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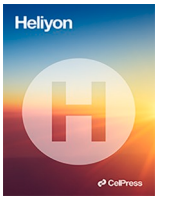

Research article

Chemical profiling and arginine kinase inhibitory activity of *Angelica dahurica* leaves

Aitong Yang [a,b,c,1], Junyan Zhang [d,1], Guangying Lv [a], Jiabao Chen [a], Long Guo [a,b,c], Yan Liu [e], Yuguang Zheng [b,c,f,**], Lei Wang [a,b,c,*]

[a] College of Pharmacy, Hebei University of Chinese Medicine, Shijiazhuang, 050200, China
[b] Traditional Chinese Medicine Processing Technology Innovation Center of Hebei Province, Shijiazhuang, 050200, China
[c] International Joint Research Center on Resource Utilization and Quality Evaluation of Traditional Chinese Medicine of Hebei Province, Shijiazhuang, 050200, China
[d] Cangzhou Hospital of Integrated Traditional Chinese and Western Medicine, Cangzhou, 061000, China
[e] Tianjin Tasly Chinese Medicine Resources Technology Development Co., Ltd., Tianjin, China
[f] Hebei Chemical and Pharmaceutical College, Shijiazhuang, 050026, China

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ABSTRACT

Angelica dahurica is a medicinal herb of the Umbelliferae family. The dried root of *A. dahurica*, also known as Angelicae dahuricae Radix, is widely used in clinical treatment. However, the aboveground part of *A. dahurica* which accounted for over 70% of the total plant was abandoned in the field. In order to develop the value of the aboveground part of *A. dahurica*, the chemical constituents and arginine kinase (AK) inhibitory activity of *A. dahurica* leaves were studied. 85 volatile components were identified from *A. dahurica* leaves by GC-MS; 39 non-volatile components including sugars, amino acids and organic acids were identified by pre-column derivatization GC-MS analysis; and 7 coumarins were qualitatively and quantitatively analyzed by HPLC. Then, an inhibitory enzyme-linked immunosorbent assay (iEIA) was applied for evaluation of AK inhibitory activity. The extracts of *A. dahurica* leaves exhibited well inhibitory effects on AK. Further, potential AK inhibitors were screened by grey relational analysis and their inhibitory activities were validated by iEIA. L-aspartic acid exhibited strongest inhibitory effect on AK with its IC_{50} value was 0.558 mM, which was much lower than that of chlorpheniramine (6.644 mM). The obtained chemical profiles displayed chemical diversity of *A. dahurica* leaves and will provide data support for the future development and utilization of *A. dahurica* leaves. The screened potential AK inhibitors from *A. dahurica* leaves could be candidates for development of antiallergic substances or insecticides.

1. Introduction

Angelica dahurica is a perennial herb of the Umbelliferae family. The dried root of *A. dahurica* which is known as Angelicae dahuricae Radix or “baizhi”, is a commonly used Traditional Chinese Medicine for expelling wind and cold, removing dampness,

* Corresponding author. College of Pharmacy, Hebei University of Chinese Medicine, Shijiazhuang, 050200, China.
** Corresponding author. Traditional Chinese Medicine Processing Technology Innovation Center of Hebei Province, Shijiazhuang, 050200, China.
E-mail addresses: zyg314@163.com (Y. Zheng), wanglei1031@126.com (L. Wang).
[1] These authors have contributed equally to this work.

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arresting leukorrhea, eliminating swelling, draining pus and relieving pain [1]. Angelicae dahuricae Radix could also be used as spice to give dishes a unique flavor. The clinical and industry demand of Angelicae dahuricae Radix is great, especially in China, Japan, Korea and other Asian counties and regions. However, the aboveground part of *A. dahurica* which accounts for over 70% of the total plant is discarded in the field. Value exploration of the aboveground part of *A. dahurica* will be friendly to the environment and resource utilization.

As early as about 1500 years ago, it was recorded in Annotation of Materia Medica (Ben Cao Jing Ji Zhu, 本草经集注) that *A. dahurica* leaves could be used in medicated bath for treatment of erysipelas and urticaria [2]. The records about application of *A. dahurica* leaves in bath continued until Ming Dynasty but rarely mentioned since the Qing Dynasty. Though *A. dahurica* leaves has not been collected in pharmacopoeia, it was reported that mashed *A. dahurica* leaves were effective for treatment of bee stings [3]. The historic and modern records about applications of *A. dahurica* leaves indicated its potential developmental values.

