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Research Article

Comparative analysis of nutritional and medicinal components of two *Artemisia* plants during different growth periods

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Abstract: *Artemisia argyi* and *Artemisia indica* are often used owing to their advantageous biological effects, such as relieving pain and itching, improving homeostasis, and regulating menstruation. However, research on their nutritional components is lacking. In this study, we evaluated the composition and differences in the primary nutritional and medicinal components of these two plants during three different growth periods. The contents of polysaccharide, total dietary fiber, protein, and volatile metabolites were determined using the phenol–sulfuric acid method, enzymatic gravimetric method, Kjeldahl method, and gas chromatography-mass spectrometry (GC-MS), respectively. The polysaccharide content in the leaves of *A. indica* was significantly higher than that in *A. argyi*, and the leaves of the two plants were rich in total dietary fiber. The contents of nine volatile ingredients exhibited significant differences between the two *Artemisia* plants. Using hydrochloric acid hydrolysis and inductively coupled plasma-mass spectrometry, 17 amino acids and 27 mineral elements, respectively, were identified. In particular, the leaves of *A. argyi* and *A. indica* were rich in amino acids, including seven essential amino acids (EAAs). Of the 27 mineral elements detected in the leaves of *A. argyi* and *A. indica* during the three growth periods, 20, 9, and 17 mineral elements were significantly different ($P < 0.05$) in the leaves at early, middle, and late growth stages of the two plants, respectively. From the perspective of multiple nutrients and volatile ingredients, *A. indica* leaves are suitable additives for food production compared with *A. argyi* leaves. This study provides a scientific basis and data-driven support for the comprehensive development and utilization of *A. argyi* and *A. indica* leaves as edible medicinal resources.

Keywords: *Artemisia argyi*; *Artemisia indica*; medicinal plants; nutrient analysis

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1 Introduction

Artemisia argyi and *Artemisia indica* are two edible medicinal plants belonging to the Asteraceae family. *A. argyi* is primarily found in China, South Korea, Japan, and Mongolia, while *A. indica* is widely distributed from the southern temperate zones to tropical Asia, with abundant wild resources in Guangdong and Guangxi[1,2]. In most other areas, *A. indica* is used as a raw material for food production[3]. As a commonly used traditional Chinese medicine, the leaves of *A. argyi* disperse the cold, relieve pain, warm the meridians, improve hemostasis, regulate qi, and calm fetuses. Made from the leaves of *A. argyi*, moxa floss is the primary material used for clinical moxibustion, and its physiotherapeutic effects are excellent[4,5]. Moreover, *A. indica* dispels wind and swelling, relieves pain and itching, and regulates menstruation and hemostasis. In some regions, *A. indica* is commonly used instead of *A. argyi* in moxibustion therapy[6,7]. Essential oils, flavonoids, phenolic acids, and terpenoids have been identified in *A. argyi*[8-11], and the secondary metabolites in *A. indica* are similar to those in *A. argyi*[7,12]. *A. argyi* and *A. indica* share many similarities in both their traditional applications and modern pharmacological treatments[13].

According to previous reports, *A. argyi* and *A. indica* are rich in proteins, lipids, dietary fiber, phenolic compounds, minerals, vitamin C, and EAAs. Their leaves are widely used as raw materials in traditional foods, such as qingtuan, dumplings,

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glutinous rice cakes, cakes, tea, soup, and porridge (Fig. 1)[14]. However, the harvest times of the two plants for food and medicinal applications differ. For example, the young leaves of *A. argyi* and *A. indica* are typically harvested as raw food materials, whereas their mature leaves are typically harvested as raw medicinal materials. Thus, how the nutritional components of *A. argyi* and *A. indica* change during the growth process from young to mature leaves and the differences in nutritional components between them at the same harvest time should be further investigated.

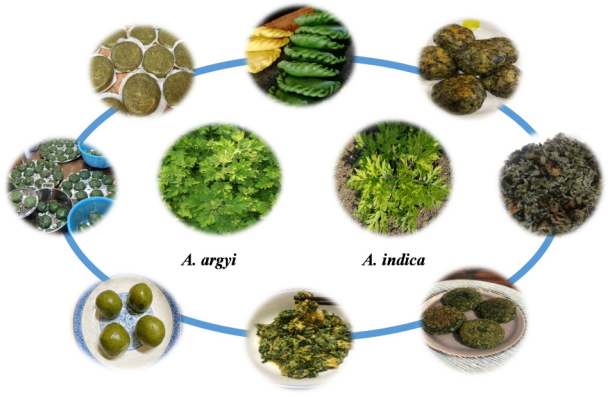

Figure 1 *A. argyi* and *A. indica* are used as raw materials of foods.

Researchers have investigated the chemical components and pharmacological effects of *A. argyi* and *A. indica*[15-22], but there are limited reports on their nutritional components. In particular, a comparative analysis between the nutritional components of the two plants and a dynamic difference analysis of their nutritional components at different developmental stages have not been reported. In this study, the contents of polysaccharides, total dietary fiber, proteins, amino acids, mineral elements, and volatile ingredients in *A. argyi* and *A. indica* were determined during three different growth periods, and the obtained data were analyzed and evaluated using econometric methods. This study provides a scientific basis and data support for the comprehensive development and utilization of edible and medicinal resources of *A. argyi* and *A. indica*.

2 Materials and methods

2.1 Materials

A. argyi and *A. indica* were collected during the growing season of 2020 from an experimental field in Nanyang County (33°03' 6.56"N, 112°49'36.91"E), Henan Province, China. *A. argyi* and *A. indica* leaves were obtained at three different growth stages: the early growth stage (April 6, 2020), middle growth stage (May 16, 2020), and later growth stage (June 26, 2020). They were labeled AAE, AAM, and AAL, respectively, for *A. argyi* and AIE, AIM, and AIL, respectively, for *A. indica*. The impurities were removed, the samples were selected, dried, cut, and mixed from the same batch for further analyses.

2.2 Reagents and instrumentation

The following instruments and reagents were used in this study: ICAP-7200 ICP-AES instrument (Thermo Fisher Scientific, USA), Thermo X series - II ICP-MS instrument (Thermo Fisher Scientific, USA), Mars-5 microwave digestion system (CEM Corporation), K-360 Kjeldahl N_2 determinator (Buchi, Switzerland), UV-1102 ultraviolet spectrophotometer (Shanghai Tianmei Scientific, China), SECAM amino acid analyzer (SYKAM, Germany), desktop high-speed centrifuge (SORVAL, Germany), AR5120 electronic balance (AHOMS, USA), multi-element standard solution (SPEX, USA), and high-purity nitric acid (Merk, Germany). All the solutions were prepared using high-purity deionized water.

