A comparative study of growth, biological efficiency, antioxidant activity and molecular structure in wild and commercially cultivated *Auricularia cornea* strains

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ABSTRACT

Auricularia cornea, jelly mushroom, is a popular ingredient of traditional Chinese cuisine. This study aimed at evaluating the growth, yield, biological efficiency, total phenolic and flavonoid contents, the antioxidant activity, elemental composition, and molecular structure of the wild and domesticated strain Ac24 and the commercially cultivated edible mushroom *A. cornea* strains Ac1, Ac3 and Ac15. Based on the weight of the fresh fruiting bodies of *A. cornea* strains, the maximum yield was obtained from commercial strain Ac1 (237.10 g), followed by Ac3 (224.47 g), Ac15 (158 g) and Ac24 (132.37 g), while the biological efficiency range of *A. cornea* strains was 52.94–94.84%, with significant differences among the *A. cornea* strains. Our results revealed that Ac24 contained the highest phenolic content (20.10 mg GAE · g⁻¹), while the highest flavonoid content was found in Ac1 (35.13 mg CE · g⁻¹). The maximum mineral contents and the strains were as follows: copper (7.2 mg · kg⁻¹) and zinc (310 mg · kg⁻¹) in Ac1, manganese (788 mg · kg⁻¹) in Ac3 and iron (310 mg · kg⁻¹) in Ac24. DPPH assay found maximum antioxidant activity in Ac24 (IC₅₀ 0.233 mg TX · mL⁻¹), FRAP (591 mg TX · g⁻¹) in Ac15, and erythrocyte haemolysis in Ac24. SEM-EDX and FTIR analyses verified the differences among *A. cornea* strains. The results revealed that wild, domesticated *A. cornea* strain Ac24 is a promising dietary source of natural antioxidants and is of high nutritional value, compared to commercially cultivated strains.

Keywords: antioxidant, *Auricularia cornea*, energy-dispersive x-ray, Fourier-transform infrared spectroscopy, scanning electron microscopy

INTRODUCTION

Auricularia cornea belongs to Family Auriculariaceae under Division Basidiomycota. This is an edible fungus with high nutritional value, as its fruiting bodies are abundant in amino acids, polysaccharides, vitamins and minerals (Sheu et al., 2004). *Auricularia cornea* is widely used as a functional food and oriental stimulant, especially in Korea and China (Abd Razak et al., 2013). Due to its significant medicinal value

and high economic returns, the cultivation area of *A. cornea* and *Auricularia heimuer* is continuously increasing, especially in China, with an annual production of about 8 million tons in the past 5 years. A variety of agro-industrial wastes, such as cotton waste, wheat straw and sawdust, can be utilised to cultivate *A. cornea* (Ghorai et al., 2009; Sánchez, 2010; Zhang et al., 2016; Dai et al., 2019).

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Contemporary pharmacological studies have revealed that *A. cornea* has anti-nociceptive functions, reduces blood fat, and has anti-tumour property and aphrodisiac activity (Yang et al., 2002; Arora et al., 2013; Gupta et al., 2018). Moreover, *A. cornea* can also nourish the lungs so that it breathes easily, enriches and circulates blood, and restrains pain and bleeding (Song and Du, 2012). Recently, *A. cornea* was shown to exhibit hypoglycaemic effect by reducing blood glucose levels, modulating glucose tolerance and recovering the serum level in experimental mice (Wang et al., 2019). Based on the massive data reporting its medicinal values, *A. cornea* can be exploited as an antioxidant and antimicrobial agent.

Oxidation is a natural process in which energy is produced by living organisms. Recent studies have revealed that numerous bioactive compounds obtained from biological sources play a crucial role in the prevention of oxidative mutilation in living organisms by free radical-scavenging and might serve as innovative potential antioxidants (Matkowski et al., 2008; Yuan et al., 2008). Similarly, the ethyl acetate and aqueous extracts of *A. cornea* showed high efficiency as antioxidants in scavenging all kinds of radicals (Cai et al., 2015). However, knowledge about the antioxidant potential of Chinese germplasm resources of *A. cornea* is scarce. The present study was designed to evaluate the phenolic and flavonoid contents, antioxidant activity, elemental composition and molecular structure of a wild domesticated and commercially cultivated strains of *A. cornea* from China.