Arginine kinase (AK) catalyzes the reversible transphosphorylation between ATP and arginine. The generated phosphoarginine containing high-energy phosphate could serve as energy reserve. AK plays critical role in energy metabolism in invertebrates [4]. The expression of AK was reported to be closely related with flight activities, host identification, growth and development in insects. Evidences indicated that knocking down AK led to retarded development and increased mortality in insects [5–8]. AK was also reported to be one of the major allergens of shrimps and crabs [9,10]. Furthermore, AK exists only in invertebrates and shared low homology with creatine kinase, which plays the same role in vertebrates, indicating it is a suitable potential target for development of insecticides [11] and anti-allergic medicines.

To develop potential values of *A. dahurica* leaves, an integrated chemical and bioactivity research strategy was applied. Firstly, GC-MS and HPLC were applied for chemical profiling. Then, the inhibitory capacities of *A. dahurica* leaves extracts on AK were evaluated by an inhibitory enzyme-linked immunosorbent assay (iEIA). Grey relational analysis was further applied for the screening of potential AK inhibitors. The inhibitory activities of the selected potential AK inhibitors were validated by the iEIA. The systematic chemical profiling of *A. dahurica* leaves provides foundation for its further value development. The screened potential AK inhibitors could be candidates for development of pesticide and antiallergic drugs which would be safer to mammals.

2. Materials

2.1. Chemicals and reagents

HPLC-grade methanol, acetonitrile and formic acid were from Fisher Scientific (Pittsburgh, PA, USA). *n*-Hexane (purity ≥98%) and anhydrous pyridine (purity ≥99.8%) were purchased from Aladdin biochemical Technology Co. Ltd (Shanghai, China). Salicylic acid (purity ≥99.5%) was from Ron Reagent Co. Ltd (Shanghai, China). Methoxyamine hydrochloride (purity ≥97.5%) and *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA, purity ≥98.5%) were obtained from Sigma Aldrich Company (USA). Xanthotoxol, oxypeucedanin hydrate, byakangelicin, oxypeucedanin, imperatorin, phellopterin and isoimperatorin with their purities over 98% were purchased from Chengdu Push Bio-Technology Co. Ltd (Chengdu, China). L-aspartic acid, lactic acid and citric acid with their purities over 98% were purchased from Beijing Solarbio Science & Technology Co.,Ltd. (Beijing, China). Malic acid (purity ≥99.5%), shikimic acid and galactose (purity ≥98%) were obtained from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). AK enzyme-linked immunosorbent assay kit was purchased from Jiangsu Enzyme-linked Biotechnology Co., Ltd. (Jiangsu, China).

2.2. Plant materials

Five batches of fresh mature *A. dahurica* leaves (A、B、C、D、E) were collected from Qizhou Campus of Hebei University of Chinese Medicine and dried in shade. The voucher specimens were identified by Professor Yuguang Zheng and deposited in the Traditional Chinese Medicine Processing Technology Innovation Centre of Hebei Province with their specimen numbers were from 202210AD01 to 202210AD05.

3. Methods

3.1. Analysis of the volatile metabolites by GC-MS

3.1.1. Sample pretreatment

1 g of fully ground *A. dahurica* leaves powder was mixed with 1 mL *n*-hexane. After ultrasonication (300 W, 40 kHz) 15 min at room temperature, the extraction mixture was centrifuged at 13000 rpm at room temperature for 10 min. 100 μL of the supernatants was transferred to clean vials for GC-MS analysis.

3.1.2. Instrument parameters

The headspace injection conditions were as follows: heater temperature was set at 150 °C; quantitative ring temperature was 160 °C; transmission line temperature was 170 °C; sample bottle equilibrium time was 15 min; continuous injection time was 0.5 min.

The GC-MS analysis was performed with an Agilent 7890B5977B GC-MS (Agilent, CA, USA) coupled with a HP-5MS capillary column (30 m × 0.25 mM, 0.25 μm, Agilent, CA, USA). Helium (≥99.999%) was used as carrier gas. Evaporated samples were injected in split-mode with the split ratio set to 5:1 at a temperature of 250 °C. The oven temperature program was initially set at 45 °C, then increased to 170 °C at a rate of 4 °C/min. The electronic ionization voltage of electron-impact (EI) ion source was 70 eV. The interface

temperature was 250 °C, the ion source temperature was 230 °C and the quadrupole temperature was 150 °C. The mass spectrometer was operated in full scan mode with a scanning range of 50–500 *m/z*. Solvent delay time was set as 2 min.

3.2. Analysis of non-volatile metabolites by pre-column derivatization GC-MS

3.2.1. Sample pretreatment

Extraction and derivatization of non-volatile metabolites were performed according to a reported protocol [12].

3.2.2. Instrument parameters

The headspace injection conditions are as follows: heater temperature was 110 °C; quantitative ring temperature was 120 °C; transmission line temperature was 130 °C; sample bottle equilibrium time was 10 min; continuous injection time was 1 min.

