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ORIGINAL RESEARCH PAPER

Exploring antioxidant potential in two basidiomycetous mushrooms using high-performance thin-layer chromatography–DPPH–videodensitometry

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Abstract
The antioxidant activity of the methanol extracts of fruiting bodies of two basidiomycetous mushroom species (*Cyclocybe cylindracea* and *Leccinum duriusculum*) was evaluated by high-performance thin-layer chromatography (HPTLC) combined with 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and videodensitometry. The HPTLC separation was achieved on a silica gel layer with acetonitrile–water–acetic acid (75:25:3, *V/V*). The results were compared with those obtained by the conventional microplate-based DPPH assay. The two methods provided similar results showing that the extract of the *C. cylindracea* had higher total antioxidant activity than the extract of the *L. duriusculum*, and the antioxidant activities of both extracts were much weaker than the antioxidant activities of ascorbic acid or gallic acid. Mushroom components in one zone of *L. duriusculum* extract and three zones of *C. cylindracea* extract assigned using HPTLC–DPPH–videodensitometry were mainly responsible for the antioxidant activity. Based on a previous study the β-carboline alkaloid C1-*S* diastereomer of brunnein B in *C. cylindracea* at hR_F 49 contributed to the antioxidant effect the most, which corresponded to 52.0 ± 3.1 ng of ascorbic acid equivalent and 51.7 ± 3.7 ng of gallic acid equivalent.

Keywords Antioxidant · *Cyclocybe* · 1,1-diphenyl-2-picrylhydrazyl (DPPH) · High-performance thin-layer chromatography (HPTLC) · *Leccinum* · Videodensitometry

1 Introduction

High-performance thin-layer chromatography (HPTLC) provides an affordable, high-throughput, and fast analysis of complex matrices. The HPTLC layer, especially the silica gel layer, is compatible with various chemical-based and biological assays, including antioxidant, antimicrobial, and different enzyme inhibitions. The different reactions/actions proceed directly in situ in the adsorbent bed enabling researchers to obtain bioactivity profiles of the extracts [1, 2]. Therefore, the HPTLC–effect-directed analysis (EDA) is suitable and expansively used for nontarget screening of the bioactive constituents from complex environmental [3], food [4], plant [5, 6], or mushroom [7] samples. Furthermore, HPTLC–EDA can also be applied to quantitative analysis using the bioactivity response as a signal measured by densitometry [8, 9] or videodensitometry [10]. To quantify unknown components, an equivalency calculation can be done by comparing their efficiency to that of known bioactive compounds [9–11].

There is an increasing interest in the pharmaceutical, food, and cosmetic industries in searching for natural antioxidants in connection with various fields of human life (e.g., nutrition, stress management) [12]. The assays that determine the antioxidant activity and its linked parameters are based on different mechanisms, for example, electron or proton transfer [13]. One of the most popular antioxidant assays employs an artificial radical molecule, 1,1-diphenyl-2-picrylhydrazyl (DPPH). A DPPH assay in a 96-well microplate can be carried out quickly and effectively at a low price [14], but it only provides information about the

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total antioxidant activity of the whole extracts. However, the easy-to-perform HPTLC–DPPH–videodensitometry allows determination of the content of both total antioxidants and the separated antioxidant spots when compared with the signal of the codeveloped analyzed compounds [8–15] or a known antioxidant (e.g., Trolox [16], ascorbic acid, or gallic acid [17]) in the appropriate amount range.

Among the basidiomycete mushrooms, many species produce numerous bioactive compounds (e.g., antioxidant, antimicrobial, and antitumor) via secondary metabolism [18]. Mushrooms have many constituents, but we know little about their antioxidant and/or antimicrobial effects [19]. The quality control of different plant, food, or mushroom extracts is based on the identification and/or quantification of bioactive compounds, which requires chromatographic and/or microplate-based methods [16]. This study aims to assess the total antioxidant capacity of extracts of two mushroom species, *Cyclocybe cylindracea* and *Leccinum duriusculum*, by HPTLC–DPPH–videodensitometry and microplate-based DPPH assays. The results were expressed as ascorbic acid and gallic acid equivalents and compared. Furthermore, the strength of the main antioxidant zones selected according to the HPTLC–DPPH radical scavenger antioxidant profile was also evaluated.