MATERIALS AND METHODS

Mushroom strains and cultivation experiment

Four strains of *A. cornea* were used in this study (Table 1 and Figure 1). Three commercial strains were obtained from the Mushroom Breeding and Genetics Laboratory, the Engineering Research Centre for Edible and Medicinal Fungi, Jilin Agricultural University, Changchun, while one wild strain was collected by Prof. Yao Fangjie from the Dashushan Forest Park, Hefei City, Anhui Province, China, and then domesticated in the Mushroom Breeding and Genetics Laboratory. The multiplication of *A. cornea* strains was performed on potato dextrose agar medium (PDA; 200 g · L⁻¹

chopped potatoes, 12 g · L⁻¹ agar, 20 g · L⁻¹ glucose) at 25–26°C for regular subculture and maintained on PDA slants at 4°C. The sawdust spawn was prepared in 800 mL polypropylene plastic bottles filled with 240 g of sawdust, supplemented with 20% wheat bran, 1% calcium carbonate and 1% gypsum (w/w). The sterilised sawdust mixture was then inoculated with 100 × 15 mm mycelial agar discs and incubated at 25°C until the substrate was fully colonised (Wang et al., 2019).

The pre-wet sawdust substrate was mixed uniformly with wheat bran (20%), CaCO₃ (1%) and CaSO₄ (1%). The water content of the sawdust mixture was adjusted to approximately 55–60%. Each polythene bag (height: 30 cm; diameter: 10 cm) was filled with 0.5 kg of a sawdust-based substrate, sterilised (121°C, 120 min) and then inoculated with 4 g · bag⁻¹ of prepared spawn. The inoculated bags were kept in a spawn running room (25 ± 1°C temperature and 80% relative humidity) under dark conditions. When the mycelium had fully colonised, the bags were shifted to the cropping room (20 ± 2°C; relative humidity: >90%) to stimulate primordia formation. When the fruiting bodies were fully grown, they were then harvested for further analysis with clean hands by the twisting method to show the waveform margin (Liang et al., 2019).

Preparation of methanolic extract

Samples (400 g) of fresh fruiting bodies for each mushroom strain were dried at room temperature, followed by grinding to a fine powder. Twenty grams of the dried material was loaded into a Soxhlet apparatus and extracted in methanol (80%) for 48 h at 30°C. The methanolic extracts were then filtered through Whatman No. 4 paper and concentrated using a rotary evaporator at 36°C to dryness. The dried extracts were re-dissolved in 80% methanol at a concentration of 5 mg · mL⁻¹ and stored in the dark at 4°C for further use.

Total phenolic content

The total phenolic content (TPC) of the mushroom methanolic extracts was measured by the colourimetric assay (Chowdhury et al., 2015). In brief, 0.5 mL mushroom sample extract was mixed with 0.5 mL phenol reagent (Folin and Ciocalteu). After 5 min, 0.5 mL of sodium carbonate (14%) was added to the mixture and adjusted to 5 mL with sterilised distilled water. The

Table 1. List of *Auricularia cornea* strains used in this study.

Original reference	Strain name	Origin	Colour	Strain type
Ac1	WMAC001	Mushroom Breeding and Genetics Laboratory, Jilin Agricultural University, Changchun, P. R. China	White	Commercial
Ac15	WMAC012	Mushroom Breeding and Genetics Laboratory, Jilin Agricultural University, Changchun, P. R. China	White	Cross breeding
Ac3	JAUACP064	Mushroom Breeding and Genetics Laboratory, Jilin Agricultural University, Changchun, P. R. China	Pink	Cross breeding
Ac24	JAUACP001	Dashushan Forest Park, Hefei City, Anhui Province, P. R. China	Brown	Wild domesticated

Figure 1. Fruiting bodies of strains of *Auricularia cornea*: (A) Ac1, (B) Ac3, (C), Ac15 and (D) Ac24.

final solution was mixed properly, kept in the dark for 90 min for colour development and the absorbance was read spectrophotometrically at 750 nm. Gallic acid was used as the standard, and the TPC was calculated as milligrams of gallic acid equivalents (GAE) per gram of the sample.