Aforementioned GC-MS instrument and column (see 2.1.2) was also applied for analysis of derivatized samples. 5 μL of the derivatized sample was evaporated in a 20 mL vial and injected using 5:1 split-mode at a temperature of 250 °C. The temperature gradient program and other parameters were set the same as in Ref. [12].

3.3. Analysis of the coumarins by HPLC

3.3.1. Preparation of samples and standard solutions

Standard solutions of xanthotoxol (0.05 μg/mL, 0.17 μg/mL, 0.425 μg/mL, 0.85 μg/mL, 4.25 μg/mL, 8.5 μg/mL, 17 μg/mL, 85 μg/mL, 0.85 mg/mL), oxypeucedanin hydrate (0.05 μg/mL, 0.10 μg/mL, 1.4375 μg/mL, 2.875 μg/mL, 14.375 μg/mL, 28.75 μg/mL, 57.5 μg/mL, 115 μg/mL, 1.15 mg/mL), byakangelicin (0.08 μg/mL, 0.24 μg/mL, 0.6 μg/mL, 3 μg/mL, 6 μg/mL, 30 μg/mL, 60 μg/mL, 120 μg/mL, 1.2 mg/mL), oxypeucedanin (0.06 μg/mL, 0.18 μg/mL, 2 μg/mL, 4 μg/mL, 20 μg/mL, 40 μg/mL, 80 μg/mL, 400 μg/mL, 4 mg/mL), imperatorin (0.09 μg/mL, 0.28 μg/mL, 0.55 μg/mL, 1.1 μg/mL, 5.5 μg/mL, 11 μg/mL, 22 μg/mL, 220 μg/mL, 2.20 mg/mL), phellopterin (0.13 μg/mL, 0.32 μg/mL, 1.275 μg/mL, 2.55 μg/mL, 12.75 μg/mL, 25.5 μg/mL, 51 μg/mL, 255 μg/mL, 2.55 mg/mL) and isoimperatorin (0.07 μg/mL, 0.16 μg/mL, 0.325 μg/mL, 0.65 μg/mL, 3.25 μg/mL, 6.5 μg/mL, 13 μg/mL, 260 μg/mL, 2.6 mg/mL) were prepared by gradient dilution with 80% (v/v) methanol.

50 mg of each pulverized samples were extracted exhaustively with 1 mL of 80% (v/v) methanol by ultrasonication (40 kHz, 300 W) 50 min at room temperature. After extraction, the mixed suspension was centrifuged 10 min. The supernatant was filtered through a 0.22 μm nylon filter membrane for HPLC analysis.

3.3.2. Instrument parameters

Chromatographic separation was conducted on an Agilent ZORBAX SB C18 column (4.6 × 50 mm, 1.8 μm). The mobile phases were consisted of ultrapure water (A) and acetonitrile (B). The HPLC chromatographic condition was as follows: 0–1 min, 18% B; 1–15 min, 18%–35% B; 15–18 min, 35%–60% B; 18–25 min, 60%–85% B. The flow rate was maintained at 0.5 mL/min. 5 μL of the prepared samples was injected into the column. The column temperature was maintained at 25 °C and the ultraviolet detection wavelength was set at 310 nm.

3.4. Inhibition ELISA for evaluating AK inhibition activity

200 μL extracts of *A. dahurica* leaves that obtained under the condition of "3.2.1" were dried using a SpeedVac (Thermo Scientific, Inc., Bremen, Germany) at 5000 rpm and 40 °C for 180 min and then redissolved in 100 μL dimethyl sulfoxide (DMSO).

50 μL AK (20 U/L) was mixed with 5 μL the redissolved *A. dahurica* leaves extracts or hexane extracts from 3.1.1 or candidate compounds at various concentrations. After incubation at 37 °C for 10 min, 50 μL of the mixture was added to a 96-well immunoplate (Jianglanchun, Jiangsu, China) which was coated with purified crab AK antibody. The plate was then incubated at 37 °C for 30 min. After incubation, the mixtures were discarded and washed 5 times. Subsequently, HRP-labeled AK antibody was added and the mixtures were incubated for 30 min at 37 °C. After washing completely, the tetramethyl benzidine (TMB) substrate solution was added and the plate was incubated for another 15 min at room temperature in dark. Color development was stopped by adding 50 μL stopping solution. The luminescence intensity of each well was measured within 15 min using a microplate reader (Victor Nivo multiplate reader, PerkinElmer, USA) at 450 nm wavelength. In the blank groups, neither extract-AK mixture nor the HRP-labeled AK antibody was added. In the negative and positive groups, 5 μL of solvents (DMSO or hexane) or chlorpheniramine (2 mM) instead of *A. dahurica* leaves extracts or candidate compounds were applied and measured under the same condition. The inhibition rate was calculated as follows:

$$\text{Inhibition rate } (\%) = (A_S\text{-}A_B)/(A_N\text{-}A_B) \times 100\%$$

A_S, A_B, A_N are the absorbances of sample wells, blank wells and negative control wells, respectively.