2 Experimental

2.1 Materials

Gallic acid (purity ≥ 98%), ascorbic acid (purity ≥ 99%), and 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) were purchased from Merck (Budapest, Hungary). Analytical grade methanol and acetic acid were purchased from Molar Chemicals (Halásztelek, Hungary). Gradient-grade acetonitrile was from Fisher Scientific (Pittsburgh, PA) and pure water was from a Millipore Direct-Q 3 UV System (Merck).

2.2 Samples and preparation of extracts

The wild-growing macrobasidiomycetous mushrooms, poplar fieldcap (*Cyclocybe cylindracea*) and slate bolete (*Leccinum duriusculum*) were collected in Eger and Buda Hills, respectively, in Hungary. The fresh fruiting bodies were cleaned, sliced, frozen at −18 °C, and carefully dried using a freeze dryer (Alpha-1–2 D, Christ, Osterode am Harz, Germany). The dried fruit bodies were pulverized (Bosch MKM6000, Stuttgart, Germany) and kept in a hermetically sealed sample holder at room temperature until analysis. The mushroom samples (total mass of the dried mushroom samples 1 g) were extracted with methanol (concentration: 1 mL per 100 mg dried mushroom sample) by ultrasonication for 1 h [20], and the supernatant was filtered through filter paper (Macherey–Nagel, MN 85/70 BF; Düren, Germany). The extraction was repeated twice with the residual material, and the resulting filtrates were combined. The merged extracts were filtered through a 0.22-µm pore diameter polytetrafluoroethylene (PTFE) syringe filter (Nantong FilterBio Membrane Co., Zhongnan, P.R. China) and stored in vials at 5 °C for further use. The concentrations of mushroom extracts were set to 10 mg/mL with methanol (for dry weight). Methanol solutions of gallic acid and ascorbic acid were prepared at concentrations of 10 µg/mL for HPTLC–DPPH and 100 µg/mL for the microplate-based DPPH assay.

2.3 HPTLC–DPPH

The HPTLC–DPPH method was implemented on HPTLC silica gel 60 F_{254} 10 × 10 cm glass-backed plates (art. no. 5629, Merck, Darmstadt, Germany). The mushroom extracts were applied on the HPTLC layer by an automatic TLC sampler (ATS 3, CAMAG, Muttenz, Switzerland) with the following parameters: a 5.0 mm band length, a 4.0 mm distance between tracks, and applied volumes of the mushroom extracts of 8, 10, 12, and 14 µL, with an 8.0 and 10 mm distance from the bottom and left/right sides, respectively. The first (application at 7.5 mm) and last (application at 92.5 mm) tracks were used for calibration series of the standards, gallic acid and ascorbic acid. The HPTLC layers were developed with acetonitrile–water–acetic acid (75:25:3, *V/V*) up to 75 mm in a twin-trough chamber (CAMAG) without saturation [21]. After development, the plates were dried with a stream of cold air using a hair dryer and the standards were applied with volumes of 1, 2, 4, 6, 8, 10, and 12 µL and at heights of 8, 18, 28, 38, 48, 58, and 68 mm. Then, the plates were documented under an ultraviolet (UV) lamp (CAMAG) at 254 and 366 nm by a digital camera (DSC-WX350, Sony, Tokyo, Japan). After documentation, the plates were dipped into the 1 mM DPPH solution (19.7 mg of the DPPH was dissolved in 50 mL methanol) and dried quickly with a stream of cold air. Then the antioxidant activity was measured based on scavenging the stable artificial radical molecule (DPPH). The zones with an antioxidant effect appeared as yellow spots on the dark purple background and were documented by the camera in visible light after the plates dried (2 min). Images of the autograms were processed by ImageJ [22] software [National Institutes of Health, Laboratory for Optical and Computational Instrumentation (LOCI), University of Wisconsin, Madison, WI]. Initially, the images were converted to black and white, inverted, and denoised (reduction of the background noise). First, the rectangular selection tool was chosen and a small part of the chromatogram background was marked to provide the median

value that was used for background subtraction (Process/Math/Subtract). Each track was designated as rectangles with the same size. Then, line profile plots (videodensitograms) were generated and the peak areas were determined, including the peaks at the application zones. Developments and treatments were performed in triplicate on separated plates, and these results are expressed as the mean ± standard deviation (mean ± SD). As the results of the HPTLC–DPPH–densitometry were appropriately repeatable for plate by plate, which was also indicated by the low standard deviation values, for quantification, the calibration curves were constructed by plotting averages of the peak areas obtained from the three plates *versus* the amount of gallic acid or ascorbic acid. Quantitative results were expressed as a gallic acid equivalent (GAE, mg of GA/g of extract) or ascorbic acid equivalent (AAE, mg of AA/g of extract).