Total flavonoid content

The total flavonoid content (TFC) was estimated by the AlCl_3 method (Zhang et al., 2015). Generally, 250 μL of the methanolic extract of the mushrooms was mixed with 1.25 mL sterilised distilled water, followed by addition of 75 μL NaNO_2 . After 5 min of reaction, 150 μL of 10% AlCl_3 was added into the solution and 0.5 mL of 1 M NaOH was added after some time. The total volume was adjusted up to 2.5 mL with sterilised distilled water, the solution was mixed well and the intensity of pink colour (at 510 nm) was measured using a spectrophotometer. Catechin was used as the standard compound, and the TFC was stated as milligrams of catechin equivalents (CE) per gram of sample.

Elemental composition

Elemental composition of the powdered samples of the fruiting body of the *A. cornea* strains was determined by using scanning electron microscopy–energy-dispersive x-ray (SEM-EDX) analysis (Liu et al., 2015). The dried mushroom powder sample was placed on the copper stub with the carbon tape and, then, a sputter coater (Hitachi E-1010) was used to coat the samples with gold for 20 s at 20 mA. Afterwards, the mushroom samples were analysed by SEM (Hitachi S-4800 (Japan) and EDX (Genesis-2000; Ametek, USA) at 8 mm working distance with 03 KV and $\times 1.0$ K magnification.

Antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay of mushroom samples was performed according to a procedure described by Teoh et al. (2018). Accordingly, 200 μL mushroom methanolic extract sample and control (methanol) were mixed with 2 mL DPPH (0.2 mm) solution. After 30 min incubation in the dark at 30°C, the absorbance was measured at 517 nm using a 96-well microplate reader. The antioxidant activity was

expressed as inhibition percentage ($I\%$) and calculated by using the following equation:

$$I\% = [(A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}}] \times 100$$

DPPH inhibition (50% inhibitory concentration) represents the IC_{50} value. It was calculated by constructing a curve between sample concentration versus inhibition percentage, and the results were presented as milligrams of sample per millilitre.

The 50% inhibitory concentration (half-maximal inhibitory concentration or IC_{50}) was the parameter used to compare the radical-scavenging activity. A lower IC_{50} means better radical scavenging activity.

Ferric-reducing antioxidant power assay method for determining antioxidant capacity

The ferric-reducing antioxidant power (FRAP) assay was used, according to Teoh et al. (2018). In brief, the methanol extract of the sample (100 μL) of mushroom and distilled water (blank) were combined with 100 μL of 0.2 M phosphate buffer (6.6 pH) and 100 μL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 30 min, and 10% trichloroacetic acid solution was added after the mixture had cooled to room temperature. Afterwards, 125 μL was transferred to 96-well plates from the upper surface and 20 μL freshly prepared ferric chloride (0.1%) was added. Finally, the absorbance value was measured at 620 nm. Trolox was used as a standard, and the FRAP activity was expressed as milligrams of Trolox equivalents (TE) per gram of sample.

Erythrocyte haemolysis analysis

The erythrocyte haemolysis for *A. cornea* mushroom strains was assessed with certain modifications through the method of Liao et al. (2014) and Zhao et al. (2015). Briefly, erythrocytes were collected from the blood of healthy adult male volunteers aged 24–30 years and body weight 64–71 kg. Blood from the healthy male volunteers was collected by venipuncture in citrate-containing tubes. The obtained blood was centrifuged (1,800 rpm, 10 min and 4°C) and washed with phosphate-buffered saline (PBS) (20 mM and pH 7.4) thrice; then, 20% erythrocyte suspension was made with the same buffer

solution. A 300 µL-aliquot of the 20% erythrocyte suspension was thoroughly mixed with an equal volume of PBS (absorbance A) or diosmetin (absorbance B) at various concentrations. The resultant solution was stored for 20 min at 37°C with continuous shaking at 60 rpm, and 600 µL of 2,2'-azobis (2-amidinopropane) di-hydrochloride (AAPH) was added and stored for 2 h at the same temperature. The reaction solution was diluted with 12 mL PBS and centrifuged (1,800 rpm, 10 min and 4°C) before measuring the absorbance at 540 nm. To obtain complete haemolysis, 8 mL of sterilised water was supplemented to the mixture, which was then centrifuged at 1,200 rpm for 10 min at 4°C, and the absorbance of the supernatant was measured at 540 nm. The percentage of haemolysis inhibition was calculated as follows:

$$\% \text{ Haemolysis inhibition} = (1 - A/B) \times 100\%$$

Mineral quantification

Minerals present in the *A. cornea* mushroom strains were quantified according to the methods used by Khan et al. (2019). The fruiting body samples were oven-dried at 35°C for 24h and made powder to pass through 1 mm sieve. To quantify mineral content in samples, 1 g mushroom from each sample was weighed. Afterwards, using the "wet" method mixture of nitric and perchloric acids (3:1) for digestion. At the end, solution was used for quantification of Zn, Cu, Mn, and Fe by atomic absorption spectrometer.