2.3 Determination of polysaccharide, total dietary fiber, and protein contents

The polysaccharide content was determined using the phenol–sulfuric acid method. The total dietary fiber content was determined using the enzymatic gravimetric method according to the national standard of the People's Republic of China (GB/5009) and GB 88-2004 National Food Safety Standards. The sample was digested with a sulfuric acid catalyst, and the protein content was determined using the Kjeldahl method.

2.4 Determination of amino acid content

An accurately weighed plant sample (approximately 0.2 g) was placed in a hydrolysis tube, and 10 mL of 6 mol/L hydrochloric acid solution was added. Subsequently, 3–4 drops of phenol were added to the hydrolysis tube, which was placed in the refrigerant, frozen for 3–5 min, and connected to the extraction pipe of a vacuum pump, vacuumed, and filled with N_2. Vacuum pumping was repeated thrice, and the tube was filled with N_2 and sealed under N_2-filled conditions. The sealed hydrolysis tube was placed in an electric blast incubator or a hydrolysis furnace at 110 ± 1 °C, removed after 22 h, and cooled to room temperature. The hydrolysis pipe was opened, and the hydrolysate was filtered through a 0.45 μm aqueous phase filter membrane. Subsequently, 1.0 mL of filtrate was suctioned from the tube, and a tube concentrator or parallel evaporator was used to dry the filtrate under reduced pressure in a heated environment at 40–50 °C. The dried residue was redissolved with 1 mL amino acid diluent and filtered through a 0.22 μm membrane. The filtrate was transferred to an instrument injection bottle as a sample solution for instrument determination, and gradient elution was performed. The experimental conditions were as follows: separation column temperature, 57 °C; reaction column temperature, 130 °C; buffer flow rate, 0.45 mL/min; ninhydrin flow rate, 0.25 mL/min; detection wavelength of channel 1,570 nm; detection wavelength of channel 2,440 nm; and injection volume, 50 μL.

2.5 Determination of mineral element content

An accurately weighed sample (approximately 0.4 g) was added to the inner digestion tank, followed by the addition of 5 mL of HNO_3 and overnight soaking. The inner cover of the tank was positioned, and the stainless-steel jacket was tightened. The tank was placed into a constant temperature drying oven, which was held at 80 °C for 1–2 h, 120 °C for 1–2 h, and then 160 °C for 4 h to dry the acid. The digestion solution was washed with a washing solution in a 25 mL volumetric flask and fixed with 1% HNO_3, and the solution was mixed well and set aside. Simultaneously, a blank reagent test was performed using a computer. The radiofrequency (RF) power was 1,200 W, the atomizer pressure was 1.0 bar, the auxiliary gas (Ar) flow rate was 0.7 L/min, the plasma gas flow rate was 13 L/min, and the peristaltic pump speeds were 30 and 70 r/min for analysis and flushing, respectively. The primary working parameters for ICP-AES were as follows: RF power, 1,150 W; peristaltic pump speed, 50 r/min;

auxiliary air flow, 0.50 L/min; and atomizer gas flow, 0.7 L/min. The contents of 27 mineral elements were determined, including K, Ca, Mg, Al, Fe, Mn, Co, Na, Cu, Sr, Zn, Ba, Ti, Ni, Cr, S, Mo, Cd, Pb, Be, Si, Zr, As, Se, Li, B, and P.

2.6 GC-MS analysis of volatile ingredients

The identification and quantification of volatile compounds were performed using an Agilent Model 7890 BGC and a 7000 D mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The test samples and GC-MS procedures were performed as previously reported[13]. The compounds were identified by comparing the mass spectra with the MWGC database (Metware Biotechnology Co., Ltd., Wuhan, China) and a linear retention index. The relative contents of the compounds were determined via the area normalization method[23].

2.7 Statistical analysis

The data were sorted and summarized using Microsoft Office 2019 software, and the polysaccharide, total dietary fiber, protein, amino acid, and mineral element contents in the different samples were analyzed using a one-way ANOVA and SPSS 19.0 software. Data are presented as the mean ± standard deviation. Additionally, Pearson's correlation analysis was conducted. The comprehensive evaluation score F was calculated using SPSS via the following formula:

$$F = X1 \times F1 + X2 \times F2 + X3 \times F3 + X4 \times F4 + X5 \times F5 \cdots\cdots$$

where $X1$ is the variance contribution rate of the first principal component, $F1$ is the factor score of each principal component in the first principal component, $X2$ is the variance contribution rate of the second principal component, and $F2$ is the factor score of each principal component in the second principal component.

The Shapiro–Wilk test was performed on the data of *A. argyi* and *A. indica* from different growth periods using R version 4.4.1. Spearman's rank correlation analysis was performed using GraphPad Prism version 9.5.0 (GraphPad Software Inc., La Jolla, CA, USA).

3 Results

3.1 Analysis of polysaccharide, total dietary fiber, and protein contents

The polysaccharide content ranged from 22.52 to 32.32 mg/g in the leaves of *A. argyi* and *A. indica* in the three growth stages, with *A. indica* having a significantly higher content than *A. argyi*. The polysaccharide content in AIL exceeded that in AIE, and the polysaccharide content in AAM exceeded that of AAE and AAL (Table 1). The leaves of *A. argyi* and *A. indica* at different growth stages were rich in total dietary fiber, with contents ranging from 48.05% to 62.91%. The total dietary fiber contents in AAM and AIL, respectively, were significantly higher than those in the leaves harvested during other periods, with AIL exhibiting the highest content. The protein content in the leaves of *A. argyi* and *A. indica* at the three growth stages was between 13.17% and 26.61%. The protein contents in AAE and AIE were significantly higher than those in the leaves harvested during the other periods, with AIE exhibiting the highest content.

