European Journal of Nutrition & Food Safety

11(4): 175-186, 2019; Article no.EJNFS.2019.024
ISSN: 2347-5641

Proximate Compositions and Bioactive Compounds of Cultivated and Wild *Auricularia auricular* from Northeastern China

Hong Shan[1,2], Xiang dong Sun[1,2*], Huan Chun Yang[1,2], Fang Yuan Liu[1,2], Bing Wang[1,2], Li Chai[1,2] and Jun Luan[1,2]

[1]Quality and Safety Institute of Agricultural Products, Heilongjiang Academy of Agricultural Sciences, Harbin-150086, China.
[2]Laboratory of Quality and Safety Risk Assessment for Agro-products (Harbin), Ministry of Agriculture, Harbin-150086, China.

Authors' contributions

This work was carried out in collaboration among all authors. Author HS designed the study and performed the statistical analysis. Author XDS wrote the protocol and wrote the first draft of the manuscript. Authors HCY, FYL, BW, LC and JL conducted experiment and managed the analyses of the study. Author XDS managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJNFS/2019/v11i430160
Editor(s):
(1) Dr. Johnson Akinwumi Adejuyitan, Senior Lecturer, Department of Food Science and Engineering, Ladoke Akintola University of Technology (LAUTECH), Nigeria.
Reviewers:
(1) Rajaa Seghiri, Ibn Tofail University, Morocco.
(2) Tajini Reda, National School of Mineral Industry, Morocco.
Complete Peer review History: http://www.sdiarticle4.com/review-history/53594

Original Research Article

Received 20 October 2019
Accepted 26 December 2019
Published 31 December 2019

ABSTRACT

Auricularia auricular is a traditional Chinese edible and medicinal fungus containing several bioactive compounds that have been proven to possess various healing effects. Around 98% of the global yield is provided by China and Heilongjiang province produces more than 52% of the total yield for the country. Meanwhile, *Auricularia auricular* harvested in this province is very famous for its superior quality. However, chemical compositions and bioactive compounds of *Auricularia auricular* grown in this region have not yet been investigated.

Proximate compositions and bioactive compounds in 36 cultivated and wild *Auricularia auricular* samples collected from Northeastern China were compared and analyzed. The average contents

**Corresponding author: Email: xdsun65@yahoo.com, samson65@qq.com;*

of protein, fat, fiber, ash, moisture, polysaccharide, total saccharide, and total flavonoid were determined to be 11.42%, 1.46%, 5.45%, 3.85%, 13.30%, 38.91%, 47.37%, and 2.18 mg 100 g^{-1}, respectively. Amino acid compositions and nutritive element contents were also detected. Results showed that no significant difference exists for protein, fat, fiber, ash, moisture, polysaccharide, and total saccharide contents between wild and cultivated *A. auricular* samples. While wild *A. auricular* samples contained over three times more content than cultivated samples. Some varieties with high calcium and iron contents were found in this study. White *A. auricular* was determined to have a very high fiber content of 16.39%.
This study reveals proximate compositions and bioactive compounds of cultivated and wild *Auricularia auricular* from Northeastern China and people will benefit from our results due to the special nutrition and bioactive compounds of *Auricularia auricular* harvested in this region.

Keywords: Auricularia auricular; proximate composition; total flavonoid; polysaccharide.

1. INTRODUCTION

Auricularia auricular is a traditional Chinese edible and medicinal fungus containing several bioactive compounds that have been proven to help in lowering blood pressure, reducing blood vessel fat, inhibiting bacterial quorum sensing, as well as detoxification [1-4]. Nowadays, this fungus is believed to possess many healthy functions such as antitumour [5], anti-virus [2], anticoagulant [6], anti-inflammatory [7], and immuno-enhancing [3] characteristics. As a result, *A. auricular* has become increasingly popular in China. In 2014, the annual yield of *A. auricular* in China reached 3.4 million tons and accounted for 98% of the global yield. A large percentage of this fungus was exported to Japan, the USA, Russia, Southeastern Asian countries, and Europe. Among the regions where this fungus is cultivated, Heilongjiang province, located in northeastern China, produces more than 52% of the total yield for the country. This is due to its adequate climate conditions and abundant sawdust. The *A. auricular* produced in Heilongjiang is famous for its high quality as the shortage of basswood causes less and less wild *A. auricular* to be found in the forest. Cultivated *A. auricular* on basswood is also very rare and as such almost all *A. auricular* sold in the markets are cultivated in saw dust. Wild and cultivated *A. auricular* is shown in Fig. 1. The fungus grows on basswood and sawdust, as well as in growing fields and natural drying areas. Although some studies [8-15] have detected the proximate composition and bioactive compound contents in *A. auricular* cultivated in the northeast of China, small sample sizes and limited testing index [16-17] have resulted in an incomplete understanding of this fungi. In addition, modern food processing technologies enable researchers to extract bioactive substances such as polysaccharide, total flavonoid, and black pigment from *A. auricular* [18-22], and these bioactive compounds may be utilized as healthy food or biological medicine ingredients. These are all depending on chemical composition and biological compound contents of *A. auricular* materials. The proximate composition and bioactive compound contents of *A. auricular* cultivated in Heilongjiang province have not yet been widely detected and elucidated until now. Consequently, it is necessary to conduct a comprehensive study to investigate the proximate composition and bioactive compounds of *A. auricular* grown in the Northeastern China and compare the difference in nutritional composition between cultivated and wild *A. auricular*.

The objective of this study is to investigate the proximate compositions and bioactive compounds of cultivated and wild *A. auricular* from Heilongjiang, China and to compare the difference in nutritional properties between cultivated and wild *A. auricular.*

2. MATERIALS AND METHODS

2.1 Materials

A. auricular samples were purchased from farmers' markets, supermarkets, and farmers' warehouses. All samples were naturally dried under sunlight near the fungi's growing area (Fig. 1), ground using a traditional Chinese medicine grinder (Model DFY-500, 500 g Swing Type Chinese Medicine Pulverizer, Wenling Linda Machinery Limited Company, Zhejiang, China) to fine flour, sieved through a 60-mesh screen, sealed in plastic bags, and stored in a freezer for the upcoming analysis. All chemicals used were of analytical grade.