2.4 Microplate-based DPPH assay

The standards and the mushroom extracts were tested using a microplate-based DPPH assay. Ascorbic and gallic acid (0.1 mg/mL) and the extracts (10 mg/mL) were prepared for twofold serial dilutions. Ascorbic acid or gallic acid or extracts (50 µL) were mixed with 150 µL of methanol and 50 µL of a 1 mM DPPH solution in wells of a 96-well microplate in triplicate. The concentrations in the wells were as follows: 2, 1, 0.5, 0.25, 0.125, 0.063, and 0.031 mg/mL (extracts) and 20, 10, 5, 2.5, 1.25, 0.625, and 0.31 µg/mL (standards). After loading, the microplate was kept at room temperature in the dark for 30 min. After that, the absorbance at 517 nm was measured by a microplate reader (LabSystems Multiskan MS 4.0, Thermo Fischer Scientific, Budapest, Hungary). The capability to scavenge the DPPH radical was calculated using this equation:

$$\text{Radical scavenging activity}\,(\%) = \left(\left(A_{\text{blank}} - A_{\text{sample}}\right)/A_{\text{blank}}\right) \times 100 \quad (1)$$

where A_{blank} is the absorbance of the control (the reaction mixture contains 150 µL of methanol and 50 µL of DPPH solution, plus 50 µL of methanol instead of the mushroom extract) and A_{sample} is the absorbance of the mixture with mushroom extracts or ascorbic/gallic acid. Each sample/standard in the microplate experiments was measured three times (in three microplates), each time in triplicate. The results were reported as the mean ± standard deviation (mean ± SD). The calibration curves (radical scavenging activity against the concentrations of the standards at 5, 2.5, 1.25, 0.625, and 0.31 µg/mL) provided the basis for calculating the amounts of antioxidants in the crude extracts and the half maximal inhibitory concentration (IC_{50}) values of the extracts.

3 Results and discussion

3.1 HPTLC–DPPH–videodensitometry

The HPTLC–DPPH assay was used to detect the separated zones responsible for the antioxidant activity, and the videodensitometric evaluation of the autogram (Fig. 1C) made their quantification possible. HPTLC separation of the antioxidant zones of *C. cylindracea* and *L. duriusculum* extracts was achieved with an acetonitrile–water–acetic acid (75:25:3, *V/V*) mobile phase and a 75 mm development distance. In the first experiment, different volumes of the extracts were applied (4–10 µL, not shown), and 8 µL (80 ng) was found for both extracts as the lowest volume that gave any detectable antioxidant zone(s). It has been reported that DPPH staining is affected by many factors that distort the results. These factors are the mobile and stationary phases, temperature, light, and the elapsed time till the documentation [23]. To avoid false positive results, after the immersion of the chromatoplates into a DPPH solution, the autograms were dried in the air for 2 min and documented immediately at white light (Fig. 1). The UV activity of the bands showing the antioxidant effect can also be significant, since the spots (Fig. 1B) are visible at 366 nm but not at 254 nm.