3.2 Amino acid composition and content analysis

The leaves of *A. argyi* and *A. indica* were rich in amino acids, including seven EAAs. The contents of 17 amino acids, total amino acids (TAAs), EAAs, and non-essential amino acids (NEAAs) in *A. argyi* and *A. indica* are presented in Table 2. The TAA contents of *A. argyi* and *A. indica* in the three growth periods were 31.23–46.03 and 24.84–49.58 mg/g, respectively. Significant differences were observed in the amino acid content of the leaves of *A. argyi* and *A. indica* at different growth stages. For example, the amino acid content in AAE was the highest, whereas that in AIL was the highest. During the growth of *A. argyi*, the amino acid content in its leaves decreased, whereas during the growth of *A. indica*, the amino acid content in its leaves increased. According to the content pattern spectrum and scoring standard for ideal protein EAAs proposed by the World Health Organization, the EAA/TAA and EAA/NEAA contents were 40% and 60%, respectively, and the nutritional value of the protein was high[24]. Table 2 indicates that the EAA/TAA and EAA/NEAA contents in the *A. argyi* leaves at the three growth stages were 31.00%–38.36% and 44.93%–62.23%, respectively. For the *A. indica* leaves at the three growth stages, the EAA/TAA and EAA/NEAA contents were 30.23%–35.14% and 43.34%–54.19%, respectively.

3.3 Mineral element composition and content analysis

Regression analysis was performed by plotting the standard curve using the elemental mass concentration as the abscissa and the ratio of the peak value of the element to be measured to that of the internal standard element as the ordinate. Each of the elements to be analyzed exhibited a strong linear correlatioln, with a linear correlation coefficient $>0.997,2$, indicating that the linear range and quantification limit met the analysis requirements. The results are summarized in Table 3.

Twenty-seven mineral elements were detected in the leaves of *A. argyi* and *A. indica* during the three different growth periods (Table 4). The results of ANOVA and multiple comparisons revealed that the contents of 17 elements, including Li, Be, and B, differed significantly between AAE and AAM ($P < 0.05$). Significant differences were observed in the contents of 17 elements, including Si, Zn, and Al, between AAE and AAL. The contents of 15 elements, including Mg, Fe, and Cd, differed significantly between AAM and AAL. Between AIE and AIM, significant differences were observed in the contents of 16 elements, including Fe, Ba, and K. Significant differences were observed in the contents of 23 elements, including Na, Ca, and Cd, between AIE and AIL, and between AIM and AIL, significant differences were observed in the contents of 25 elements, including As, Mn, and Ti. The levels of Li, Be, Mn, and 20 other

Table 1 Polysaccharide, total dietary fiber and protein contents ($\bar{x} \pm s$, $n = 3$)

Component	AAE	AAM	AAL	AIE	AIM	AIL
Polysaccharide (mg/g)	25.03 ± 0.36c	28.84 ± 0.64b	22.52 ± 0.51c	29.44 ± 0.66ab	31.80 ± 1.40ab	32.32 ± 2.44a
Dietary fiber (g/100g)	50.46 ± 1.17c	58.94 ± 1.03b	51.44 ± 1.90c	48.05 ± 1.49c	49.89 ± 0.92c	62.91 ± 0.39a
Protein (g/100g)	20.75 ± 0.35b	16.47 ± 0.41c	14.82 ± 0.44cd	26.61 ± 1.62a	16.91 ± 1.36c	13.17 ± 1.55d

Note: Different lowercase letters in the same line indicate significant difference ($P < 0.05$), the same notation applies below.

Table 2 Amino acid composition and content ($\bar{x} \pm s$, $n = 3$) (mg/g)

Component	AAE	AAM	AAL	AIE	AIM	AIL
Aspartate	7.27 ± 0.11a	4.30 ± 0.18b	3.39 ± 0.08c	3.01 ± 0.38c	4.79 ± 0.27b	7.19 ± 0.61a
Threonine*	2.04 ± 0.01ab	1.75 ± 0.09bc	1.76 ± 0.04bc	1.44 ± 0.22c	1.85 ± 0.09b	2.26 ± 0.22a
Serine	2.09 ± 0.04b	1.90 ± 0.04bc	1.71 ± 0.02c	1.60 ± 0.16c	1.91 ± 0.09bc	2.62 ± 0.21a
Glutamate	5.44 ± 0.03b	4.24 ± 0.18cd	4.18 ± 0.11cd	3.35 ± 0.43d	4.58 ± 0.21bc	6.79 ± 0.64a
Glycine	2.41 ± 0.01ab	2.13 ± 0.06bc	1.97 ± 0.05c	1.68 ± 0.20d	2.08 ± 0.09c	2.60 ± 0.07a
Alanine	2.62 ± 0.04ab	2.31 ± 0.07b	2.32 ± 0.04b	1.80 ± 0.23c	2.29 ± 0.11bc	3.10 ± 0.35a
Cystine	0.12 ± 0.02b	0.11 ± 0.00b	0.07 ± 0.00c	0.13 ± 0.01b	0.15 ± 0.01b	0.24 ± 0.01a
Valine*	2.20 ± 0.04a	1.49 ± 0.18bc	1.80 ± 0.11ab	1.18 ± 0.25c	1.91 ± 0.11ab	2.27 ± 0.25a
Methionine*	0.16 ± 0.02c	0.32 ± 0.03b	0.17 ± 0.03c	0.20 ± 0.01c	0.17 ± 0.03c	0.48 ± 0.03a
Isoleucine*	1.44 ± 0.13a	0.98 ± 0.13bc	1.31 ± 0.08ab	0.92 ± 0.17c	1.31 ± 0.09ab	1.31 ± 0.18ab
Leucine*	3.27 ± 0.10ab	2.77 ± 0.15b	2.93 ± 0.08b	2.13 ± 0.32c	2.84 ± 0.15b	3.56 ± 0.35a
Tyrosine	1.49 ± 0.04a	1.51 ± 0.04a	1.21 ± 0.05b	1.16 ± 0.13b	1.31 ± 0.07ab	1.37 ± 0.10ab
Phenylalanine*	2.30 ± 0.03a	1.77 ± 0.07b	1.79 ± 0.05b	1.38 ± 0.18c	1.90 ± 0.09b	2.45 ± 0.20a
Histidine	0.90 ± 0.01ab	0.66 ± 0.03c	0.66 ± 0.03c	0.47 ± 0.08d	0.81 ± 0.02bc	0.98 ± 0.08a
Lysine*	2.86 ± 0.03a	2.12 ± 0.15c	2.22 ± 0.12bc	1.48 ± 0.29d	2.44 ± 0.14bc	2.66 ± 0.27ab
Arginine	2.63 ± 0.04a	1.75 ± 0.11b	1.82 ± 0.05b	1.32 ± 0.22c	2.07 ± 0.11b	2.99 ± 0.20a
Proline	6.79 ± 0.26a	2.38 ± 0.16c	1.92 ± 0.05cd	1.59 ± 0.23d	5.78 ± 0.26b	6.71 ± 0.09a
TAA	46.03	32.49	31.23	24.84	38.19	49.58
EAA	14.27	11.20	11.98	8.73	12.42	14.99
NEAA	31.76	21.29	19.25	16.11	25.77	34.59
EAA/TAA	31.00%	34.47%	38.36%	35.14%	32.52%	30.23%
EAA/NEAA	44.93%	52.61%	62.23%	54.19%	48.20%	43.34%

Note: *: EAAs. TAA: The total amount of amino acids. EAA: The total amount of EAAs. NEAA: The total amount of NEAAs.