2.2 Analysis for Proximate Composition

Dried *A. auricular* samples were analyzed for moisture, protein, fat, ash, fiber, and total

saccharide contents. Moisture content was determined by drying the samples in a forced air oven at 105ºC for 3 h. Protein content was determined using the Kjeldahl method with an N to protein conversion factor of 6.25. Crude fat content was detected using Soxhlet's extraction method by extraction through petroleum ether. Ash content was determined using the residue on ignition method in accordance with the Chinese National Standard GB/T 5009.4-2016 [23] by using a furnace (Labotery LX 1811, Tianjin Labotery Instrument & Equipment Co., Ltd.) heated to 550ºC overnight (about 16 h). Fiber content was determined using an Auto Fiber Analysis System (Foss Fibertec^{TM} 2010, Denmark) in accordance with the Chinese National Standard GB/T 6434-2006 [24].

2.3 Amino Acids Determination

Amino acid compositions of the fungi samples were quantified using a Hitachi L-8900 fully automatic amino acid analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan) which used ion-exchange chromatography to separate amino acids in accordance with the Chinese National Standard GB/T 5009.124-2003 [25] with some modifications. In brief, samples were hydrolyzed in 6 mol L^{-1} HCl solution with drops of phenol for 24 h at 110ºC after 10 min of nitrogen blowing. Then, 1 mL hydrolysate was centrifuged at 6000 × g for 5 min and 200 µL of supernatant was evaporated via nitrogen blowing at 50ºC. The residue was dissolved in 1.5 mL of 0.2 mol L^{-1} HCl solution and passed through a 0.45 µm membrane filter. 20 µL of the hydrolysates were injected using an auto-sampler. Amino acids were determined and quantified at 570 nm for primary amines and at 440 nm for proline after a post-column reaction with ninhydrin at 135ºC. Standard amino acids were mixed with standard taurine and analyzed prior to sampling. The system was calibrated with a Hitachi amino acid standard mixture to which l-2,4-diaminobutyric acid (Dab) had been added. The amino acids were identified and quantified through comparison of peak profiles of the fungi samples with standard amino acid profiles.

2.4 Minerals Determination

Minerals were determined using the method developed by Chen et al. [26] with slight modifications. In brief, 0.25 g of *A. auricular* powder was accurately weighed into a polytetrafluoroethylene microwave digestion inner tank along with 5.0 mL of concentrated nitric acid. The tank was allowed to rest for 30 min, after which 2.0 mL of H_2O_2 was added to the tank. The tank was again set aside for several minutes. Finally, the inner covers and protective covers were installed and the tank was placed into an airtight microwave digestion workstation (High pressure airtight microwave chemical workstation (Mars X System, CEM, Mathews, NC, USA). Microwave digestion was conducted according to the procedures described by Chen et al. [26] After digestion, the microwave system was cooled down naturally and the digested solution was transferred into a 50 mL sample bottle by repeatedly rinsing the tank with ultrapure water (Human up 900 Ultrapure Water Treatment System) until the bottle contained 50 mL of solution. Then, the bottle was shaken to ensure the solution was homogenous. Minerals were detected using an ICP-MS (iCAP Q, Thermo Scientific, USA). The detection procedures and instrument parameters were adapted from Chen et al. [26]. The reagent blank and national standard material tests were also performed at the same time.

2.5 Polysaccharide Determination

Polysaccharide was detected in accordance with the Chinese Agricultural Standard NY/T 1676-2008 [27] with some modifications. In brief, 0.5 g of *A. auricular* ground samples was weighed with an accuracy of 0.001 g into a 50 mL screw cap centrifuge tube along with 5 mL of distilled water to soak the samples. Then, 20 mL of absolute alcohol was added slowly. Then, the tube was shaken for approximately 1 minute using a scroll oscillator. The samples were then treated using ultrasonic extraction 30 minutes, and then centrifuged at 4000 rpm for 10 minutes. The supernatants were discarded and the undissolved substances were washed using 10 mL 80% ethanol. The samples were centrifuged again at 4000 rpm for 10 minutes and the supernatants were discarded again. The undissolved substances were suspended in 50 mL of distilled water and transferred into grinding mouth Erlenmeyer flasks along with 15 mL of concentrated hydrochloric acid. The flask was shaken and air condensers were installed. Then, the flask was placed into a boiling water bath for 2 hours. The suspensions were cooled to room temperature through natural cooling and the pH value was adjusted to neutral. The suspension was filtered through filter paper, and the filtrate was transferred into a 100 mL volumetric flask. The residues were washed with distilled water and filtered again. This procedure was repeated

2-3 times. Distilled water was added to the volumetric flask until the volume reached 100 mL to form the sample solution.

5.0 mL of Fehling's A solution and 5.0 mL of Fehling's B solution were transferred into a 100 mL beaker and shaken well. The beaker was placed on a hot plate and the Fehling's solution mixture was heated to boiling. Then the mixture was titrated using a 1 mg/mL glucose solution until the titrand turned yellow. The volume of glucose solution was recorded as variable A. Then, the same Fehling's solution mixture was prepared and titrated using the sample solution until the titrand turned yellow. The volume of sample solution consumed was recorded to calculate polysaccharide content.