The HPTLC–UV analysis (Figs. 2A and B) showed that the methanol extracts of *C. cylindracea* (tracks 2–5)

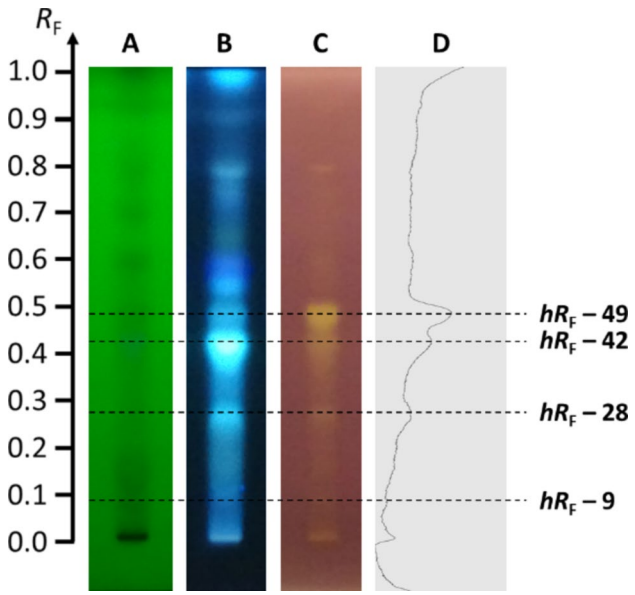

Fig. 1 HPTLC chromatograms of *Cyclocybe cylindracea* (10 mg/mL) extract (14 µL) after development at 254 nm (**A**) and 366 nm (**B**) and after DPPH assay at white light from the bottom (**C**), as well as a videodensitogram (**D**) obtained by image analysis of chromatogram (**C**). The HPTLC silica gel 60 F_{254} plate was developed with acetonitrile–water–acetic acid (75:25:3, *V/V*)

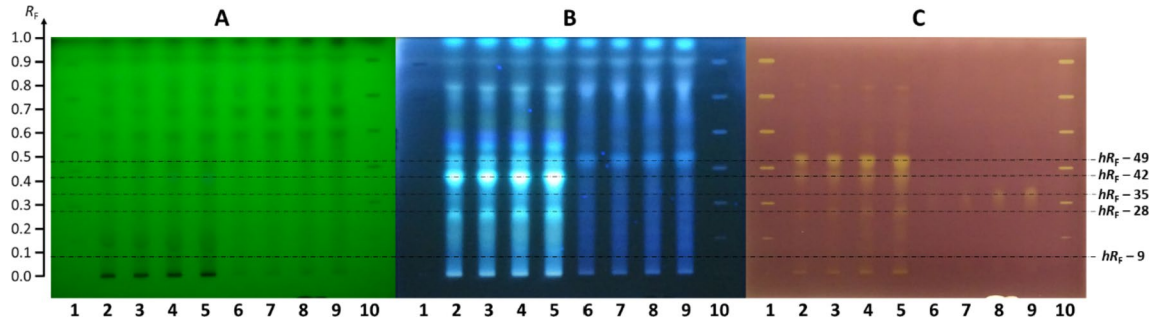

Fig. 2 HPTLC chromatograms of *Cyclocybe cylindracea* (tracks **2–5**; 8, 10, 12, and 14 µL, respectively) and *Leccinum duriusculum* (tracks **6–9**; 8, 10, 12, and 14 µL, respectively) mushroom extracts (10 mg/mL) and the standard series of gallic acid (track **1**; 10, 20, 40, 60, 80, 100, and 120 ng from bottom to top, respectively) and ascorbic acid (track **10**; 10, 20, 40, 60, 80, 100, and 120 ng from bottom to top, respectively) at 254 nm (**A**), at 366 nm (**B**), and at white light after DPPH assay (**C**). HPTLC silica gel 60 F_{254} plate was developed with acetonitrile–water–acetic acid (75:25:3, *V/V*)

and *L. duriusculum* (tracks 6–9) mushrooms have diverse matrices. The HPTLC–DPPH analysis of the extracts of *C. cylindracea* (Fig. 2C, tracks 2–5) revealed three characteristic antioxidant zones: the two main active zones were present at hR_F 42 and 49 and gave, along with the one at hR_F 28, blue fluorescence at 366 nm (Fig. 2B, tracks 2–5). At hR_F 9, a non-UV-active component also displayed weak antioxidant activity. In the *L. duriusculum* extract, only one antioxidant zone at hR_F 38 (Fig. 2C, tracks 6–9) was observed. Based on our previous results, the compound in the *C. cylindracea* extract at hR_F 49 has been determined as a β-carboline alkaloid, the C1-*S* diastereomer of brunnein B, and its antioxidant activity, was also confirmed by the DPPH–HPLC method [21].