3.5. Data processing and multivariate statistical analysis

For qualitative analysis, the metabolites detected by GC-MS with a similarity more than 80% to the NIST17 standard library were identified using the Agilent MassHunter analysis program (Agilent, Santa Clara, CA, USA). The coumarin components of *A. dahurica*

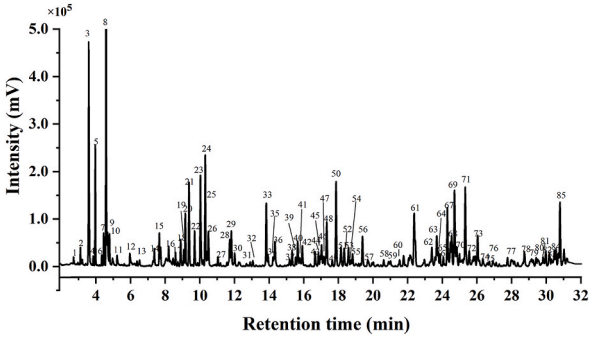

Fig. 1. TIC of volatile metabolites in *A. dahurica leaves* by GC-MS; The number of peaks was consistent with those of compounds in Table 1.

leaves were qualitatively and quantitatively analyzed by comparing the HPLC chromatograms of *A. dahurica* leaves with reference substances mixture. SPSS 24.0 statistical software was used for grey relational analysis.

4. Results

4.1. Characterization of volatile metabolites by GC-MS

The extracted volatile components of *A. dahurica leaves* were analyzed by GC-MS. A typical total ion chromatogram (TIC) shows in Fig. 1. Qualitative Navigator (B.08.00) software and the NIST17 standard mass spectrometry database were applied for compound qualification and relative quantification. A total of 85 volatile components were identified including alkanes, alkenes, aldehydes, alcohols, esters and others (Table 1). The identified volatile components accounted for 92.09% of the total peak area. Alkane accounted for 47.34% of the identified components; followed by alkenes, accounted for 15.39%; aldehyde, alcohols and esters accounted for 11.43%, 10.94% and 3.35%, respectively. The compounds with the highest relative contents were 2-hexenal, 2-cyclohexene-1-ol, farnesane, saffron, β-thujene and so on.

4.2. Characterization of non-volatile metabolites by pre-column derivatization GC-MS

The *A. dahurica* leaf extracts were derivatized according to the experimental conditions of "2.2.1″ and then analyzed by GC-MS. A TIC shows in Fig. 2. The data were analyzed by Qualitative Navigator (B.08.00) software and compared with the NIST17 standard mass spectrometry database.

A total of 39 derivative components were identified including sugars, organic acids, amino acids and alcohols (Table 2). The identified components accounted for 97.18% of the total peak area. Sugars accounted for 44.75% of the total peak area of all the identified components. Organic acids, alcohols, and amino acids accounted for 32.23%, 9.00%, and 1.83%, respectively. The relative contents of malic acid, D-glucose, D-fructose, D-galactose, myo-inositol were highest in *A. dahurica* leaves.

4.3. Characterization of coumarins by HPLC

Seven coumarins, including xanthotoxol, oxypeucedanin hydrate, byakangelicin, oxypeucedanin, imperatorin, phellopterin and isoimperatorin, were identified by HPLC. The chromatograms of *A. dahurica leaves* and reference substances are shown in Fig. 3.

For the quantification of coumarins, the mixed reference solution that containing 7 coumarins was firstly diluted with 80% methanol into a series of gradient concentration solutions. Then they were analyzed under the same condition as *A. dahurica leaves*. Calibration curves were established between compound concentrations and the corresponding peak areas (Table 3). All the calibration curves showed good linearity ($R^2 > 0.997$). The contents of the 7 coumarins in *A. dahurica* leaves were then calculated according to the regression equations. Among the seven quantified coumarins, the content of oxypeucedanin (1475.58 ± 701.24 μg/g) and oxypeucedanin hydrate (498.38 ± 165.81 μg/g) were highest in *A. dahurica leaves*.

4.4. Inhibitory effects of A. dahurica leaves extracts on AK

iEIA was applied for evaluation the inhibitory effects of *A. dahurica* leaves extracts on AK. The inhibition rates of the 5 batches *A. dahurica* leaves extracts on AK were 34.78%–62.61% which were comparable with chlorpheniramine at 2 mM (30.12%) (Fig. 4).