The polysaccharide content was calculated using the following equation:

$$\text{Polysaccharide}(\%) = A \times V_1 \times 100/m \times V_2 \times 1000 \quad (1)$$

Where, A: Fehling's solution (half of Fehling's A and half of Fehling's B solution) equal to the mass of glucose, mg

- 100 : conversion factor
- V_1 : constant volume, 100 mL
- M : weight of sample, g
- V_2 : volume of the determined sample solution consumed, mL
- 1000 : conversion factor

2.6 Total Saccharide Determination

Total saccharide was detected in accordance with the Chinese National Standard GB/T 15672-2009 [28] with some modifications. In brief, 0.5 g of *A. auricular* ground samples were weighed into grinding mouth Erlenmeyer flasks, accurate to 0.001 g. 50 mL of distilled waters were added into the flasks, shaken well, and then 15 mL of concentrated hydrochloric acids were added and shaken well again. Air condensers were installed to the flasks and the flasks were put into boiling water baths to react for 2 h. The suspensions in the flasks were cooled down to room temperature naturally. Then the pH values of the suspensions were adjusted to neutral, followed by filtration through filter papers. The filtrates were transferred into 100 mL volumetric flasks; the residues were washed with distilled water and filtrated through filter papers. The above washing and filtering procedure was repeated for 2-3 times. The filtrates were merged into the former filtrates in the flasks and distilled waters were added to the volumetric flasks to constant volume. These are sample solutions to be determined later.

As described in 2.5, A value was obtained by titration of the Fehling's solution mixture using 1 mg/mL glucose solution. Then the Fehling's solution mixture was titrated using sample solution instead of glucose solution, and the volumes of sample solutions were recorded for calculating total saccharide content. The total saccharide content was calculated also using equation (1) as described in 2.5.

2.7 Total Flavonoid Determination

Total flavonoid was determined using the method developed by Zhang et al. [29] with some modifications. In brief, 5.00 g of *A. auricular* samples was accurately weighed into a 250 mL Erlenmeyer flask along with 50 mL of 95% ethanol. The flask was shaken at 100 rpm, 70ºC for 30 min using a shaking water bath. The supernatants were centrifuged at 12,000 rpm, 4ºC for 25 min. Then, the resulting residues were extracted with 50 mL of 95% ethanol and were centrifuged again. The procedure was performed three times to extract three supernatants, which were merged and heated to reduce the volume to 15 mL. The resulting concentrate was transferred to a 25 mL volumetric flask. The rutin standard curve was prepared by adding 0.1, 0.5, 1.0, 2.0, and 3.0 mL of 0.20 g L^{-1} rutin standard solution into five individual 25 mL volumetric flasks along with 10 mL of absolute ethanol and 0.5 mL of 10% $Al(NO_3)_3$ each. The solutions were mixed, and distilled water was added until all flasks contained 25 mL of solution. The flasks were allowed to rest for 20 minutes, then absorbance was detected at 415 nm using a spectrometer (Double-beam Ultraviolet Visible Spectrophotometer, TU-1901, Beijing Puxi General Instrument Co., Ltd.). The regression equation and coefficient of the standard curve were calculated for upcoming use. Then, 0.5 mL of 10% $Al(NO_3)_3$ solution was added into the sample concentrate, and 95% ethanol was added until the sample concentrate reached a volume of 25 mL. The sample concentrate was allowed to rest for 20 minutes, then absorbance was detected at 415 nm. Total flavonoid content was calculated using linear regression.

2.8 Statistical Analysis

Replicate data on proximate composition, amino acid, mineral composition, total flavonoid, as well

Fig. 1. Wild and cultivated *A. auricular*. a. wild *A. auricular*; b. *A. auricular* cultivated on basswood logs; c. white *A. auricular*; d. *A. auricular* cultivated on saw dust; e. *A. auricular* growing field; f. *A. auricular* natural drying area

as polysaccharide contents were treated with a GraphPad Prism 5 software (GraphPad Software Inc. La Jolla, CA, USA). One-way analysis of variance (ANOVA) was employed to analyze significant differences among fungi samples using Tukey's test with a minimum significance at 5% level ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Proximate Compositions and Bioactive Compounds

Different varieties of *A. auricular* collected from various sources were detected for proximate compositions and bioactive compounds, and the results are shown in Table 1. As seen in the table, the protein contents of *A. auricular* varies between 8.08% and 13.22% with a mean value of 11.42%, the fat contents varies between 0.8% and 2.5% with a mean value of 1.46%, the crude fiber content varies between 3.43% and 7.43% with a mean value of 5.45%, the ash content varies between 2.36% and 5.27% with a mean value of 3.85%, the moisture contents varies between 10.61% and 16.34% with a mean value of 13.30%, the polysaccharide contents varies between 31.68% and 38.91% with a mean value of 47.8%, the total saccharide contents varies between 31.68% and 38.91% with a mean value of 47.8%, and the total flavonoid contents varies between 0.98 mg 100 g^{-1} and 6.93 mg 100 g^{-1} with a mean value of 2.18 mg 100 g^{-1}. It was observed that No. 34 is a special variety of white *A. auricular*; its fiber content is much higher than that of the traditional black *A. auricular*. Therefore, its fiber content value has not been included in the statistical calculation. In general, our results for protein, fat, and fiber contents are consistent with the results of Wu [8], Yuan et al. [9], and Zhang et al. [10]. However, our results of protein and fat content differ from the results of Lin & Wu [11] who determined the protein, fat, fiber, ash, moisture, and total saccharide content

for wild *A. auricular* to be 23.20%, 12.21%, 5.19%, 6.34%, 9.29%, and 43.77% and for cultivated *A. auricular* to be 20.12%, 10.61%, 10.40%, 8.26%, 9.90%, and 40.71%, respectively. Great differences were observed for protein, fat, ash, and moisture contents between our results and their data. This may be caused by the investigation of different varieties of *A. Auricular*. The accuracy of determination might also be an important reason. After careful consideration and discussion we believe our results are more reliable in comparison with their higher values.

For the determination of polysaccharide, total saccharide, and total flavonoid contents, there seem to be some problems with the detection methods. In most of the literature published in Chinese, polysaccharide in *A. auricular* is detected in accordance with the Chinese Agricultural Standard NY/T 1676-2008 [26] in which the Phenol Sulfuric Acid Colorimetry Method is applied. Due to low solubility of polysaccharide in *A. auricular*, the polysaccharide content in *A. auricular* was usually detected at unreasonably low values.