Molecular structure

The molecular spectra of the fruiting bodies from various strains of *A. cornea* were obtained using Fourier-transform infrared (FTIR) spectroscopy (Vertex-70; Bruker). Ground (1 mg) samples were mixed with 100 mg potassium bromide powder and pressed into tablet-shaped pellets under pressure, and the spectra were recorded at a frequency range of 500–4,000 cm⁻¹ (Idrees et al., 2019).

Statistical analysis

The data were analysed using the well-known statistical method: Fisher's analysis of variance (ANOVA), and the treatment means were compared by using the least significant difference (LSD) test at the 5% probability level.

RESULTS AND DISCUSSION

Cultivation experiment

Significant differences were noticed among the strains of *A. cornea* based on mycelium growth (days), pinhead's initiation (days), completion of mycelium growth (days), completion of formation of fruit bodies (days), yield, dry matter contents and biological efficiency (BE) (Table 2). The maximum mycelium growth per day was recorded for Ac3 (2.09 mm), followed by Ac1 (1.98 mm), Ac24 (1.96 mm) and Ac15 (1.24 mm). For complete mycelium colonisation of the substrate, a minimum number of days was noticed for Ac3 and Ac24 (50 days), followed by Ac1 (51 days) and Ac15 (75 days). Primordia formation was observed at the second week after the completion of colonisation of the mycelium bags. In general, the shortest time for completion of formation of fruit bodies was observed for wild strain Ac24 (91 days), followed by Ac3 (99 days), Ac1 (101 days) and Ac15 (115 days).

Yield, dry matter content and BE

Based on the weight of the fresh fruiting bodies of the *A. cornea* strains, the maximum yield was obtained from the commercial strain Ac1 (237.10 g), followed by Ac3 (224.47 g), Ac15 (158 g) and Ac24 (132.37 g), as shown in Table 2. According to our observations, the dry matter contents of the freshly harvested fruiting bodies of the *A. cornea* strains ranged from 11.56% to 18.15%. The Ac15 and Ac24 strains possessed the maximum dry matter content (18.15% and 17.42%, respectively), while Ac1 and Ac3 exhibited the minimum dry matter content (11.56% and 12.60%, respectively). The BE is the ratio of the weight of the fresh fruiting body per dry weight of substrate, expressed as a percentage. Hence, the BE range of *A. cornea* strains was 52.94–94.84%, with significant differences among the *A. cornea* strains (Table 2) (Wu et al., 2017).

Estimation of phenolic and flavonoid contents

The TPC in the fruiting bodies of *A. cornea* ranged from 8.76 to 20.10 mg GAE · g⁻¹ of fruiting body (Figure 2). The wild *A. cornea* strain Ac24 presented the maximum TPC (20.10 mg GAE · g⁻¹), followed by commercial Ac3 (17.23 mg GAE · g⁻¹) and Ac15 (12.53 mg GAE · g⁻¹), while minimum TPC was observed in commercial

Table 2. Mycelium growth per day, pinhead initiation days, number of days for completion of formation of mycelium and fruiting bodies, yield, dry matter and biological efficiency of *Auricularia cornea* strains.