4.5. Grey relation analysis for screening of potential AK inhibitors

Grey relational analysis was applied for investigating the spectrum-effect relationships between peak areas (chemical components) and AK inhibitory activity of *A. dahurica* leaves extracts. The grey relational coefficients of all the identified non-volatile metabolites

Table 1
Identification of volatile compounds analyzed by GC-MS.

No.	t/min	Compounds	Molecular formula	Relative content/%	Class
1	2.709	2,5-Dimethylhexane	C_8H_{18}	0.13	Alkane
2	3.104	3,5-Dimethylheptane	C_9H_{20}	0.36	Alkane
3	3.582	2-Cyclohexen-1-ol	$C_6H_{10}O$	6.52	Alcohol
4	3.84	3,4,5-Trimethylheptane	$C_{10}H_{22}$	0.16	Alkane
5	3.962	2,3,4-Trimethylhexane	C_9H_{20}	2.38	Alkane
6	4.329	2,6-Dimethylhept-3-ene	C_9H_{18}	0.22	Alkene
7	4.447	2-Cyclohexen-1-ol	$C_6H_{10}O$	0.74	Alcohol
8	4.58	2-Hexenal	$C_6H_{10}O$	10.87	Aldehyde
9	4.667	*Trans*-3-hexen-1-ol	$C_6H_{12}O$	1.18	Alcohol
10	4.782	4-Methyloctane	C_9H_{20}	0.76	Alkane
11	5.218	3-Ethoxy-2-methylacrylaldehyde	$C_6H_{10}O_2$	0.31	Aldehyde
12	5.951	2-Ethylfuran	C_6H_8O	0.52	Furan
13	6.517	β-Terpinene	$C_{10}H_{16}$	0.21	Alkene
14	7.369	4-Methylnonane	$C_{10}H_{22}$	0.55	Alkane
15	7.655	Sabinene	$C_{10}H_{16}$	0.95	Alkene
16	8.196	Myrcene	$C_{10}H_{16}$	0.47	Alkene
17	8.587	β-Phellandrene	$C_{10}H_{16}$	0.38	Alkene
18	8.895	4,5-Dimethylnonane	$C_{11}H_{24}$	0.89	Alkane
19	9.072	2,5-Dimethylnonane	$C_{11}H_{24}$	0.48	Alkane
20	9.163	3,6-Dimethyloctane	$C_{10}H_{22}$	1.65	Alkane
21	9.371	β-Thujene	$C_{10}H_{16}$	2.86	Alkene
22	9.694	Ocimene	$C_{10}H_{16}$	1.13	Alkene
23	10.03	3-Carene	$C_{10}H_{16}$	3.26	Alkene
24	10.31	5-Propylnonane	$C_{15}H_{32}$	3.64	Alkane
25	10.406	Carbonic acid, Bis(2-ethylhexyl) ester	$C_{17}H_{34}O_3$	0.48	Ester
26	10.49	5-Propylnonane	$C_{12}H_{26}$	1.22	Alkane
27	11.04	*Trans*-1,4-dimethylcyclooctane	$C_{10}H_{20}$	0.3	Alkane
28	11.724	1,2-Epoxy-9-decene	$C_{10}H_{18}O$	1.09	Alkene
29	11.817	4,6-Dimethyldodecane	$C_{14}H_{30}$	1.21	Alkane
30	12.004	3-Ethyl-3-methylheptane	$C_{10}H_{22}$	0.41	Alkane
31	12.899	(+)-Trans-limonene oxide	$C_{10}H_{16}O$	0.13	Alkene
32	13.058	(−)-Trans-limonene oxide	$C_{10}H_{16}O$	0.2	Alkene
33	13.832	Cyclohexyl-dimethoxy-methylsilane	$C_9H_{20}O_2Si$	2.28	Alkane
34	13.928	3,4-Dimethylundecane	$C_{13}H_{28}$	0.41	Alkane
35	14.217	α-Pinene	$C_{10}H_{16}$	0.36	Alkene
36	14.326	Isopulegol	$C_{10}H_{18}O$	1.24	Alcohol
37	15.169	3,8-Dimethyldecane	$C_{12}H_{26}$	0.21	Alkane
38	15.324	3-Methyl-5-propylnonane	$C_{13}H_{28}$	0.56	Alkane
39	15.526	5-Methyl-5-propylnonane	$C_{13}H_{28}$	0.27	Alkane
40	15.644	3,6-Dimethylundecane	$C_{13}H_{28}$	0.91	Alkane
41	15.772	2,4-Dimethylbenzaldehyde	$C_9H_{10}O$	0.25	Aldehyde
42	15.915	4-Methyldodecane	$C_{13}H_{28}$	0.72	Alkane
43	16.658	3,8-Dimethyldecane	$C_{12}H_{26}$	0.57	Alkane
44	16.841	3,8-Dimethylundecane	$C_{13}H_{28}$	0.47	Alkane
45	16.956	Decyl heptyl ether	$C_{17}H_{36}O$	0.33	Ether
46	17.024	4,6-Dimethyldodecane	$C_{14}H_{30}$	0.68	Alkane
47	17.161	5-Propyldecane	$C_{13}H_{28}$	0.25	Alkane
48	17.326	2,6,10-Trimethyltridecane	$C_{16}H_{34}$	1.79	Alkane
49	17.649	2-Bromododecane	$C_{12}H_{25}Br$	0.19	Alkane
50	17.864	4,6-Dimethyldodecane	$C_{14}H_{30}$	3.32	Alkane
51	18.137	2,6,11-Trimethyldodecane	$C_{15}H_{32}$	0.79	Alkane
52	18.33	2,7,10-Trimethyldodecane	$C_{15}H_{32}$	0.7	Alkane
53	18.585	2-Ethyl-2-methyl-tridecanol	$C_{16}H_{34}O$	0.75	Alcohol
54	18.728	2-Isopropyl-5-methyl-1-heptanol	$C_{11}H_{24}O$	0.23	Alcohol
55	18.812	4-Ethylundecane	$C_{13}H_{28}$	0.46	Alkane
56	19.387	2,6,11-Trimethyldodecane	$C_{15}H_{32}$	1.21	Alkane
57	19.999	2,3,5-Trimethyldecane	$C_{13}H_{28}$	0.17	Alkane
58	20.612	2,9-Dimethylundecane	$C_{13}H_{28}$	0.23	Alkane
59	20.997	α-Cubebene	$C_{15}H_{24}$	0.24	Alkene
60	21.526	Helminthogermacrene	$C_{15}H_{24}$	0.26	Alkene
61	22.378	Tetradecane	$C_{14}H_{30}$	3.15	Alkane
62	23.391	Isocaryophyllene	$C_{15}H_{24}$	0.84	Alkene
63	23.68	Pristane	$C_{19}H_{40}$	1.31	Alkane
64	23.761	5,5-Dibutylnonane	$C_{17}H_{36}$	0.5	Alkane
65	23.864	2,6,11-Trimethyldodecane	$C_{15}H_{32}$	0.44	Alkane
66	24.059	3,8-Dimethylundecane	$C_{13}H_{28}$	0.3	Alkane
67	24.286	β-Cubebene	$C_{15}H_{24}$	2.19	Alkene
68	24.46	Phytane	$C_{20}H_{42}$	0.71	Alkane