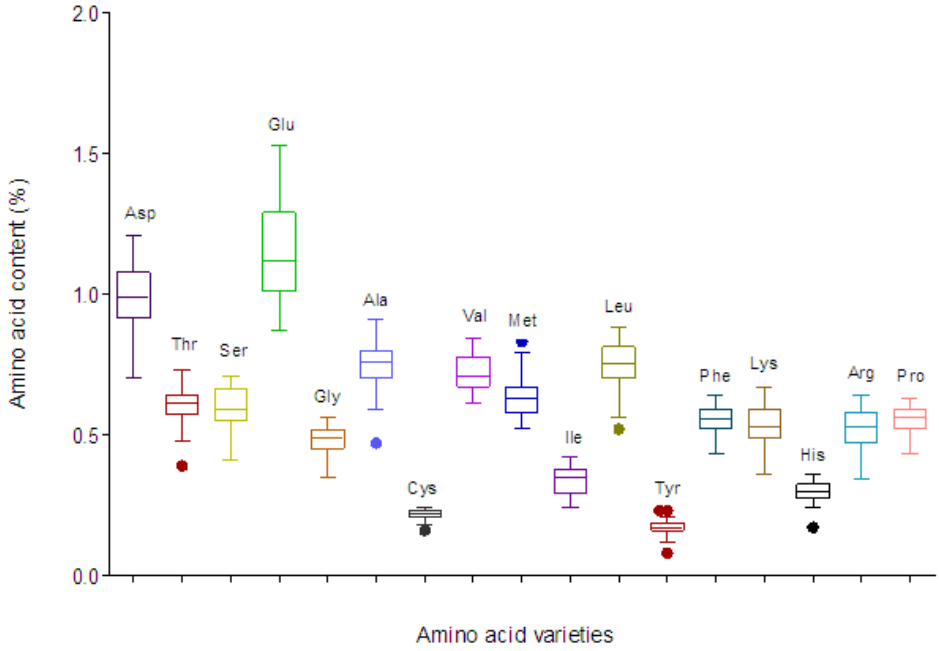

Fig. 2. Amino acid contents of *A. auricular*

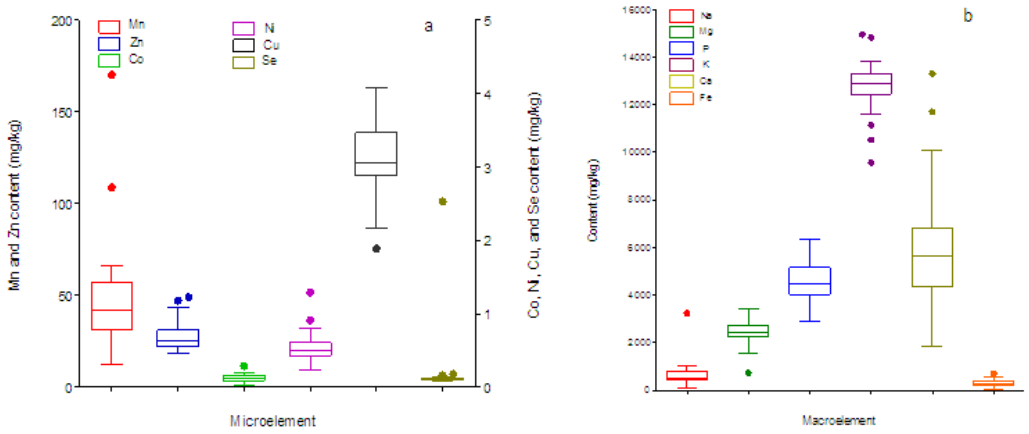

Fig. 3. Content of different microelements and microelements in *A. auricular*

These papers include Zhu [12] who reported a polysaccharide content of 2.575% in *A. auricular*, Yin et al. [13] who reported a polysaccharide content of 4.304% in basswood *A. auricular*, Ni et al. [14] who reported polysaccharide contents of 9.21% and 8.20% in wild and cultivated *A. auricular* samples respectively, Gao et al. [29] who determined polysaccharide concentrations of 4.7% and 5.2% for *A. auricular* from Northern and Southern regions respectively, Feng et al. [30] who detected polysaccharide content at 3.31%-5.95%, and Gao [31] who detected a polysaccharide content range of 13.2-16.8% for 20 *A. auricular* samples collected in Heilongjiang province. Zhang et al. [10] determined polysaccharide contents of 6 *A.auricula-judaes* samples cultivated in the Heilongjiang and Tibet region and obtained data in the range of 13.42-28.90%. All the results for polysaccharide contents in *A. auricular* listed above are lower than our data, and we believe that their low values are a result of insufficient extraction of polysaccharide resulting in a low polysaccharide recovery rate.

In a study, Luo et al. [32] reported polysaccharide contents of 41.5%, 49.22%, and 37.97% for *Auricularia heimuer*, *Auricularia cornea* var. Li., and *Auricularia cornea*, respectively. Their results for polysaccharide content are similar to ours (mean value of 38.91%, maximum 47.8%). In addition, a few studies even detected higher polysaccharide content in comparison to ours. For example, Chen et al. [33] detected polysaccharide content at a very high value of 60.30%. It is unknown why they detected such a great value; two possible reasons are investigation of a high polysaccharide variety or inaccuracies in the determination.

For total saccharides content, we determined a mean value of 47.37% for the 36 samples with maximum value at 58.03%. Our results are in general lower than the results of Yuan et al. [9], Zhu [12], and Zhang et al. [10] who reported a range of total saccharides content of 51.9-54.5%, 67.862%, and 46.7-68.6%, respectively. The difference between their data and our data may be a result of different *A. auricular* varieties. However, Lin & Wu [11] reported the total saccharides contents of 40.71% and 43.77% for cultivated and wild samples, respectively. Dong [34] even reported a total saccharides content range of only 30-35%. Their results are lower than our data (Table 2, 47.25% and 49.41% for cultivated and wild samples, respectively). We highly suspect that the above differences among different studies are induced by extraction efficiency, namely recovery rate.