The HPTLC–DPPH–videodensitometry enabled the comparison of the antioxidant content in the two different mushroom extracts. In Fig. 3A, the linear calibration curves illustrate the accuracy and reliability of the evaluation. The correlation coefficients (R^2) for both ascorbic acid and gallic acid standards exceeded 0.98. The amounts of the antioxidant components in the two mushroom extracts in AAE and GAE are presented in Table 1.

The amount of antioxidant substances in the *C. cylindracea* extract was significantly higher (on average, 2.5 times higher) than in the *L. duriusculum* extract. The less applied volumes (8–10 µL) resulted in a smaller difference (28%), and the application of more amount increased the differences between the two mushroom extracts (61%). Furthermore, among the antioxidant compounds in the extract of *C. cylindracea*, the C1-*S* diastereomer of brunnein B (hR_F 49, Fig. 2C, tracks 2–5) contributed to the total antioxidant effect with the largest proportion (67.01% ± 8.7%).

3.2 Microplate-based DPPH assay and determination of IC_{50}

The microplate-based DPPH assay was used for measuring the radical scavenging activity and IC_{50} values of the samples and standards (Table 2). The value of the IC_{50} represents the concentration required to obtain a 50% radical scavenging. The standards were used at a lower concentration than in the HPTLC–DPPH assay, since at the concentration of 5 µg/mL ascorbic acid and gallic acid scavenged about 80% of the free radicals. The sigmoid curve of the radical scavenging activity (%) was employed to determine the IC_{50} values (Fig. 4). The IC_{50} values of ascorbic acid (2.6 µg/mL) and gallic acid (1.5 µg/mL) were found to be much lower than that of the *C. cylindracea* (306.2 µg/mL) and *L. duriusculum* (852.8 µg/mL) extracts (Table 2). Several methods have been developed to determine the antioxidant activity, and the DPPH assay is among the most reliable ones [24]. The IC_{50} value of ascorbic acid is typically below 10 µg/mL, but the proportion, concentration, and volume of DPPH solution used in the reaction mixture can influence the measured values [25]. Some researchers pointed out that gallic acid is a stronger antioxidant than ascorbic acid, which is in harmony with our observation [26].

Comparing the two mushroom extracts, at lower concentrations the antioxidant activity of *C. cylindracea* extract was higher; however, the extract of *L. duriusculum* was a bit more effective at the highest concentration used (Table 2), from which a synergistic effect of the compounds or the presence of minor antioxidant components can be inferred. In the latter case, the minor constituents were not detected in the HPTLC–DPPH test (Table 1). The total antioxidant content expressed in terms of ascorbic acid or gallic acid