(*continued on next page*)

Table 1 (*continued*)

No.	t/min	Compounds	Molecular formula	Relative content/%	Class
69	24.7	Crocetane	$C_{20}H_{42}$	3.1	Alkane
70	24.986	7-Methylhexadecane	$C_{17}H_{36}$	0.18	Alkane
71	25.318	2,4-Di-*tert*-butylphenol	$C_{14}H_{22}O$	2.79	Phenol
72	25.555	β-Cedrene	$C_{15}H_{24}$	0.6	Alkene
73	26.04	Hexadecane	$C_{16}H_{34}$	1.01	Alkane
74	26.338	Sulfurous acid, 2-ethylhexyl undecyl ester	$C_{19}H_{40}O_3S$	0.34	Ester
75	26.705	5,5-Dibutylnonane	$C_{17}H_{36}$	0.17	Alkane
76	26.919	3-Isopropyl-6,10-dimethylundecane-2-ol	$C_{16}H_{34}O$	0.28	Alcohol
77	27.973	Sulfurous acid, decyl 2-ethylhexyl ester	$C_{18}H_{38}O_3$	0.89	Ester
78	28.738	3-Ethyl-2,6,10-trimethylundecane	$C_{16}H_{34}$	0.8	Alkane
79	29.139	Sulfurous acid, 2-ethylhexyl octadecyl ester	$C_{26}H_{54}O_3S$	0.85	Ester
80	29.419	9-Methylheptadecane	$C_{18}H_{38}$	0.68	Alkane
81	29.82	Sulfurous acid, butyl heptadecyl ester	$C_{21}H_{44}O_3S$	0.19	Ester
82	29.972	8-Methylheptadecane	$C_{18}H_{38}$	0.37	Alkane
83	30.165	Sulfurous acid, 2-ethylhexyl Tetradecyl ester	$C_{22}H_{46}O_3S$	0.6	Ester
84	30.466	3-Methyltetradecane	$C_{15}H_{32}$	1.29	Alkane
85	30.793	Heptadecane	$C_{17}H_{36}$	3	Alkane

were ranged from 0.605 to 0.92.34 non-volatile metabolites with their coefficients more than 0.8 are listed in Table 4 which could be possible AK inhibition candidates. The higher the coeffieiects were, the more possibilities of the corresponding candidates to show AK inhibitory effects.