For total flavonoid content, we determined a mean value of 2.18 mg 100 g^{-1} for the 36 samples with maximum value at 6.93 mg 100 g^{-1}. Our results are consistent with those of Zhang et al. [10] and Zhang et al. [35] who detected the total flavonoid content in the range of 3.19-4.91 mg 100 g^{-1} and 3.43-6.74 mg 100g^{-1}, respectively. However, Zhang et al. [36] reported their result of flavonoid content in *A. auricular* at 56 mg 100 g^{-1}, around 20 times greater than our data. Yuan et al. [9] even determined flavonoid content in a range of 140.4-213.2 mg 100 g^{-1}; their results are much higher in comparison with ours, and we believe there must be something incorrect with their detection or calculation.

Comparison of the proximate compositions and bioactive compounds between wild and cultivated *A. auricular* were conducted in Table 2. As seen in the table, no significant difference was observed for protein, fat, fiber, ash, moisture, polysaccharide, and total saccharide contents between wild and cultivated *A. auricular*. Total flavonoid content was found to be the only significant difference between wild and cultivated *A. auricular*. Total flavonoid content in wild *A. auricular* was over three times greater than those of in cultivated ones; this component might be a potential specific index for differentiation of wild and cultivated *A. auricular*.

3.2 Amino Acid Contents Analysis

Amino acid contents of the 36 *A. auricular* samples collected from various sources were determined and the results are shown in Fig. 2. As seen in the table, there are great differences in amino acid contents between cultivated samples, but there is little difference in amino acid content between the two wild samples. This is likely caused by the many cultivated varieties of *A. auricular*, each with different amino acid contents. We also calculated the mean value of total amino acids content of the 36 samples at 9.85% with maximum value at 12.03%. These results agree with that of Yuan et al. [9].

As seen in Table 3, no significant difference was observed for the 17 amino acids between cultivated and wild *A. auricular* samples, this is reasonable since cultivated *A. auricular* originated from domesticated wild varieties.

Table 1. Proximate compositions and bioactive compounds of *A. auricular* collected from various sources

No.	Sources/varieties	Protein %	Fat %	Fiber %	Ash %	Moisture %	Polysaccharide %	Total saccharide %	Total flavonoids mg/100 g
1	Keshan Farmer's Market	11.35	2.0	7.36	3.69	14.07	41.71	50.76	2.11
2	Keshan Farmer's Market	11.11	2.5	4.51	4.25	12.46	37.25	43.96	1.82
3	Keshan Farmer's Market	11.12	2.0	4.89	3.73	12.64	32.29	45.25	1.73
4	Hada Farmer's Market	11.11	2.5	5.61	3.62	15.69	42.15	49.92	1.90
5	Hada Farmer's Market	10.95	2.3	4.85	3.46	15.57	41.06	49.48	2.07
6	Hada Farmer's Market	11.69	1.5	5.51	3.60	13.48	37.48	44.86	1.25
7	Hada Farmer's Market	11.14	1.0	6.89	3.47	13.27	39.82	44.14	1.46
8	Hada Farmer's Market	11.34	2.0	5.09	3.54	13.36	39.73	45.9	1.77
9	Carrefour Supermarket/Fall *A. auricular*	10.71	1.5	4.96	3.18	11.14	38.40	44.49	1.83
10	Carrefour Supermarket/Fall *A. auricular*	11.88	2.1	4.03	3.82	11.60	40.94	48.6	1.82
11	Carrefour Supermarket/Selenium enriched *A. auricular*	12.11	1.3	ND	4.35	13.16	39.29	45.05	1.11
12	Carrefour Supermarket	11.86	1.0	4.72	4.07	13.05	39.83	43.81	1.75
13	Carrefour Supermarket	11.27	1.2	5.18	4.19	13.03	34.86	45.97	1.82
14	Carrefour Supermarket	10.44	0.9	4.88	3.87	12.87	39.47	44.82	1.26
15	Carrefour Supermarket	10.56	0.8	6.43	3.78	13.53	31.68	49.18	2.75
16	Carrefour Supermarket	11.27	0.9	5.85	3.68	13.41	35.57	49.24	2.71
17	Carrefour Supermarket	10.64	0.9	4.08	3.70	13.67	33.23	42.08	1.43
18	Carrefour Supermarket	11.64	1.7	5.85	3.35	13.69	32.57	46.41	2.18
19	Kangan Farmer's Market	12.10	0.8	4.64	4.16	13.43	36.05	48.00	1.60
20	Kangan Farmer's Market	13.22	2.0	6.75	4.17	11.54	38.15	48.05	2.90
21	Kangan Farmer's Market	12.92	1.1	5.59	4.09	11.27	35.12	48.13	1.85
22	The first crop of *A. auricular* Heifeng No. 3	12.90	1.4	6.83	3.93	14.46	41.26	47.09	2.66
23	The second crop of *A. auricular* Heifeng No. 3	12.25	1.7	4.62	3.85	13.46	39.88	48.68	1.50
24	The third crop of *A. auricular* Heifeng No. 3	12.29	1.7	5.37	3.87	14.21	38.98	48.38	1.43
25	The fourth crop of *A. auricular* Heifeng No. 3	13.16	2.2	4.81	3.69	11.30	41.15	46.46	1.85
26	The first crop of *A. auricular* Chunyu	10.81	0.9	5.85	3.91	13.37	37.83	48.59	1.50
27	The second crop of *A. auricular* Chunyu	9.26	1.5	3.43	3.06	16.34	40.69	46.38	1.80
28	The first crop of *A. auricular* Junfeng No. 28	10.12	1.0	5.11	3.88	13.97	43.20	48.97	2.08
29	The second crop of *A. auricular* Junfeng No. 28	10.62	1.1	6.21	3.89	12.42	42.63	47.82	1.69
30	The first crop of *A. auricular* Heiwei No. 15	12.46	1.3	5.15	5.27	15.29	40.90	46.59	1.35
31	The third crop of *A. auricular* Heishan	9.84	0.9	4.44	3.92	13.77	36.67	46.7	2.57
32	Basswood fall *A. auricular* of Heiwei No. 15	11.87	1.3	5.68	4.83	13.42	40.64	46.54	2.62