Strains	Mycelium growth per day (mm)	Completion of mycelium formation (days)	Pinhead initiation (days)	Completion of formation of fruiting bodies (days)	Yield (g)	Dry matter contents (%)	Biological efficiency (%)
Ac1	1.98 ± 0.09 b	51 ± 2 b	11 ± 1 b	101 ± 3 b	237.10 ± 28 a	11.56 ± 0.91 c	94.84 ± 11 a
Ac15	1.24 ± 0.07 d	75 ± 3 a	10 ± 1 c	115 ± 2 a	158.00 ± 03 b	18.15 ± 2.00 a	63.20 ± 1 b
Ac3	2.09 ± 0.04 a	50 ± 1 c	14 ± 1 a	99 ± 3 b	224.47 ± 10 a	12.60 ± 0.22 bc	89.78 ± 4 a
Ac24	1.96 ± 0.06 c	50 ± 2 c	11 ± 1 b	91 ± 3 c	132.37 ± 34 b	17.42 ± 4.03 ab	52.94 ± 14 b

Values with no letter in common in each column are significantly different ($p < 0.05$) (means ± SD, $n = 3$).

Ac1 (8.76 mg GAE). The TFC of *A. cornea* strains ranged from 10.37 to 35.13 mg CE · g⁻¹ (Figure 2). The highest TFC was observed in the commercial strain Ac1 (35.13 mg CE · g⁻¹), followed by wild Ac24 (29.04 mg CE · g⁻¹) and the commercial strain Ac15 (25.14 mg CE · g⁻¹). The lowest TFC was found in commercial *A. cornea* strain Ac3 (10.37 mg CE · g⁻¹).

The TPC of *A. cornea* extracts was estimated by the Folin–Ciocalteu method, and gallic acid was used as the standard compound; the results are expressed as milligrams per gram GAE. Mushrooms contain a variety of secondary metabolites, including various kinds of phenolic compounds that have been shown to act as antioxidants. The identification and evaluation of the phenolic compounds in mushrooms are of great importance, both in their nutritional as well as functional characterisation (Ghahremani-Majd and Dashti, 2015). Phenolics are secondary metabolites commonly found in plants and fungi, reported to exert multiple biological effects, including medicinal and antioxidant activities (Dimitrios, 2006; Kim et al., 2008). Our results of the phenolic contents are consistent with the findings of Teoh et al. (2018), who observed quantities of phenolic substances in *A. polytricha* by the Folin–Ciocalteu method (Teoh et al., 2018).

Estimation of elemental composition

The EDX spectra revealed the presence of carbon, nitrogen, iron, zinc, sodium, magnesium, phosphorus, potassium and calcium in *A. cornea* strains (Figure 3). A higher level of nitrogen, iron and phosphorus, as well as a minimum level of calcium, was present in wild strain

Ac24 (13.40%, 2.58%, 1.27% and 0.16%, respectively). In comparison, the commercial strain Ac3 contained a maximum level of sodium (0.04%) and calcium (0.78%) and a minimum level of carbon (50.99%). In addition, Ac1 strain contained high level of carbon (59%) and magnesium (0.22%), while another commercial strain Ac15 contained the maximum percentage of zinc (0.46%) and potassium (2.30%) and a minimum level of phosphorous (0.38%) and nitrogen (2.77%) (Table 3).

The elemental compositions of *Lentinula edodes* and *Pleurotus cornucopiae* var. *citrinopileatus* mushrooms were studied by Owaid et al. in different studies by SEM–EDX (Owaid et al., 2017, 2019), and they reported the presence of potassium, calcium, phosphorous and magnesium, with higher levels of oxygen and carbon. Our SEM–EDX findings of the elemental composition of various strains of *A. cornea* are in accordance with the results of Owaid et al. (2019). Apart from phosphorous, potassium, iron, calcium, zinc and magnesium, we noticed high percentage of oxygen, carbon, and nitrogen in all the strains.

Estimation of mineral contents

Table 4 indicates the mineral contents of the fruiting bodies of different strains of *A. cornea*. The copper and manganese contents of *A. cornea* ranged from 1.2 to 7.2 mg · kg⁻¹ and 371 to 788 mg · kg⁻¹, respectively. The maximum copper content was noticed in the commercial strain Ac1 (7.2 mg · kg⁻¹), followed by Ac3 (4.2 mg · kg⁻¹) and Ac15 (2.4 mg · kg⁻¹), while minimum copper content was observed in

Figure 2. Total flavonoid content (TFC) and total phenolic content (TPC) of the four *Auricularia cornea* strains. dw, dry weight.