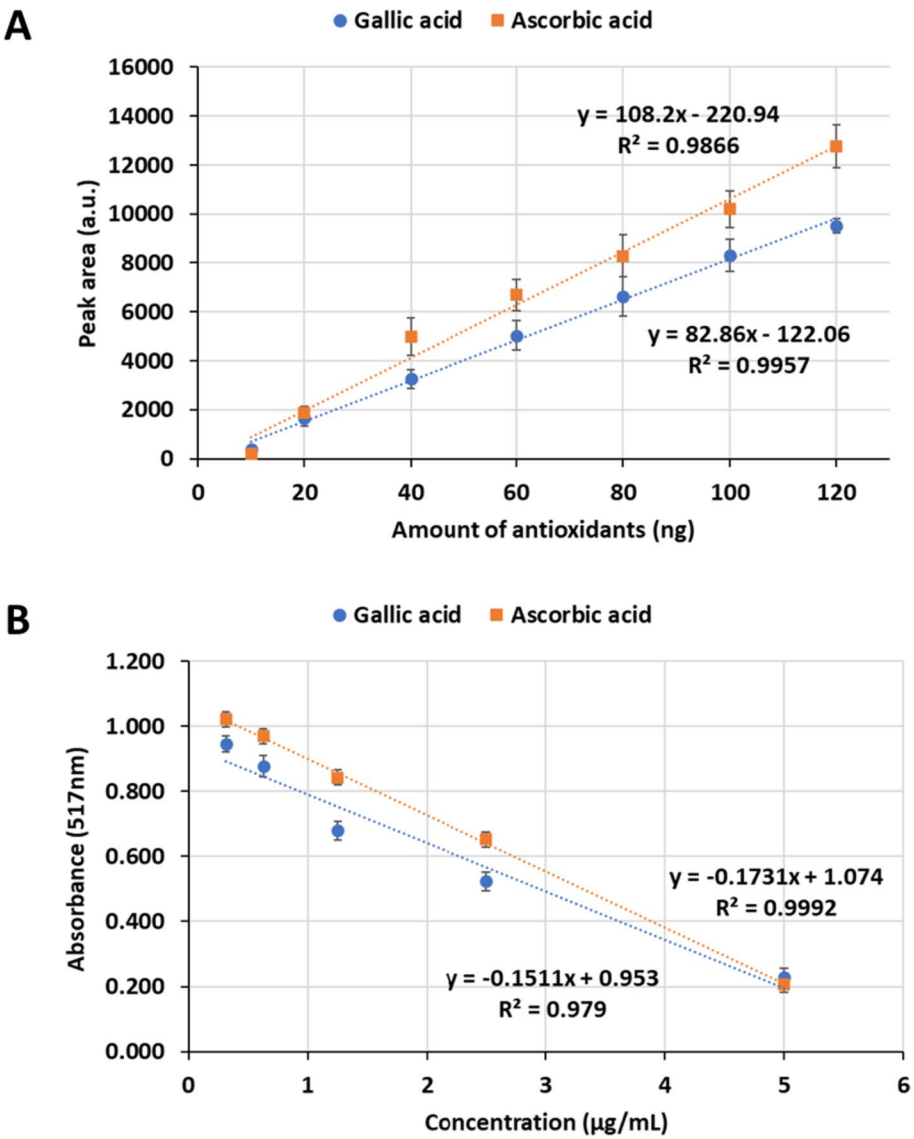

Fig. 3 Calibration curves of ascorbic acid and gallic acid obtained by the HPTLC–DPPH–videodensitometry (**A**) and the microplate-based DPPH (**B**) assay (based on triplicate experiments)

equivalent (Table 3) was calculated with the help of the calibration curves (Fig. 3B).

At the highest concentration (2 mg/mL), *C. cylindracea* and *L. duriusculum* extracts contained similar amounts of antioxidant compounds (4.41 ± 0.006 and 4.63 ± 0.01 µg, respectively). Decreasing the applied concentration (to 0.5 mg/mL and below), the amounts of the antioxidants did not follow the concentration changes (twofold dilution series), which was typical of the extract of *C. cylindracea*. This phenomenon may indicate that the compounds in the extract of *C. cylindracea* exert a synergistic effect on each other at a specific concentration, thereby maintaining a more pronounced antioxidant effect at higher concentrations.

The antioxidant content in the extract of *L. duriusculum* was not measurable at lower concentrations (0.25 mg/mL and above) compared with the gallic acid equivalent. The pattern of the amounts of antioxidant compounds followed the dilutions as ascorbic acid equivalent in this case. This change could also be observed in the case of HPTLC–DPPH, because only a characteristic band is responsible for the antioxidant activity of the extract.

3.3 Comparison of the HPTLC–DPPH and the microplate-based DPPH methods

The simultaneous use of the two methods to measure two different mushroom extracts (*C. cylindracea* and *L.*

Table 1 Amount of the antioxidant components of *Cyclocybe cylindracea* and *Leccinum duriusculum* extracts (10 mg/mL) in ascorbic acid equivalent (AAE) and gallic acid equivalent (GAE), determined by HPTLC–DPPH assay