No.	Sources/varieties	Protein %	Fat %	Fiber %	Ash %	Moisture %	Polysaccharide %	Total saccharide %	Total flavonoids mg/100 g
33	Basswood spring *A. auricular* of Heiwei No. 15	13.08	1.4	6.75	3.94	10.61	42.87	48.24	3.82
34	White *A. auricular*	8.08	1.0	16.39*	2.36	13.62	47.80	58.03	0.98
35	Wild *A. auricular*	11.58	1.7	5.99	4.07	15.46	38.36	50.16	6.93
36	Wild *A. auricular*	12.25	1.5	7.43	4.50	11.13	41.07	48.66	6.50
	Mean	11.42	1.46	5.45	3.85	13.30	38.91	47.37	2.18
	Maximum	13.22	2.50	7.43	5.27	16.34	47.8	58.03	6.93
	Minimum	8.08	0.80	3.43	2.36	10.61	31.68	42.08	0.98

*All data are mean of duplicate samples. ND, not detected. *Data is not included in the statistical calculation due to extreme value*

Table 2. Comparison of proximate compositions and bioactive compounds of wild and cultivated *A. auricular*

Sources/varieties	Protein %	Fat %	Fiber %	Ash %	Moisture %	Polysaccharide %	Total saccharide %	Total flavonoids mg/100g
Cultivated *A. auricular*	11.38±1.13^{a}	1.45±0.52^{a}	5.37±0.92^{a}	3.83±0.49^{a}	13.30±1.31^{a}	38.86±3.52^{a}	47.25±2.78^{a}	1.91±0.59^{a}
Wild *A. auricular*	11.92±0.47^{a}	1.60±0.14^{a}	6.71±1.02^{a}	4.29±0.30^{a}	13.30±3.06^{a}	39.72±1.92^{a}	49.41±1.06^{a}	6.72±0.30^{b}

$^{a-b}$ *Column values followed by the same superscript letter are not significantly different (P < 0.05)*

Table 3. Comparison of amino acid contents of wild and cultivated *A. auricular*

Sources / varieties	Asp	Thr	Ser	Glu	Gly	Ala	Cys	Val	Met
Cultivated *A. auricular*	0.99±0.12^{a}	0.60±0.07^{a}	0.60±0.07^{a}	1.15±0.18^{a}	0.48±0.06^{a}	0.75±0.09^{a}	0.22±0.02^{a}	0.72±0.06^{a}	0.63±0.07^{a}
Wild *A. auricular*	0.98±0.00^{a}	0.59±0.01^{a}	0.58±0.01^{a}	1.11±0.01^{a}	0.50±0.01^{a}	0.73±0.00^{a}	0.23±0.01^{a}	0.72±0.01^{a}	0.71±0.12^{a}
Sources / varieties	**Ile**	**Leu**	**Tyr**	**Phe**	**Lys**	**His**	**Arg**	**Pro**	
Cultivated *A. auricular*	0.34±0.05^{a}	0.74±0.09^{a}	0.17±0.03^{a}	0.55±0.05^{a}	0.53±0.08^{a}	0.30±0.04^{a}	0.52±0.08^{a}	0.55±0.05^{a}	
Wild *A. auricular*	0.34±0.02^{a}	0.75±0.04^{a}	0.15±0.01^{a}	0.54±0.01^{a}	0.54±0.04^{a}	0.28±0.01^{a}	0.53±0.04^{a}	0.56±0.00^{a}	

Table 4. Comparison of the content of different elements in wild and cultivated *A. auricular*

Sources/varieties	Na mg/kg	Mg mg/kg	P mg/kg	K mg/kg	Ca mg/kg	Mn mg/kg
Cultivated *A. auricular*	600.56±217.95^{b}	2494.24±406.65^{b}	4667.34±809.60^{b}	12693.30±979.75^{a}	5981.85±2482.30^{b}	44.38±26.31^{a}
Wild *A. auricular*	66.76±0.21^{a}	1581.16±1201.30^{a}	3566.34±573.51^{a}	13996.38±1164.10^{a}	4504.69±1580.10^{a}	87.04±30.87^{b}
Sources/varieties	**Fe mg/kg**	**Co mg/kg**	**Ni mg/kg**	**Cu mg/kg**	**Zn mg/kg**	**Se mg/kg**
Cultivated *A. auricular*	289.47±103.05^{a}	0.13±0.05^{a}	0.53±0.19^{a}	3.16±0.74^{a}	28.28±7.78^{a}	0.18±0.42^{a}
Wild *A. auricular*	461.27±166.77^{b}	0.19±0.01^{a}	0.72±0.12^{a}	3.56±0.09^{a}	24.23±7.06^{a}	0.15±0.05^{a}

3.3 Mineral Element Contents Analysis

The 36 *A. auricular* samples were detected for mineral element contents and the results are shown in Fig. 3. It can be seen in the data (not shown in this Figure) that the sodium content of the two wild varieties (No. 35 and 36) greatly differs; one sample reaches 3234.36 mg $100g^{-1}$, while the other one only reaches 66.76 mg $100g^{-1}$. The mean value of the other 34 samples is determined at 600.56 mg $100g^{-1}$. The cause for this high sodium content of 3234.36 mg $100g^{-1}$ in sample No. 35 may be because it is a counterfeit product. The vendor or the cultivator may have added some salt to the sample to increase net weight to make more money, since wild *A. auricular* sells at very high price (See Fig. S1, a mobile vending vehicle with a billboard declares that wild *A. auricular* sells for ¥600 per 500g, around USD $90 $500g^{-1}$) at the local market.

For the magnesium content, it was observed that it varies greatly between the two wild samples. The magnesium content of No. 36 is the lowest at 731.74 mg kg^{-1} among all 36 samples, while the magnesium content of No. 35 at 2430.58 mg kg^{-1} matches the average content of 2494.24 mg kg^{-1}. There are two possible reasons: one is that the two wild samples have different compositional characteristics; the other is that magnesium has been added to sample No. 35. It is difficult to confirm whether the magnesium content in sample No. 35 is original or has been altered post-harvest.