the wild strain Ac24 ($1.2 \text{ mg} \cdot \text{kg}^{-1}$). A significant difference was observed in the manganese content of the *A. cornea* strains; the maximum manganese content was obtained from the commercial strain Ac3 ($788 \text{ mg} \cdot \text{kg}^{-1}$), followed by Ac1 ($486 \text{ mg} \cdot \text{kg}^{-1}$) and Ac15 ($381 \text{ mg} \cdot \text{kg}^{-1}$), while the lowest was noticed in the wild strain Ac24 ($371 \text{ mg} \cdot \text{kg}^{-1}$). Among the various strains of *A. cornea*, the highest zinc contents were obtained from the commercial strain Ac1 ($310 \text{ mg} \cdot \text{kg}^{-1}$), followed by Ac3 ($291 \text{ mg} \cdot \text{kg}^{-1}$),

while the highest iron contents were noticed in the wild strain Ac24 ($310 \text{ mg} \cdot \text{kg}^{-1}$), followed by the commercial strain Ac15 ($275 \text{ mg} \cdot \text{kg}^{-1}$). On the contrary, the zinc contents were the lowest in the commercial strain Ac15 ($291 \text{ mg} \cdot \text{kg}^{-1}$), while the iron contents were the lowest in the commercial strain Ac3 ($77 \text{ mg} \cdot \text{kg}^{-1}$).

In general, zinc and iron contents were higher in *A. cornea*; mushrooms are good natural accumulators of zinc and iron, which are biologically very important to the human body. Our results are in line with the findings

Figure 3. EDX spectra and SEM images of the fruiting bodies of various strains of *Auricularia cornea* (A) Ac1, (B) Ac15, (C), Ac3 and (D) Ac24. EDX, energy-dispersive x-ray; SEM, scanning electron microscopy.

Table 3. Average relative values (% weight) for the elemental composition of *Auricularia cornea* strains.

Element (%)	Ac1	Ac15	Ac3	Ac24
C	$59.00 \pm 0.864 \text{ a}$	$54.62 \pm 0.453 \text{ c}$	$50.99 \pm 0.601 \text{ d}$	$57.42 \pm 0.148 \text{ b}$
N	$5.21 \pm 0.209 \text{ b}$	$2.77 \pm 0.125 \text{ d}$	$3.55 \pm 0.229 \text{ c}$	$13.40 \pm 0.075 \text{ a}$
O	$30.23 \pm 0.864 \text{ c}$	$36.89 \pm 0.453 \text{ b}$	$38.96 \pm 0.601 \text{ a}$	$22.82 \pm 0.148 \text{ d}$
Fe	$2.27 \pm 0.249 \text{ ab}$	$2.05 \pm 0.033 \text{ b}$	$2.05 \pm 0.123 \text{ b}$	$2.51 \pm 0.082 \text{ a}$
Zn	$0.18 \pm 0.012 \text{ c}$	$0.46 \pm 0.056 \text{ a}$	0.00	$0.36 \pm 0.056 \text{ b}$
Na	0.00	0.00	0.04 ± 0.008	0.00
Mg	$0.22 \pm 0.012 \text{ a}$	$0.18 \pm 0.008 \text{ b}$	$0.13 \pm 0.021 \text{ c}$	$0.09 \pm 0.012 \text{ d}$
Al	0.00	0.00	$0.24 \pm 0.026 \text{ a}$	$0.05 \pm 0.005 \text{ b}$
P	$0.77 \pm 0.017 \text{ c}$	$0.38 \pm 0.021 \text{ d}$	$0.97 \pm 0.017 \text{ b}$	$1.27 \pm 0.012 \text{ a}$
K	$1.63 \pm 0.125 \text{ c}$	$2.30 \pm 0.079 \text{ a}$	$2.28 \pm 0.059 \text{ a}$	$1.92 \pm 0.012 \text{ b}$
Ca	$0.49 \pm 0.125 \text{ b}$	$0.36 \pm 0.079 \text{ c}$	$0.78 \pm 0.059 \text{ a}$	$0.16 \pm 0.012 \text{ d}$

Values with no letter in common in each column are significantly different ($p < 0.05$) (means $\pm$ SD, $n = 3$).

Table 4. Mineral contents of wild and cultivated *Auricularia cornea* strains.