4.6. Verification of the inhibitory effects on AK

To further validate the results of grey relational analysis, the inhibitory effects of 10 compounds, including aspartic acid, lactic acid, citric acid, malic acid, shikimic acid, phellopterin, xanthotoxol, byakangelicin, galactose and oxypeucedanin at 2 mM were tested. The inhibition rates of aspartic acid, lactic acid, citric acid, malic acid, shikimic acid were higher than that of chlorpheniramine at 2 mM, while the inhibition rates of phellopterin, xanthotoxol, byakangelicin, galactose and oxypeucedanin were lower than that of chlorpheniramine at 2 mM (Fig. 5).

Further, the IC_{50} values of aspartic acid, lactic acid, citric acid, malic acid, shikimic acid and chlorpheniramine were determined by evaluating their inhibitory effects on AK at 5 concentrations. As shown in Fig. 6, all of the 6 compounds exhibited dose-dependent inhibitory effects on AK. It could be noted that aspartic acid, with the highest coefficient in grey relational analysis, exhibited strongest inhibitory effects on AK. The IC_{50} value of aspartic acid was 0.558 mM which was much lower than that of chlorpheniramine (6.644 mM). Lactic acid, citric acid, malic acid and shikimic acid also showed strong inhibitory effects on AK with their IC_{50} values were 0.823 mM, 1.008 mM, 1.755 mM and 1.881 mM, respectively.

5. Discussion

5.1. Volatile metabolites by GC-MS

The volatile oils of *A. dahurica* roots showed bioactivities such as anti-inflammation [13,14] and antioxidation [15,16]. According

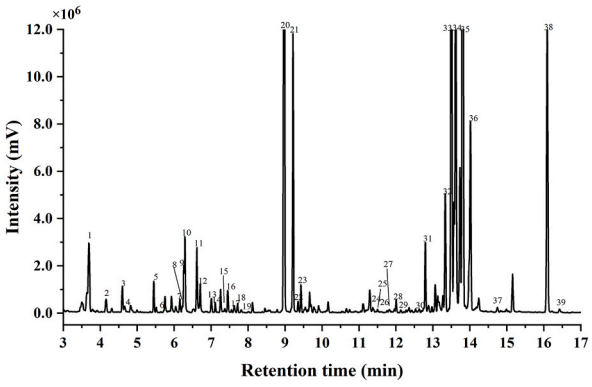

Fig. 2. TIC of nonvolatile metabolites in *A. dahurica leaves* by GC-MS after derivatization. The number of peaks was consistent with those of compounds in Table 2.

Table 2
Identification of non-volatile metabolites analyzed by pre-column derivatization combining with GC-MS.