Cyclocybe cylindracea extract ($n=3$)				
Applied volume (µL)	8	10	12	14
GAE (ng)				
hR_F 9	n.d.*	n.d	7.4 ± 0.9	10.0 ± 0.6
hR_F 28	0.8 ± 0.3	3.5 ± 0.6	4.9 ± 0.1	12.2 ± 0.4
hR_F 42	3.6 ± 0.2	9.3 ± 0.7	37.0 ± 1.4	57.0 ± 1.6
hR_F 49	15.5 ± 1.8	38.9 ± 2.4	72.3 ± 1.0	100.9 ± 2.5
Total	19.9 ± 2.0	51.7 ± 2.0	114.2 ± 2.5	180.1 ± 5.1
AAE (ng)				
hR_F 9	n.d	n.d	10.9 ± 1.2	14.3 ± 0.8
hR_F 28	2.2 ± 0.4	5.8 ± 0.8	7.5 ± 0.1	17.1 ± 0.6
hR_F 42	5.9 ± 0.2	13.4 ± 0.9	49.5 ± 1.9	75.6 ± 2.1
hR_F 49	21.4 ± 2.4	52.0 ± 3.1	95.6 ± 1.3	133.0 ± 3.2
Total	29.5 ± 3.0	71.2 ± 4.8	152.6 ± 3.3	240.0 ± 6.7
Leccinum duriusculum extract ($n=3$)				
Applied volume (µL)	8	10	12	14
GAE (ng)				
hR_F 35	8.9 ± 3	29.0 ± 7.6	44.7 ± 13.9	66.8 ± 4.3
AAE (ng)				
hR_F 35	21.8 ± 3.9	39.1 ± 9.9	59.6 ± 18.2	88.5 ± 5.6

**n.d.* not detected

Table 2 Radical scavenging activity (%) and the IC_{50} values (µg) of the antioxidant standards (ascorbic and gallic acid) and the examined mushroom extracts diluted for different concentrations, determined by microplate-based DPPH assay

Radical scavenging (%, mean ± SD; $n=3$)					
Concentration (mg/mL)	*Cyclocybe cylindracea*	*Leccinum duriusculum*	Concentration (µg/mL)	Ascorbic acid	Gallic acid
2	72.5 ± 0.5	75.8 ± 0.4	20	82.5 ± 0.9	79.9 ± 0.4
1	68.3 ± 0.1	54.4 ± 2.2	10	81.8 ± 0.2	79.8 ± 0.2
0.5	57.6 ± 2.4	24.4 ± 1.9	5	81.7 ± 0.2	79.5 ± 0.6
0.25	32.9 ± 2.5	13.6 ± 1.7	2.5	42.3 ± 1.1	64.8 ± 2.5
0.13	17.5 ± 1.6	7.9 ± 2.1	1.25	25.4 ± 0.3	39.9 ± 0.8
0.06	11.2 ± 1.2	6.2 ± 0.9	0.06	14.2 ± 0.5	22.3 ± 1.1
0.03	7.9 ± 2.4	4.3 ± 0.8	0.03	9.6 ± 1.2	16.3 ± 2.2
IC_{50} value	306.2 µg/mL	852.8 µg/mL	IC_{50} value	2.6 µg/mL	1.5 µg/mL

duriusculum) and standards (ascorbic and gallic acids) proved very useful. Both methods provided the total antioxidant contents of the mushroom extracts (Table 4), which were very similar in the case of *L. duriusculum* and in the same range in *C. cylindracea*. However, if the sample contains more antioxidant components, as in the case of the *C. cylindracea* extract, then HPTLC–DPPH can provide information about the separated zones. In addition, with a microplate-based DPPH assay, the amounts of measurable antioxidants in twofold serial dilutions (both AAE and GAE) did not change linearly (2–0.5 mg/mL). Probably due to the presence of more minor antioxidant compounds (not detected in the HPTLC–DPPH test) and/or synergistic interactions among the components, the