Table 3 Validation data of analytical procedure

Element	Linear regression	r^2	linear range	detection limit
K	$Y = 69.37X + 4.634$	0.999,6	0-5000,0 μg/L	0.065 μg/L
Ca	$Y = 10.63X - 0.104$	0.998,5	0-5000,0 μg/L	0.042 μg/L
Mg	$Y = 35.63X - 6.665$	0.999,2	0-5000,0 μg/L	0.016 μg/L
Al	$Y = 1405X + 11237$	0.998,6	0-5000,0 μg/L	0.024 μg/L
Fe	$Y = 2336X + 14673$	0.997,8	0-5000,0 μg/L	0.006 μg/L
Mn	$Y = 2754X + 3136$	0.999,4	0-5000,0 μg/L	0.008 μg/L
Co	$Y = 4168X + 630.3$	0.999,6	0-500 μg/L	0.200 μg/L
Na	$Y = 6.72X + 6.153$	0.997,6	0-5000,0 μg/L	0.039 μg/L
Cu	$Y = 856.1X + 1344$	0.999,5	0-500 μg/L	0.562 ng/L
Sr	$Y = 3158X - 747.6$	0.999,3	0-500 μg/L	0.028 ng/L
Zn	$Y = 697.2X + 2740$	0.999,6	0-500 μg/L	12.230 ng/L
Ba	$Y = 370.5X - 70.78$	0.999,6	0-500 μg/L	0.315 ng/L
Ti	$Y = 1405X + 11237$	0.998,7	0-500 μg/L	0.062 ng/L
Ni	$Y = 745.2X + 1311$	0.999,2	0-500 μg/L	0.318 ng/L
Cr	$Y = 3746X + 5377$	0.999,3	0-500 μg/L	0.086 ng/L
S	$Y = 1335X + 6328$	0.998,9	0-5000,0 μg/L	0.035 μg/L
Mo	$Y = 1130X + 792.5$	0.998,7	0-500 μg/L	0.556 ng/L
Cd	$Y = 2114X + 2321$	0.998,6	0-500 μg/L	0.001 ng/L
Pb	$Y = 8062X - 1487$	0.999,5	0-500 μg/L	0.056 ng/L
Be	$Y = 22.63X - 12.85$	0.999,3	0-500 μg/L	0.068 ng/L
Si	$Y = 3108X + 4227$	0.999,1	0-5000,0 μg/L	0.012 μg/L
Zr	$Y = 23026X - 9076.9$	0.998,8	0-500 μg/L	0.105 ng/L
As	$Y = 451.1X + 381.1$	0.999,6	0-500 μg/L	0.212 ng/L
Se	$Y = 63.34X - 14.86$	0.999,7	0-500 μg/L	0.098 ng/L
Li	$Y = 325.6X + 525.2$	0.999,5	0-500 μg/L	0.116 ng/L
B	$Y = 869.5X - 239.5$	0.999,2	0-500 μg/L	0.055 ng/L
P	$Y = 1694X + 9358$	0.997,2	0-5000,0 μg/L	0.022 μg/L

elements differed significantly between AAE and AIE. The contents of Mo, Cd, Pb, and nine other elements differed significantly between AAM and AIM. Significant differences were observed in the contents of 17 elements, including Mg, Sr, and Ni, between AAL and AIL.

3.4 Principal component analysis of *A. argyi* and *A. indica*

Principal component analysis (PCA) uses dimension reduction to fit multiple original variables into a few principal components while retaining as much information as possible. To further explore the relationship between the amino acids and mineral elements in *A. argyi* and *A. indica* during different growth periods, PCA was performed using SPSS 19.0. Two principal components (eigenvalues >1) were used for the 17 amino acids of *A. argyi* and *A. indica* during the different growth periods, and the cumulative contribution rate was 90.10%. This result revealed that the extracted principal component factors comprehensively reflected the amino acid information among the six groups of samples. The factor analysis revealed that for the first principal component, 15 amino acids, including aspartate, threonine, serine, glutamate, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, and proline had high load values, indicating that the first principal component reflected the information of these 15 amino acids, accounting for 78.67% of the variance. Similarly, for the second principal component, cysteine and methionine had high load values, accounting for 11.43% of the variance. The first and second principal components were used to draw a PC1/PC2 scatter diagram (Fig. 2A). The distribution of *A. indica* was relatively scattered in this diagram, and each group of samples was independent, indicating that the

Table 4 Mineral elements of *A. argyi* and *A. indica* ($\bar{x} \pm s$, $n = 3$)