For the contents of phosphorus and potassium, the mean values of the two elements are 4606.17 and 12765.70 mg kg^{-1}, small differences exist among the 36 samples.

For the content of calcium, the average value of the 36 samples is 5899.79 mg kg^{-1}. Sample Nos. 19, 30, and 33 were detected for high calcium content; they all exceeded 10000 mg kg^{-1}. For wild samples, calcium content of No. 35 is a little bit lower at 3387.41 mg kg^{-1}, while No. 36 is close to the mean value at 5621.97 mg kg^{-1}.

For the manganese content, No. 33 has the highest value of 170.31 mg kg^{-1}, whereas the mean value of the other 35 samples is only 43.23 mg kg^{-1}. It is unclear why this sample was detected for such a high content.

For the content of iron, the mean value of the 34 samples is 299.58 mg kg^{-1} with the maximum value at 579.19 mg kg^{-1}, and the minimum value at 129.77 mg kg^{-1}. Iron content of sample No. 30 reaches 700.34 mg kg^{-1}, whereas No. 31 only reaches 47.15 mg kg^{-1}. As such, they were not included in the mean value calculations. *A. auricular* with high iron content is a good food for those with iron deficiency anemia. Consequently, high iron content varieties such as sample No. 30 should be produced in large scale.

Cobalt, nickel, copper, zinc, and selenium are trace elements which have important physiological functions for the human body. Although great differences exist among the samples, the mean values for these samples are very low. It is noticeable that No. 11, a selenium enriched *A. auricular* sample, has a high selenium content of 2.53 mg kg^{-1}, around 23 times greater than the mean value of the other 35 samples.

As internationally acknowledged, 2/3 of the regions in China are selenium deficient, while 1/3 of them are severely selenium deficient [36] Selenium enrichment of *A. auricular* is a good approach to supplement selenium for people with selenium deficiency, since most of the selenium in *A. auricular* exists in organic forms [36].

Mineral element contents of wild and cultivated *A. auricular* were compared in Table 4. As seen in the table, Na, Mg, P, and Ca contents in cultivated *A. auricular* samples were greater than those in wild samples. However, Mn and Fe contents in cultivated samples were smaller than those in wild samples. No significant difference was found for K, Co, Ni, Cu, Zn, and Se contents between wild and cultivated *A. auricular* samples.

4. CONCLUSION

As a major cash crop, *A. auricular* cultivated in Heilongjiang province is famous for its high sensory quality and well welcomed all over the country as well as the world for its healthy characteristics. Proximate compositions and bioactive compounds of 34 major cultivated variety samples and 2 wild *A. auricular* samples were determined. Based on our results, high calcium, high iron, and high polysaccharide, and high total flavonoid varieties have been identified. Total flavonoid content in wild samples was detected to be significantly higher than that of the cultivated ones. This is the only advantage of the wild samples. It is necessary to promote cultivation of these high nutritional content varieties as wild *A. auricular* is not well received due to the poor taste, unappetizing appearance,

scarcity, and high price. Consequently, there is a great prospect for the cultivated *A. auricular* produced in Heilongjiang province, especially those varieties with high contents of nutritional components. Through the development of high quality *A. auricular,* the fungi industry has been accelerating local economic growth.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ma H, Xu X, Feng L. Responses of antioxidant defenses and membrane damage to drought stress in fruit bodies of *Auricularia auricula-judae*. World J Microb Biot. 2014;30:119–124.
2. Nguyen TL, Chen J, Hu YL, Wang DY, Fan YP, Wang JM, Abula S, Zhang J, Qin T, Chen X Y, Chen XL, Khakame SK, Dang BK. In vitro antiviral activity of sulfated *Auricularia auricula* polysaccharide. Carbohydr Polym. 2012;90:1254–1258.
3. Nguyen TL, Wang D, Hu YL, Fan Y, Wang J, Abula S, Guo LW, Zhang J, khakame SK, Dang BK. Immuno-enhancing activity of sulfated *Auricularia auricula* polysaccharide. Carbohydr Polym. 2012; 89:1117–1122.
4. Reza MA, Hossain MA, Lee SJ, Yohannes SB, Damte D, Rhee MH, et al. Dichlormethane extract of the jelly ear mushroom *Auricularia auricular-judae* (higher Basidiomycetes) inhibits tumor cell growth in vitro. Int J Med Mushrooms. 2014;16:37–47.
5. Reza A, Choi MJ, Damte D, Jo WS, Lee SJ, Lee JS, Park SC. Comparative antitumor activity of different solvent fractions from an *Auricularia auricula-judae* ethanol extract in P388D1 and sarcoma 180 cells. Toxicol Res. 2011;27:77–83.
6. Yoon SJ, Yu MA, Pyun YR, Hwang JK, Chu DC, Juneja LR, Mourao PA. The nontoxic mushroom *Auricularia auricula* contains a polysaccharide with anticoagulant activity mediated by antithrombin. Thromb Res. 2003;112:151 158.
7. Damte D, Reza MA, Lee SJ, Jo WS, Park SC. Anti-inflammatory activity of dichloromethane extract of *Auricularia auricula-judae* in RAW264.7 cells. Toxicol Res. 2011;27:11–14.
8. Wu XR. Quality standard and nutritional components of *Auricularia auricular*. Forest By-Product and Speciality China. 1996; 36:21-22 (In Chinese).
9. Yuan WD, Tao X, Wang WK, Lu N, Yu JM, Wang SH, Feng FQ, Zhou ZF, Wang SB, Yan J, Song JL. Effect of different growth substrates on selected nutritional components of *Auricularia auricula* fruit bodies, Acta Edulis Fungi. 2011;18:45-48 (In Chinese).
10. Zhang YL, Mu Y, Li YJ, Wan P, Feng L, Lei H. Research on the effects of nutritional contents and quality structure of *Auricularia auricula-judae* influenced by geographical environment and genotype. Food Sci. 2018;39:233-239. (In Chinese).
11. Lin M, Wu DQ. Comparison of nutritional components of wild and cultivated *Auricularia auricular*. Edible Fungi Supplement. 2003;6-7. (In Chinese).
12. Zhu XQ. Study on the extraction, separation and functional activity of the *Auricularia auricular* polysaccharide. Master thesis. Nanning, Guangxi University (In Chinese); 2014.
13. Yin BB, Zhao S, Liu Y, Geng XL, Xu F, Wang SX, Meng LL. The content of protein and hydrophilic polysaccharide in different kinds of Auriculariain market. Northern Hortic. 2011;23:147-149. (In Chinese).
14. Ni ZL, Shen YY, Bai L, Chen HY, Li RB. Extraction and determination of polysaccharide from Yunlong *Auricularia auricular*. J Dali Univ. 2012;11:31-34. (In Chinese).
15. Guo X, Li ZJ, Liu JY, Zhang Y. Comparison of nutritional factors of various *A. auricular* species in Yichun region. Edible Fungi. 2019;41(3):73-74,78. (In Chinese).
16. Guo X, Liu JY, Li ZJ. Comparative analysis of product quality of Auricularia heimuer in 10 provinces and cities. Prot. Forest Sci. Tech. 2019l182(11):53-54,58. (In Chinese).
17. Zhang P, Xu W, Zhang LG, Bai BW, Ma HY, Yang SJ, Zhu KC. Physicochemical properties of *Auricularia auricula* and extraction and purification of its polysaccharide. Modern Foods. 2019;21: 80-82. (In Chinese).
18. Zeng LX, Zhou JQ, Cui SW, Luo SQ, Gao YJ. Study on the extraction of polysaccharides from *Auricularia auricula* by dynamic ultra-high pressure microjet