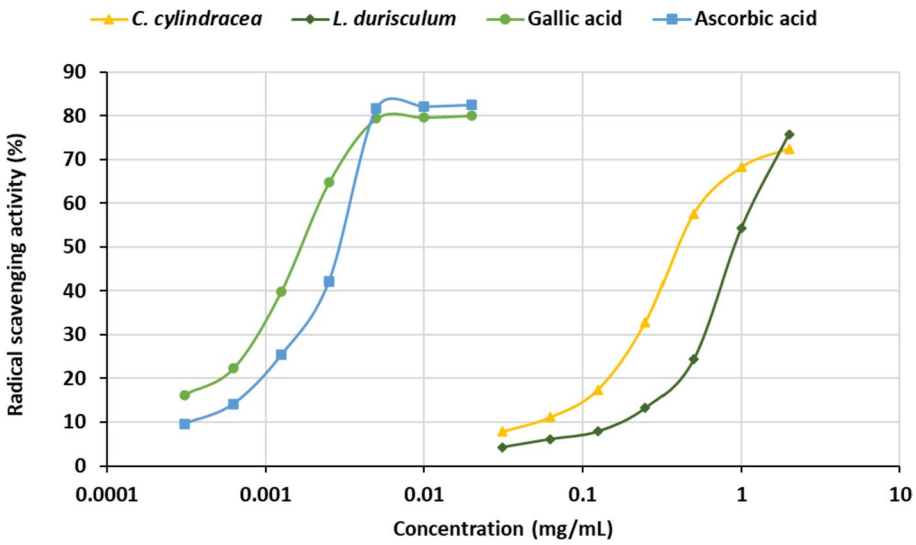

Fig. 4 Determination of the radical scavenging activity (%) of *Cyclocybe cylindracea* and *Leccinum duriusculum* extracts, ascorbic acid, and gallic acid

Table 3 The ascorbic acid (AAE) and gallic acid (GAE) equivalent amounts of antioxidant compounds of different concentrations of the mushroom extracts in the microplate-based DPPH assay

Concentration (µg/mL)	*Cyclocybe cylindracea* extract ($n=3$)		*Leccinum duriusculum* extract ($n=3$)	
	AAE (µg)	GAE (µg)	AAE (µg)	GAE (µg)
2	4.4 ± 0.1	4.3 ± 0.1	4.6 ± 0.1	4.5 ± 0.1
1	4.1 ± 0.1	3.9 ± 0.1	3.2 ± 0.1	2.9 ± 0.2
0.5	3.4 ± 0.3	3.1 ± 0.4	1.3 ± 0.1	0.7 ± 0.1
0.25	1.8 ± 0.2	1.3 ± 0.2	0.5 ± 0.1	n.d
0.125	0.8 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	n.d
0.063	0.4 ± 0.1	n.d.*	0.1 ± 0.0	n.d

**n.d.* not detected

microplate-based DPPH assay gave about 1.25–1.75 times higher total antioxidant content for the *C. cylindracea* extract. A synergistic effect has already been demonstrated by several studies, including the synergistic interactions linked to alpha-tocopherol with other organic compounds (e.g., myricetin [27], ascorbic acid [28], and lutein [29]). Other phenolic acids and terpenes can interact synergistically with ascorbic acid as well as phenolics with each other [30]. Further comparing the applicability of the two methods, only the microplate-based DPPH is eligible for the determination of IC_{50} values of the samples, while only the HPTLC–DPPH can provide information about the components responsible for the effect.

4 Conclusions

This work demonstrated that HPTLC–DPPH–videodensitometry can successfully be utilized to evaluate the antioxidant potential of complex samples such as extracts of the basidiomycetous mushrooms (*Cyclocybe cylindracea* and *Leccinum duriusculum*). The method enabled the determination of the amounts of antioxidant compounds of the crude extracts in standard (as known antioxidants, ascorbic acid, and gallic acid) equivalent units and pointed to the zones responsible for the antioxidant effect. Compared with the HPTLC–DPPH assay, the generally used microplate-based DPPH assay gave similar results for the *L. duriusculum* extract. Based on this knowledge, we suggest the HPTLC–DPPH method for the discovery of mushroom antioxidant components that are in great demand as possible preservatives in the future in the food industry.

Table 4 Ascorbic acid equivalent (AAE) and gallic acid equivalent (GAE) amounts of antioxidant compounds of the *Cyclocybe cylindracea* and *Leccinum duriusculum* extracts, determined by HPTLC–DPPH and microplate-based DPPH methods

	Cyclocybe cylindracea($n=3$)		*Leccinum duriusculum* ($n=3$)	
	AAE (µg/mg)	GAE (µg/mg)	AAE (µg/mg)	GAE (µg/mg)
HPTLC–DPPH	7.1 ± 0.5	5.2 ± 0.4	3.9 ± 0.8	2.9 ± 0.8
Microplate-based DPPH	4.1 ± 0.1	3.9 ± 0.1	3.2 ± 0.1	2.9 ± 0.2

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Declarations

Conflicts of interest The third author, Ágnes M. Móricz is a member of the editorial board of the journal. Therefore, the submission was handled by a different member of the editorial board, and she did not take part in the review process in any capacity.

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