Type	Unit	AAE	AAM	AAL	AIE	AIM	AIL
Li	μg/kg	472.79 ± 54.66c	712.90 ± 67.93b	276.22 ± 19.65d	1365.5 ± 36.41a	815.40 ± 52.83b	432.82 ± 13.39c
Be	μg/kg	8.33 ± 1.12c	12.75 ± 0.59b	4.22 ± 1.17c	25.08 ± 2.71a	15.34 ± 1.26b	8.15 ± 1.83c
B	mg/kg	30.21 ± 3.80c	19.04 ± 2.35d	39.49 ± 1.31b	19.49 ± 2.08d	19.21 ± 2.04d	59.41 ± 3.80a
Ti	mg/kg	39.16 ± 5.19c	40.75 ± 3.39bc	44.16 ± 3.94bc	51.60 ± 0.24ab	39.41 ± 2.60c	60.80 ± 5.84a
Cr	mg/kg	3.44 ± 0.19c	6.30 ± 0.75ab	4.10 ± 0.26bc	3.46 ± 0.94c	4.29 ± 1.18bc	6.70 ± 1.55a
Mn	mg/kg	169.57 ± 18.26d	240.07 ± 20.41c	141.74 ± 2.68d	554.20 ± 38.56a	362.07 ± 23.89b	243.66 ± 10.07c
Co	μg/kg	255.36 ± 13.92d	484.29 ± 39.17b	402.88 ± 38.20c	635.95 ± 6.61a	457.39 ± 36.65b	323.23 ± 29.49cd
Ni	mg/kg	2.72 ± 0.17b	2.68 ± 0.24b	1.61 ± 0.09c	4.04 ± 0.30a	2.78 ± 0.17b	1.88 ± 0.06c
As	μg/kg	254.70 ± 16.10c	315.17 ± 21.36b	161.96 ± 23.68d	419.83 ± 17.03a	317.53 ± 13.12b	242.31 ± 13.69c
Se	μg/kg	113.40 ± 10.33b	167.70 ± 10.97a	37.20 ± 7.34c	150.85 ± 7.92a	166.94 ± 9.34a	108.10 ± 14.29b
Sr	mg/kg	32.72 ± 1.51c	36.32 ± 0.49bc	42.54 ± 2.52b	31.01 ± 2.72c	29.75 ± 4.21c	51.39 ± 2.04a
Zr	μg/kg	148.75 ± 30.40b	227.25 ± 27.07a	39.30 ± 7.14c	275.13 ± 15.47a	223.00 ± 14.49a	119.92 ± 13.04b
Mo	μg/kg	105.37 ± 4.15f	179.00 ± 17.91e	389.68 ± 25.54c	282.36 ± 7.78d	780.22 ± 40.81a	633.51 ± 26.39b
Cd	μg/kg	279.07 ± 9.68a	113.44 ± 13.35c	73.91 ± 4.78d	305.93 ± 24.57a	206.96 ± 16.64b	75.31 ± 7.59cd
Pb	μg/kg	121.55 ± 13.29b	99.01 ± 4.72b	103.12 ± 6.48b	119.62 ± 13.81b	305.86 ± 21.52a	97.16 ± 8.25b
Na	mg/kg	317.00 ± 26.62a	135.30 ± 16.06b	136.91 ± 12.97b	93.91 ± 2.96c	132.05 ± 8.49bc	115.55 ± 12.34bc
Ca	g/kg	12.70 ± 0.67c	14.84 ± 0.04b	14.38 ± 0.60bc	14.15 ± 0.67bc	12.82 ± 0.58bc	19.63 ± 1.27a
K	g/kg	29.53 ± 0.83b	21.51 ± 0.39e	21.75 ± 0.72de	39.40 ± 0.58a	27.04 ± 1.28c	23.95 ± 0.84d
Mg	g/kg	4.41 ± 0.10cd	5.04 ± 0.21c	3.74 ± 0.37d	6.16 ± 0.11b	6.17 ± 0.52b	7.42 ± 0.29a
Fe	mg/kg	340.01 ± 18.28d	559.36 ± 28.76b	140.09 ± 15.60e	707.26 ± 16.01a	448.80 ± 14.08c	354.12 ± 3.31d
Al	g/kg	3.56 ± 0.15a	3.23 ± 0.22a	0.59 ± 0.13b	4.15 ± 0.81a	3.55 ± 0.36a	1.17 ± 0.15b
P	g/kg	4.95 ± 0.09bc	3.91 ± 0.17d	4.13 ± 0.11cd	6.75 ± 0.23a	3.86 ± 0.15d	5.53 ± 0.74b
Cu	mg/kg	12.80 ± 1.32bc	13.29 ± 1.07bc	9.66 ± 0.44c	22.22 ± 1.45a	11.00 ± 2.03c	16.94 ± 2.49b
Zn	mg/kg	34.71 ± 0.58a	22.64 ± 2.14de	19.69 ± 0.89e	29.26 ± 1.84bc	26.13 ± 2.13cd	30.53 ± 0.25b
Ba	mg/kg	18.92 ± 1.16c	19.62 ± 1.47c	16.90 ± 1.40c	61.04 ± 0.50a	30.31 ± 3.51b	20.80 ± 3.76c
S	g/kg	3.04 ± 0.01ab	2.99 ± 0.05b	2.19 ± 0.02d	3.21 ± 0.06a	2.60 ± 0.09c	2.33 ± 0.14d
Si	g/kg	15.61 ± 0.83a	13.87 ± 0.52ab	8.75 ± 0.57c	8.46 ± 0.46c	12.21 ± 1.64b	14.38 ± 1.10ab

Note: Different lowercase letters indicate significant difference ($P < 0.05$).

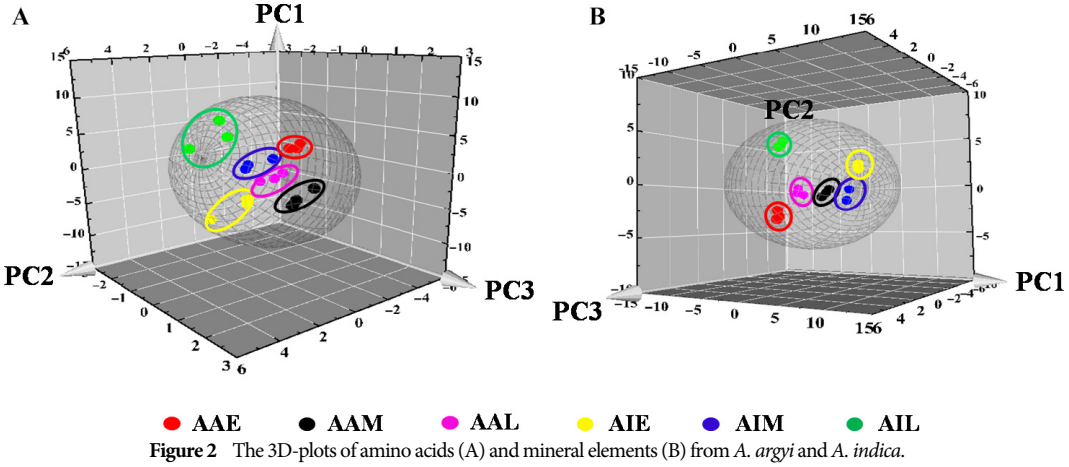

Figure 2 The 3D-plots of amino acids (A) and mineral elements (B) from *A. argyi* and *A. indica*.