Strains	Copper ($\text{mg} \cdot \text{kg}^{-1}$)	Manganese ($\text{mg} \cdot \text{kg}^{-1}$)	Zinc ($\text{mg} \cdot \text{kg}^{-1}$)	Iron ($\text{mg} \cdot \text{kg}^{-1}$)
Ac1	$7.2 \pm 0.1 \text{ a}$	$486 \pm 5 \text{ b}$	$310 \pm 13 \text{ a}$	$229 \pm 5 \text{ c}$
Ac15	$2.4 \pm 0.2 \text{ c}$	$380 \pm 7 \text{ c}$	$291 \pm 21 \text{ c}$	$275 \pm 16 \text{ b}$
Ac3	$4.2 \pm 0.3 \text{ b}$	$788 \pm 19 \text{ a}$	$215 \pm 12 \text{ ab}$	$77 \pm 3 \text{ d}$
Ac24	$1.2 \pm 0.2 \text{ d}$	$371 \pm 25 \text{ c}$	$261 \pm 12 \text{ b}$	$310 \pm 6 \text{ a}$

Values with no letter in common in each column are significantly different ($p < 0.05$) (means $\pm$ SD, $n = 3$).

Figure 4. Assays of the extracts of the four strains of *A. cornea*: (A) DPPH radical-scavenging activity; (B) ferric-reducing antioxidant power assay; and (C) erythrocyte haemolysis assay. DPPH, 2,2-diphenyl-1-picrylhydrazyl; IC₅₀, 50% inhibitory concentration.

of Wang et al. (2019), who studied the mineral contents in different *A. cornea* strains (Wang et al., 2019).

Antioxidant activity of *A. cornea*

The antioxidant activity of wild and commercial *A. cornea* strains was determined using the DPPH assay, as shown in Figure 4A. Significant variations were observed among the different strains studied.

The antioxidant activity, represented by the DPPH-radical scavenging activity, of the *A. cornea* strains are presented as the IC₅₀ in Figure 4A. The maximum DPPH radical-scavenging inhibition was noticed for the wild strain Ac24 (IC₅₀ = 0.2333 mg · mL⁻¹), followed by the commercial strains Ac3 (IC₅₀ = 0.8133 mg · mL⁻¹), Ac15 (IC₅₀ = 1.0867 mg · mL⁻¹) and Ac1 (IC₅₀ = 1.3733 mg · mL⁻¹). The capacity of Trolox (IC₅₀ = 0.0367 mg · mL⁻¹) to scavenge DPPH radical was significantly higher than all the *A. cornea* strains.

DPPH radical is extensively used to evaluate the free radical-scavenging activity of natural products. The mechanism of scavenging DPPH radicals is that the natural compounds can transfer an electron or a hydrogen atom to DPPH. Based on this phenomenon, the DPPH radical-scavenging effect of *A. cornea* strains was determined. Our results agree with the studies of Teoh et al. (2018), who noticed that the radical-scavenging activity of the ethyl acetate extract of *A. polytricha* was better probably due to the higher phenolic contents (Teoh et al., 2018).

The antioxidant capacity of the wild and commercial strains of *A. cornea*, estimated by the FRAP assay, are presented in Figure 4B. The FRAP values of the free fraction in the four *A. cornea* strains ranged from 266 to 591 mg TE · g⁻¹. The commercial strain Ac15 and the wild strain Ac24 showed the maximum antioxidant activity, while the commercial strains Ac3 and Ac1 exhibited a lower antioxidant activity in the FRAP assay.

The FRAP assay was used to evaluate the capacity of the antioxidant compound to reduce ferric ions (Fe³⁺) to ferrous ions (Fe²⁺) by electron transfer. An enhancement in the absorbance showed an increase in antioxidant activity. Similar results were obtained by Teoh et al. (2018), showing that *A. polytricha* has the ability to reduce ferric ions (Teoh et al., 2018).

Erythrocyte haemolysis assay

The oxidation-reducing potential of *A. cornea* strains, i.e. usefulness as an antioxidant, was further evaluated by the erythrocyte haemolysis assays (Figure 4C). All the wild and commercial strains of *A. cornea* attenuated the erythrocyte haemolysis induced by AAPH (Figure 4C). The maximum inhibition was observed in the wild strain Ac 24 (85%), followed by the commercial strains Ac 15 (83%), Ac 3 (77%) and Ac 1 (74%).

