacios et al. [39]
P. ostreatus	Gallic acid, *p*-hydroxy benzoic acid, caffeic acid	Ethiopia	Woldegiorgis et al. [45]
P. ostreatus	Protocatechuic acid, catechin, vanillic acid, *p*-coumaric acid, cinnamic acid	Trabzon-Turkey	Kolayli et al. [50]
P. ostreatus	Gallic acid, homogentisic acid, protocatechuic acid, chlorogenic acid	Seoul-Korea	Kim et al. [40]
P. ostreatus	*p*-Hydroxy benzoic acid, *p*-coumaric acid, cinnamic acid	Australia	Taofiq et al. [41]
R. delica	*p*-Hydroxy benzoic acid, syringic acid, vanillic acid, caffeic acid, cinnamic acid, chlorogenic acid, ferulic acid, *p*-coumaric acid	Greece	Kalogeropoulos et al. [51]

been studied [45]. In other studies, on *L. sulphureus* collected different area, caffeic acid, gallic acid, *p*-hydroxy benzoic acid, protocatechuic acid compositions have been reported [46–48]. The phenolic acid contents i.e. protocatechuic acid, *p*-hydroxy benzoic acid, *p*-coumaric acid of *L. nuda* have been studied by Barros et al. [25]. Catechin and *p*-coumaric acid contents of *L. leucothites* have been reported [49]. In another recent study, *p*-hydroxy benzoic acid, *p*-coumaric acid, vanilic acid, salicylic acid compositions of *M. oreades* using LC–ESI–MS/MS have been studied by Nowacka et al. [47]. The phenolic acid compositions such as gallic acid, protocatechuic acid, *p*-hydroxy benzoic acid, *p*-coumaric acid, chlorogenic acid, ferulic acid, epicatechin, vanillic acid, caffeic acid, cinnamic acid of *M. esculenta* have been reported by Yildiz et al. [50], Islam et al. [44], and Taofiq et al. [42]. There are several publications of phenolic acid compositions of *P. ostreatus.* Protocatechuic acid, *p*-hydroxy benzoic acid, *p*-coumaric acid, cinnamic acid by Reis et al. [2], *p*-coumaric acid, ferulic acid, gallic acid, gentisic acid, homogentisic acid, *p*-hydroxy benzoic acid, protocatechuic acid by Palacios et al. [40], gallic acid, *p*-hydroxy benzoic acid, caffeic acid by Woldegiorgis et al. [46], protocatechuic acid, catechin, vanillic acid, *p*-coumaric acid, cinnamic acid by Kolaylı et al. [51], gallic acid, homogentisic acid, protocatechuic acid, chlorogenic acid by Kim et al. [41], *p*-hydroxy benzoic acid, *p*-coumaric acid, cinnamic acid by Taofiq et al. [42] have been reported. Another study, Kalogeropoulos et al. [52] reported phenolic acids such as *p*-hydroxy benzoic acid, syringic acid, vanilic acid, caffeic acid, cinnamic acid, chlorogenic acid, ferulic acid, *p*-coumaric acid in *R. delica*. The results obtained with this study show similarities and differences with the literature. Cultivation techniques, growing conditions, ripening process, processing and storage conditions, extraction methods, different species, as well as stress conditions such as UV radiation, infection by pathogens and parasites, wounding air pollution and exposure to extreme temperatures are important factors that can explain these similarities and differences between the obtained results and the literature [53, 54].

Phenolic acids are found in the human diet in different foods such as mushrooms, plants, vegetables, fruits, spices and cereals. Because of their bioactive importance, phenolic acids are extensively studied and there is evidence of their role in disease prevention [55]. As it seen Table 3, *S. granulatus* (71.79 µg/g) and *L. nuda* (68.38 µg/g) revealed the highest concentration of total phenolic compounds among the studied mushrooms. Total amount of phenolic compounds of the methanol extracts of sea-buckthorn (*Hippophaë rhamnoides*), hawthorn (*Crataegus monogyn*), wild grown European blackberry (*Rubus fruticosus*), Cornelian cherry (*Cornus mas*), blackthorn (*Prunus spinosa*), dog rose (*Rosa canina*), hackberry (*Prunus padus*) wild fruits collected from Romania were reported as 77.01, 36.79, 292.44, 55.33, 71.11, 45.57 and 109.09 mg/100 g FW, respectively [56]. The phenolic profiles of the methanol extracts of twelve cruciferous vegetables, pakchoi (*Brassica. rapa* var. *chinensis*) (2472.00 ± 433.94 µg/g), choysum (*B. rapa* var. *parachinensis*) (4377.62 ± 400.24 µg/g), Chinese cabbage (*B. rapa* var. *pekinensis*) (9608.09 ± 3614.89 µg/g), kailan (*B. oleracea* var. *alboglagra*) (22,591.87 ± 1755.35 µg/g), Brussels sprout (*B. oleracea* var. *gemmifera*) (38,487.84 ± 2681.78 µg/g), cabbage (*B. oleracea* var. *capitata*) (46,021.61 ± 16,141.09 µg/g), cauliflower (*B. oleracea* var. *botrytis*) (7090.92 ± 1980.38 µg/g), broccoli (*B. oleracea* var. *italica*) (34,947.07 ± 7592.93 µg/g), rocket salad (*Eruca sativa*) (9253.61 ± 3175.72 µg/g), red cherry radish (*Raphanus sativus*) (1.68 ± 0.24 µg/g), daikon radish (*Raphanus sativus*) (1.02 ± 0.32 µg/g), and watercress (*Nasturtium officinale*) (6777.73 ± 3252.94 µg/g) purchased from various supermarkets in Singapore were reported by Li et al. [57]. The total amount of phenolic compounds of medically important plants *Sideritis albiflora* and *Sideritis leptoclada* collected from Turkey were determined as 22.257 and 39.245 µg/g in our previous research [58]. In a study of our group on the phenolic compounds of mushrooms carried out by Çayan et al. [59], the total amount of phenolic compounds of *Agrocybe cylindracea*, *Coprinus comatus*, *Clathrus rubber*, *Hypholoma fasciculare*, *Lentinus tigrinus*, *Mitrophora semilibera* were calculated as 16.18, 84.63, 115.90, 11.68, 146.55 and 40.84 µg/g, respectively. It is supported by this study that mushrooms have a significantly high amount of phenolic compounds when compared to different food, fruit, plant and mushroom species.

Conclusions

Phenolic compounds of 26 mushrooms collected from Anatolia were detected by using HPLC–DAD. To the best our knowledge, phenolic acids of *A. vaginata*, *A. tabescens*, *C. odora*, *C. confluens*, *C. dryophila*, *L. personata*, *L. leucothites*, *L. tricolor*, *M. elata*, *R. flava*, *R. aurora*, *R. azurea*, *S. granulatus* and *T. panuoides* have been studied for the first time. The total amount of phenolic compounds of the studied mushroom species was determined to be comparable to other foods. It can be suggested that the differences in the qualitative and quantitative composition of phenolic compounds can be caused by genetic differences between species, habitats, growth conditions, and maturation of mushrooms. Our findings suggest that these mushroom species can be used as an alternative source of natural phenolic compounds for the food, cosmetic and pharmaceutical industries.

Compliance with ethical standards

Conflict of interest No potential conflict of interest was reported by the authors.

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