PCA results based on amino acid data were feasible for classifying and evaluating the different growth stages of *A. argyi* and *A. indica*.

Five principal components (eigenvalues >1) were used for the 27 mineral elements of *A. argyi* and *A. indica* in the three growth periods, and the cumulative contribution rate was 96.07%. This result indicated that the extracted principal component factors comprehensively reflected the mineral element information among the six groups of samples. In the factor analysis, for the first principal component, the 16 elements Li, Be, Mn, Co, Ni, As, Se, Zr, Cd, K, Fe, Al, P, Cu, Ba, and S had high load values, indicating that the first principal component reflected the information of these 16 elements, accounting for 48.43% of the variance. Similarly, for the second principal component, the five elements B, Ti, Sr, Ca, and Mg had high load values, accounting for 19.52% of the variance. For the third principal component, Na and Zn had high load values, accounting for 11.96% of the variance. The distributions of the element load values of the fourth

and fifth principal components were not evident, and the contribution rates of the variance were 9.56% and 6.60%, respectively. The first and second principal components with high variance contribution rates were used to construct a PC1/PC2 scatter diagram (Fig. 2B). In this diagram, the distribution of the six groups of samples was relatively concentrated and independent, indicating that the PCA results based on mineral element data were feasible for classifying and evaluating the different growth stages of *A. argyi* and *A. indica*.

3.5 Correlation analysis of nutritional content in *A. argyi* and *A. indica*

The polysaccharide, dietary fiber, protein, EAA, NEAA, and mineral element contents of *A. argyi* and *A. indica* were analyzed. The results revealed that the dietary fiber content in *A. argyi* leaves was significantly positively correlated with the Co content (0.86). The polysaccharide content was significantly positively correlated with the Zr, Mg, Fe, Mn, and Se contents. The Zn content was significantly positively correlated with the protein (0.98) and NEAA (0.88) contents, and the EAA content was significantly positively correlated with the Pb (0.91) content (Fig. 3). The dietary fiber content in *A. indica* leaves was significantly positively correlated with the Sr (0.88) and Mg (0.86) contents. The polysaccharide content was negatively correlated with the dietary fiber, protein, and mineral element contents, the leaves of *A. indica* with a higher polysaccharide content had lower dietary fiber, protein, and mineral element contents. The protein content was significantly positively correlated with the content of various mineral elements, such as Mn, K, Fe, Ni, Zr, and Cd (Fig. 4).

Using all measured indicators, a correlation analysis was performed on the nutrient contents of *A. argyi* and *A. indica* leaves (Fig. 5). The nutrient contents in AAE and AIE were highly positively correlated (0.88), along with those in AAM and AIE (0.96), those in AAL and AIL (0.92), and those in AIM and AAL (0.90). These results indicated substantial differences in the nutrient contents of *A. argyi* and *A. indica* leaves at different growth stages. Compared with other growth stages, minimal differences were observed in the nutrient content of *A. argyi* and *A. indica* leaves at the same growth stage (early and late stages).

3.6 Comprehensive evaluation of nutritional quality of *A. argyi* and *A. indica*

For the amino acid analysis results, the sum of the product of the principal component factor scores and the variance contribution rate was used to obtain a comprehensive evaluation score: $F = 0.7867 \times F1 + 0.1143 \times F2$. The comprehensive score for each group was calculated. The results revealed that the order of the weighted values (n = 3) of the evaluation function was as follows: AIL (1.30) > AAE (0.52) > AIM (−0.05) > AAM (−0.24) > AAL (−0.49) > AIE (−1.04). Similarly, for the mineral element analysis results, the evaluation scores were $F = 0.4843 \times F1 + 0.1952 \times F2 + 0.1196 \times F3 + 0.0956 \times F4 + 0.066 \times F5$. The results revealed that the order of the weighted values (n = 3) of the comprehensive evaluation function was as follows: AIE (0.91) > AIM (0.13) > AIL (0.10) > AAE (−0.09) > AAM (−0.15) > AAL (−0.90). Furthermore, based on the results of all the measured indicators, the comprehensive evaluation score was calculated using the following formula: $F = 0.476 \times F1 + 0.193 \times F2 + 0.146 \times F3 + 0.074 \times F4 + 0.637 \times F5$. The comprehensive scores for the nutritional quality of the *A. argyi* and *A. indica* leaves at the three growth stages are presented in Table 5. The comprehensive analysis of multi-element nutritional components indicated that *A. indica* leaves are more suitable than *A. argyi* leaves as additives for food production.