In erythrocyte haemolysis, alkyl radicals used as initiators are generated through decomposition by AAPH. Peroxyl radicals are produced in the presence of oxygen because of alkyl radicals, which leads to lipid peroxidation and the loss of membrane integrity, eventually leading to haemolysis. Plasma oxidation is a crucial factor that causes many life-threatening diseases. Oxidation of both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) may lead to atherosclerosis and hypercholesterolemia, respectively. Copper induces the formation of dienes, which may increase over time, and it is known as a hallmark of lipid peroxidation (Liu et al., 2019). Therefore, haemolysis inhibition is an indirect way to attenuate the antioxidant ability of *A. cornea*.

FTIR spectra of *A. cornea* strains

FTIR spectra were obtained to investigate the functional groups of the active components of *A. cornea* mushroom strains, as well as to evaluate their bioactivity and structural characteristics. As shown in Figure 5, *A. cornea* strains revealed variation in the spectral region of the biochemical elements, i.e. carbohydrates, proteins and fatty acids. The FTIR spectra revealed the inclusive nutritional profile of the four strains of *A. cornea* mushroom (4,000–500 cm⁻¹). The following important broad bands were identified in the *A. cornea* strains; commercial strain Ac1 contained 3,400 cm⁻¹ (O–H and C–H), 2,300 cm⁻¹ (C–H), and 1,630 cm⁻¹ (proteins); commercial strain Ac15 showed 3,400 cm⁻¹ (O–H and C–H), 3,263 cm⁻¹ (C–H), 2,855 cm⁻¹ (CH₃–CH₂ lipids), 1,401 cm⁻¹ (polysaccharides), 1,303 cm⁻¹ (O–H bending polysaccharides, amide III), 1,025 cm⁻¹ (C–O bond, β (1→3) glucan, cell wall, polysaccharide), 803 cm⁻¹; commercial strain Ac3 showed 3,400 cm⁻¹

Figure 5. FTIR spectra of wild and cultivated strains of *Auricularia cornea* mushroom. FTIR, Fourier-transform infrared.

(O–H and C–H), $1,578\text{ cm}^{-1}$ (amide II, chitosan), 900 cm^{-1} (α - and β -glycosides); and wild strain Ac24 contained $3,400\text{ cm}^{-1}$ (O–H and C–H), $3,174\text{ cm}^{-1}$ (C–H), $2,850\text{ cm}^{-1}$ ($\text{CH}_3\text{--CH}_2$, lipids), $1,375\text{ cm}^{-1}$ (β -glucan), $1,402\text{ cm}^{-1}$ (polysaccharides), 800 cm^{-1} (α - and β -glycosides).

The occurrence of different functional groups of various compounds was detected by FTIR spectroscopy. Mushrooms are a hidden treasure of proteins, carbohydrates, as well as macro- and microelements, with minimum fat contents. Compositional analysis based on the functional group was well-elaborated by the peaks and spectra created by FTIR (Khan et al., 2019). Some early studies outlined the chemical characteristics for *Agaricus bisporus* and various strains of *Pleurotus eryngii* using peaks and functional groups detected by FTIR spectra (Idrees et al., 2019; Khan et al., 2019). One of the objectives of this study was to evaluate the differences between various strains of *A. cornea* mushroom, as FTIR is a non-destructive, quick and phase-interval method that is highly sensitive to dissimilarities occurring in the molecular structure and identifies a wide range of functional groups (Muhammad et al., 2019).

CONCLUSIONS

The current study showed that wild and commercial cultivated strains of *A. cornea* mushroom are a promising source of complete phenolic, flavonoid, FRAP, and erythrocyte haemolysis. The SEM–EDX and FTIR analyses verified the differences among the *A. cornea*

strains based on different peaks at different positions. The present investigation on the methanolic extracts of *A. cornea* strains revealed the nutritional as well as therapeutic potential of *A. cornea*. In general, the wild domesticated brown strain Ac24, as a new germplasm of *A. cornea*, showed respectable potential for further development and investigation as a commercial strain, as well as to add genetic diversity in the commercial cultivation of mushroom.

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AUTHOR CONTRIBUTIONS

Conceptualisation was done by F.Y. and Y.M.Z.; methodology was by L.L. and M.F.; A.A.K. and W.J. worked on software and statistics; writing and preparation of the original draft was by A.A.K.; F.Y. and M.I. reviewed and edited the paper. P.W. helped in data collection. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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