method. Food Res. Dev. 2019;40(19):155 159. (In Chinese).

19. Hou RL, Yuan Y, Xiang KK, Wu XP, Liu WX, Zheng MF, Fu JS. Cellulase and ultrasonic wave synergistic extraction technology of melanin from *Auricularia heimuer* and analysis of antioxidant activity of the melanin product. Mycosystema. 2018; 38(3):414-427. (In Chinese).
20. Wang P, Guo L, Jiang Z, guo YL, Ma X, Li Y. Synergistic antioxidation of polysaccharides and flavonoids from *Auricularia auricular* by oxygen-resistant extraction. Sci. Tech. Food Ind. 2018;39(7): 54-58,63. (In Chinese).
21. Zhai YQ. Study on extraction, structure identification and antioxidant activity of flavonoids from *Auricularia auricula*. Master Thesis, Shanxi Normal University, Taiyuan, Shanxi (In Chinese); 2018.
22. Chinese National Standard GB 5009.4-2016. National Food Safety Standard-Ash Detection in Foods. Standards Press of China, Beijing, China (In Chinese); 2016.
23. Chinese National Standard GB/T 6434-2006/ISO 6865: 2000. Feeding stuffs – Determination of Crude Fiber Content – Method with Intermediate Filtration. Standards Press of China, Beijing, China (In Chinese); 2006.
24. Chinese National Standard GB/T 5009.124-2003. Method for determination of amino acids in foods. Standards Press of China, Beijing, China (In Chinese); 2004.
25. Chen GY, Ma YH, Liu F. Study on synchronization determination of 20 kinds of elements in green hay by microwave digestion and ICP-MS. J Jilin Agr Univ. 2007;29(6):673-678. (In Chinese).
26. Chinese Agricultural Standard NY/T 1676-2008. Determination of Crude Mushroom Polysaccharide. Chinese Agricultural Press, Beijing, China (In Chinese); 2008.
27. Chinese National Standard GB/T 15672-2009. Determination of Total Saccharide in Edible Mushroom, China Standard Press, Beijing, China (In Chinese); 2009.
28. Zhang PQ, Kong XH, Liu JN, Zhang JC, Yu DS. Extraction and detection of total flavonoid in *Auricularia auricular*. Edible Fungi. 2010;4:71-72. (In Chinese).
29. Gao GF, Luo JJ, Lu HL, Niu YL, Wei BY Morphological characteristics and nutrient content of black fungus 916 from South and North of China. Hunan Agr Sci. 2014; 21:18-21. (In Chinese).
30. Feng XF, Chen YH, Zhao N. Comparison of the detection of water soluble polysaccharide and protein in *Auricularia auricular* cultivated in different regions. Edible Fungi. 2016;4:57-58. (In Chinese).
31. Gao YF. The differences analysis of Heilongjiang estate *Auricularia* quality and development of instant *Auricularia*. Master thesis, Harbin, Harbin University of Commerce (In Chinese); 2014.
32. Luo JW, Si FL, Gu ZX, Wang LL, Meng XX. Antioxidant and antimicrobial activities of polysaccharide from three species of *Auricularia* genus. Food Sci in press. Available:http://kns.cnki.net/kcms/detail/11.2206.TS.20171013.1103.112.html/Accessed 28 January 2018 (In Chinese).
33. Chen YQ, Zhou LP, Yin YM. Study on extraction techniques of water-soluble polysaccharide from fruit bodies of *Auricularia auricular*. J Jilin Agr Univ. 2003; 25:470-472. (In Chinese).
34. Dong DD. The analysis of nutrients of main mushrooms in Zhejiang province and the quality and structural studies of polysaccharide. Master thesis, Hangzhou, Zhejiang Industrial University (In Chinese); 2012.
35. Zhang HL, Li FS, Ma H, Cui XT, Wang X, Liu D, Zhai H, Xiao ZG. Process of ultrasound-assisted hot water extraction of *Auricularia auricular* polysaccharide and flavonoids. Farm Prod Processing. 2016;9: 1-4,12. (In Chinese).
36. Wu Y. A preliminary study on selenium distribution and existent morphology of selenium enriched *Auricularia auricular*. Edible Fungi. 2008;5:5-6. (In Chinese).

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/53594