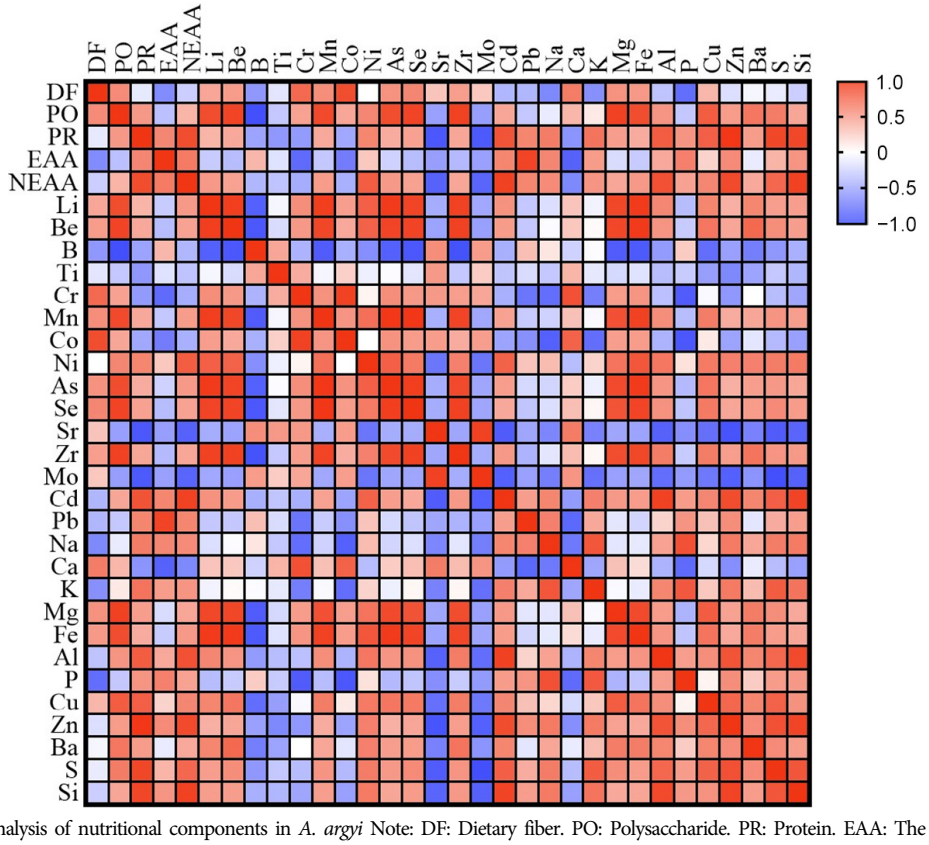

Figure 3 Correlation analysis of nutritional components in *A. argyi* Note: DF: Dietary fiber. PO: Polysaccharide. PR: Protein. EAA: The total amount of EAAs. NEAA: The total amount of NEAAs.

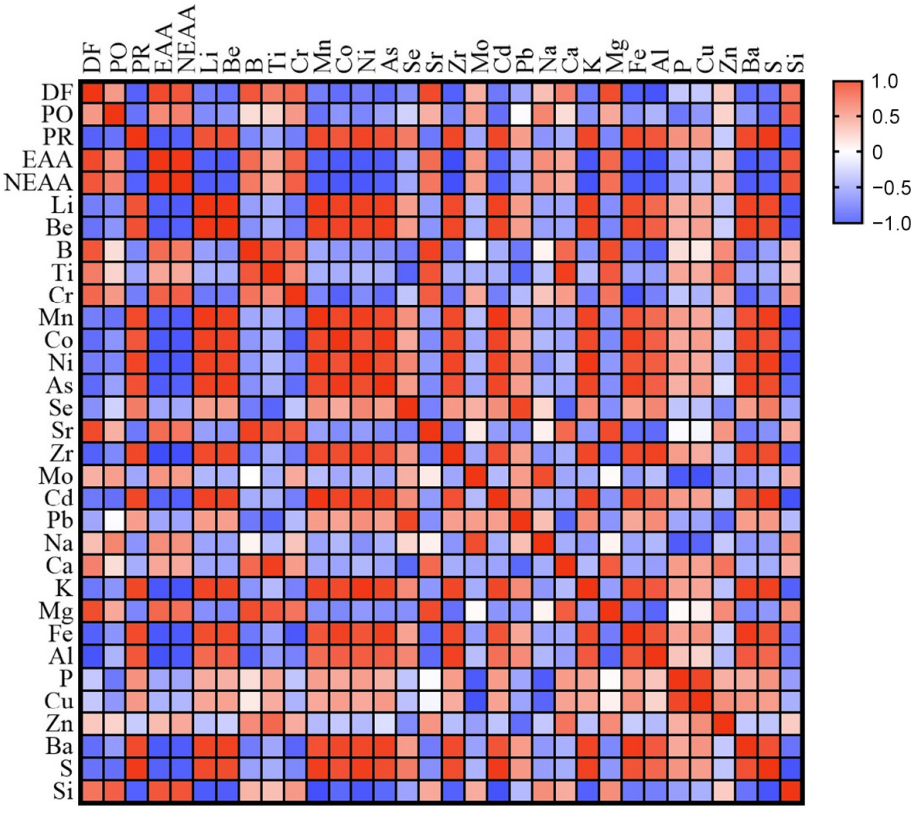

Figure 4 Correlation analysis of nutritional components in *A. indica* Note: DF: Dietary fiber. PO: Polysaccharide. PR: Protein. EAA: The total amount of EAAs. NEAA: The total amount of NEAAs.

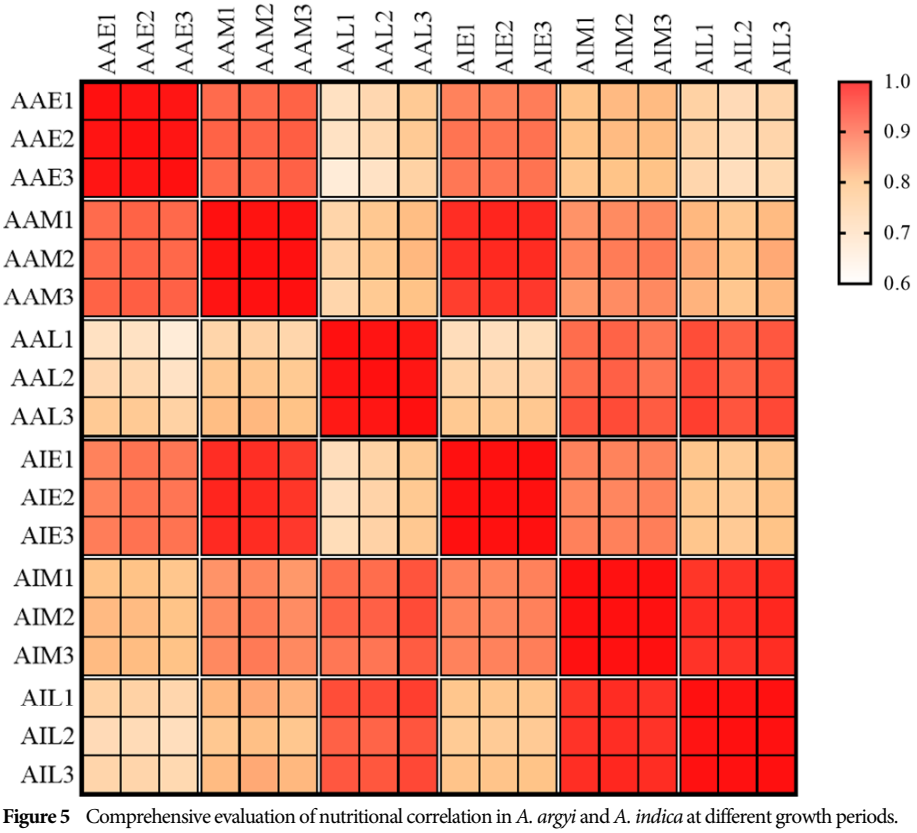

Figure 5 Comprehensive evaluation of nutritional correlation in *A. argyi* and *A. indica* at different growth periods.

3.7 Content analysis of volatile ingredients in *A. argyi* and *A. indica*

The components of volatile oil were the active ingredients in the leaves of *A. argyi* and *A. indica*. GC-MS analysis was performed to determine the contents of nine volatile components in the two plants, including seven medicinal components and two toxic components (Table 6). Seven medicinal ingredients have anti-

Table 5 Comprehensive evaluation of nutritional quality from *A. argyi* and *A. indica*

	PC1	PC2	PC3	PC4	PC5	Overall score
AAE	0.51	−0.07	1.86	0.81	−0.36	0.26
AAM	−0.22	−0.17	−0.25	−1.14	−1.78	−0.32
AAL	0.32	−1.86	−0.79	0.57	0.43	−0.26
AIE	−1.78	0.68	−0.33	0.95	0.15	−0.69
AIM	−0.21	0.12	0.49	−1.52	1.44	−0.07
AIL	1.38	1.28	−0.99	0.34	0.12	0.79

Note: PC: principal component.

Table 6 The relative content of nine volatile ingredients in *A. argyi* and *A. indica*

No	Retention time (min)	Compound name	Formula	Relative content (%)					
				AAE	AAM	AAL	AIE	AIM	AIL
1	9.00	(+)-α-Pinene	$C_{10}H_{16}$	0.67	5.01	2.63	0.13	1.49	1.94
2	10.70	Carveol	$C_{10}H_{16}O$	0.36	0.57	0.47	0.02	0.04	0.13
3	12.83	γ-Terpinene	$C_{10}H_{16}$	2.55	2.31	4.61	0.23	0.21	0.40
4	13.24	Eucalyptol	$C_{10}H_{18}O$	0.99	0.83	2.20	0.09	0.07	0.15
5	14.30	Thujone	$C_{10}H_{16}O$	1.54	N	N	N	N	0.03
6	15.64	Camphor	$C_{10}H_{16}O$	1.54	0.90	1.14	3.12	4.01	5.23
7	17.01	(−)-α-Terpineol	$C_{10}H_{18}O$	2.35	2.49	2.38	0.17	0.19	0.18
8	19.45	(−)-Borneol	$C_{10}H_{18}O$	1.97	1.56	0.12	0.31	0.22	0.31
9	23.87	Caryophyllene	$C_{15}H_{24}$	0.69	0.69	0.01	2.30	2.67	1.22

Note: N: represented not detected.

inflammatory, antibacterial, anti-asthmatic, and analgesic effects[25]. The quality of *A. argyi* leaves is mainly evaluated according to eucalyptol and borneol contents[5]. With the exception of caryophyllene, the contents of the other six medicinal ingredients in *A. argyi* are higher than those in *A. indica*, and the eucalyptol content is significantly higher. These results provide a basis for the clinical use of *A. argyi* for the treatment of diseases.

4 Discussion

The mineral element contents of *A. argyi* and *A. indica* leaves between the different growth periods exhibited their own characteristics, and significant differences were observed in the contents of certain elements in the leaves of the two plants during the same growth period and in different growth periods of the same plant. The 27 mineral elements identified in this study included four harmful heavy metals: Pb, Cd, Cu, and As. When these elements accumulate to a certain level in the human body, they can cause physiological damage. The draft of the 2019 National Pharmacopoeia General Principles for the determination of medicinal materials and decoction ingredients clearly stipulates that the Pb, Cd, Cu, and As contents in medicinal materials should not exceed 5, 1, 20, and 2 mg/kg, respectively. In this study, the highest concentrations of Pb, Cd, Cu, and As in *A. argyi* and *A. indica* leaves at different growth stages were 0.31, 0.31, 22.22, and 0.42 mg/kg, respectively. The results indicated that except for Cu, their contents were within the specified limits. The Cu content only exceeded the limit in AIE; it did not exceed the limit in AIM, AIL, or the three growth stages of *A. argyi*. We speculate that *A. indica* may have a strong Cu-enrichment ability, which will be the focus of future research.

Genetic and environmental factors significantly affect the types and contents of nutrients in plants. The efficiency of absorption, transformation, and utilization of nutrients is influenced by genetic characteristics, while environmental factors directly affect the growth conditions and availability of nutrients in plants[26,27]. In this study, the two plants grew in the same external environment, including climate, soil, light, temperature, and moisture. We attribute the differences in nutritional composition to genetic factors of the two plants. According to the literature, *A. indica* is used as a raw material for food production in many countries, such as Nepal, India, Japan, Pakistan, and China[28-32]. Meanwhile, *A. argyi* is widely used as a medicine, whereas its use as a food is relatively limited[13]. In this study, AIE had higher polysaccharide and protein contents than AAE and a lower dietary fiber content. Overall, the evaluation of mineral elements indicated that *A. indica* is superior to *A. argyi*. This explains why *A. indica* is widely used in the food industry due to its good taste and high nutritional value.

Thujone is the main toxic component in *A. argyi* and *A. indica*, with significant toxicity to the liver, kidneys, reproductive organs, and fetus. Camphor has neurotoxicity, liver toxicity, and fetal toxicity[25,33,34]. The thujone content gradually decreased with the growth of *A. argyi* leaves, and thujone was not detected in AAM or AAL. Low levels of thujone were only detected in AIL. The camphor content was higher in AAL than in AAE and AAM, and it increased with the growth of *A. argyi*. According to these results, the thujone and camphor contents in AAE are relatively high, while those in AIE are relatively low. Generally, it is recommended to avoid consuming or using *A. argyi* or *A. indica* with high levels of toxic components.

5 Conclusion

The types and contents of nutritional components are used to determine the quality of traditional medicines and food and are closely related to their quality and curative effects. During the three growth periods, the leaves of *A. argyi* and *A. indica* were rich

in polysaccharides, total dietary fibers, amino acids, and mineral elements. The contents of nine volatile ingredients differed significantly between the leaves of *A. argyi* and *A. indica* during the three growth periods. From the perspective of multiple nutrients and volatile ingredients, our findings suggest that *A. indica* leaves are more suitable additives for food production than *A. argyi* leaves. This study provides a scientific basis and data support for the comprehensive development and utilization of edible and medicinal resources of *A. argyi* and *A. indica*. In the future, we will combine the nutritional and pharmacodynamic components of the two plants for a comprehensive quality evaluation to lay a foundation for the high-quality development of the *A. argyi* and *A. indica* industries.

Conflict of interest

The authors declare that there are no conflicts of interest in this work.

Acknowledgments

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