No.	t/min	Compounds	Molecular Formula	Relative content/%	Class
1	3.691	Lactic acid, 2TMS	$C_9H_{22}O_3Si_2$	2.21%	Organic acid
2	4.155	L-Alanine, 2TMS	$C_9H_{23}NO_2Si_2$	0.29%	Amino acid
3	4.593	Oxalic acid, 2TMS	$C_8H_{18}O_4Si_2$	0.46%	Organic acid
4	4.658	L-Proline, 2TMS	$C_{11}H_{25}NO_2Si_2$	0.14%	Amino acid
5	5.512	L-Valine, 2TMS	$C_{11}H_{27}NO_2Si_2$	0.08%	Amino acid
6	5.642	Pinacol, 2TMS	$C_{12}H_{30}O_2Si_2$	0.01%	Alcohol
7	6.154	Ethanolamine, 3TMS	$C_{11}H_{31}NOSi_3$	0.20%	Amine
8	6.219	L-Leucine, 2TMS	$C_{12}H_{29}NO_2Si_2$	0.07%	Amino acid
9	6.258	Glycerol, 3TMS	$C_{12}H_{32}O_3Si_3$	0.66%	Alcohol
10	6.288	1-Hexadecanol, TMS	$C_{19}H_{42}OSi$	1.21%	Alcohol
11	6.613	2-Butenedioic acid, (Z)-, 2TMS	$C_{10}H_{20}O_4Si_2$	1.10%	Organic acid
12	6.704	Glycine, 3TMS	$C_{11}H_{29}NO_2Si_3$	0.50%	Amino acid
13	7.003	Glyceric acid, 3TMS	$C_{12}H_{30}O_4Si_3$	0.26%	Organic acid
14	7.107	2-Butenedioic acid, (E)-, 2TMS	$C_{10}H_{20}O_4Si_2$	0.14%	Organic acid
15	7.368	L-Serine, 3TMS	$C_{12}H_{31}NO_3Si_3$	0.04%	Amino acid
16	7.45	Lavandulol, TMS	$C_{13}H_{26}OSi$	0.36%	Alcohol
17	7.623	Arachidonic acid, TMS	$C_{23}H_{40}O_2Si$	0.13%	Organic acid
18	7.714	L-Threonine, 3TMS	$C_{13}H_{33}NO_3Si_3$	0.14%	Amino acid
19	7.814	(−)-Myrtenol, TMS	$C_{13}H_{24}OSi$	0.05%	Alcohol
20	8.976	Malic acid, 3TMS	$C_{13}H_{30}O_5Si_3$	24.46%	Organic acid
21	9.214	Salicylic acid, 2TMS	$C_{13}H_{22}O_3Si_2$	4.26%	Internal standard
22	9.353	L-Aspartic acid, 3TMS	$C_{13}H_{31}NO_4Si_3$	0.17%	Amino acid
23	9.427	4-Aminobutanoic acid, 3TMS	$C_{13}H_{33}NO_2Si_3$	0.41%	Amino acid
24	11.373	Xylose, 4TMS	$C_{17}H_{42}O_5Si_4$	0.12%	Sugar
25	11.499	Arabinitol, 5TMS	$C_{20}H_{52}O_5Si_5$	0.06%	Sugar Alcohol
26	11.759	D-Threitol, 4TMS	$C_{16}H_{42}O_4Si_4$	0.04%	Sugar Alcohol
27	11.816	Ribitol, 5TMS	$C_{20}H_{52}O_5Si_5$	0.08%	Sugar Alcohol
28	12.006	Erythritol, 4TMS	$C_{16}H_{42}O_4Si_4$	0.21%	Sugar Alcohol
29	12.128	D-Gluconic acid, 6TMS	$C_{24}H_{60}O_7Si_6$	0.05%	Organic acid
30	12.631	Shikimic acid, 4TMS	$C_{19}H_{42}O_5Si_4$	0.06%	Organic acid
31	12.791	Citric acid, 4TMS	$C_{18}H_{40}O_7Si_4$	1.20%	Organic acid
32	13.333	Quininic acid,5TMS	$C_{22}H_{52}O_6Si_5$	2.17%	Organic acid
33	13.498	D-Fructose, 5TMS	$C_{21}H_{52}O_6Si_5$	13.35%	Sugar
34	13.615	D-Galactose,5TMS	$C_{21}H_{52}O_6Si_5$	10.84%	Sugar
35	13.806	D-Glucose, 5TMS	$C_{24}H_{62}O_6Si_6$	20.35%	Sugar
36	14.014	Galactose oxime, 6TMS	$C_{24}H_{61}NO_6Si_6$	4.43%	Oxime
37	14.742	L-Rhamnose, 4TMS	$C_{18}H_{44}O_5Si_4$	0.10%	Sugar
38	16.09	Myo-Inositol, 6TMS	$C_{24}H_{60}O_6Si_6$	6.72%	Alcohol
39	16.424	D-Allose, oxime, 6TMS	$C_{24}H_{61}NO_6Si_6$	0.09%	Oxime

to literatures, the volatile oil extracted from the root of *A. dahurica* were mainly alkenes [17]. Present study about the volatile components of *A. dahurica* leaves indicated that they also contained variety of alkenes and alcohols. For instance, α-pinene, β-phellandrene, β-terpinene, 3-carene, β-cubebene and ocimene and so on existed both in *A. dahurica* leaves and roots but with their relative contents differed in these two parts. Gao et al. [18] applied GC-MS for comparing the volatile profiles of *A. dahurica* roots, leaves and stems during different growth periods. However, methanol instead of hexane was used for chemical extraction that making the results incomparable with the present study. They identified more alcohols, aldehydes and ketones.

5.2. Non-volatile metabolites by GC-MS

Malic acid was the most abundant primary metabolite identified in *A. dahurica* leaves. It participates in the energy metabolism of organisms and has the effects of anti-fatigue [19] and antioxidation [20]. Malic acid can improve the ability of oxidizing phosphate and energy metabolism in the processes of cell metabolism [21]. *A. dahurica* leaves contained 9 kinds of common amino acids, which are roughly the same as those detected in the root of *A. dahurica* [22]. Among them, leucine, threonine and valine are essential amino acids.

5.3. Coumarins in A. dahurica leaves

Coumarins were considered to be the main bioactive components of *A. dahurica* roots. They showed anti-inflammation, anti-cancer effects [23,24]. In the present study, seven coumarins, namely xanthotoxol, oxypeucedanin hydrate, byakangelicin, oxypeucedanin, imperatorin, phellopterin and isoimperatorin were detected in *A. dahurica* leaves. In the 2020 edition of Chinese Pharmacopoeia,

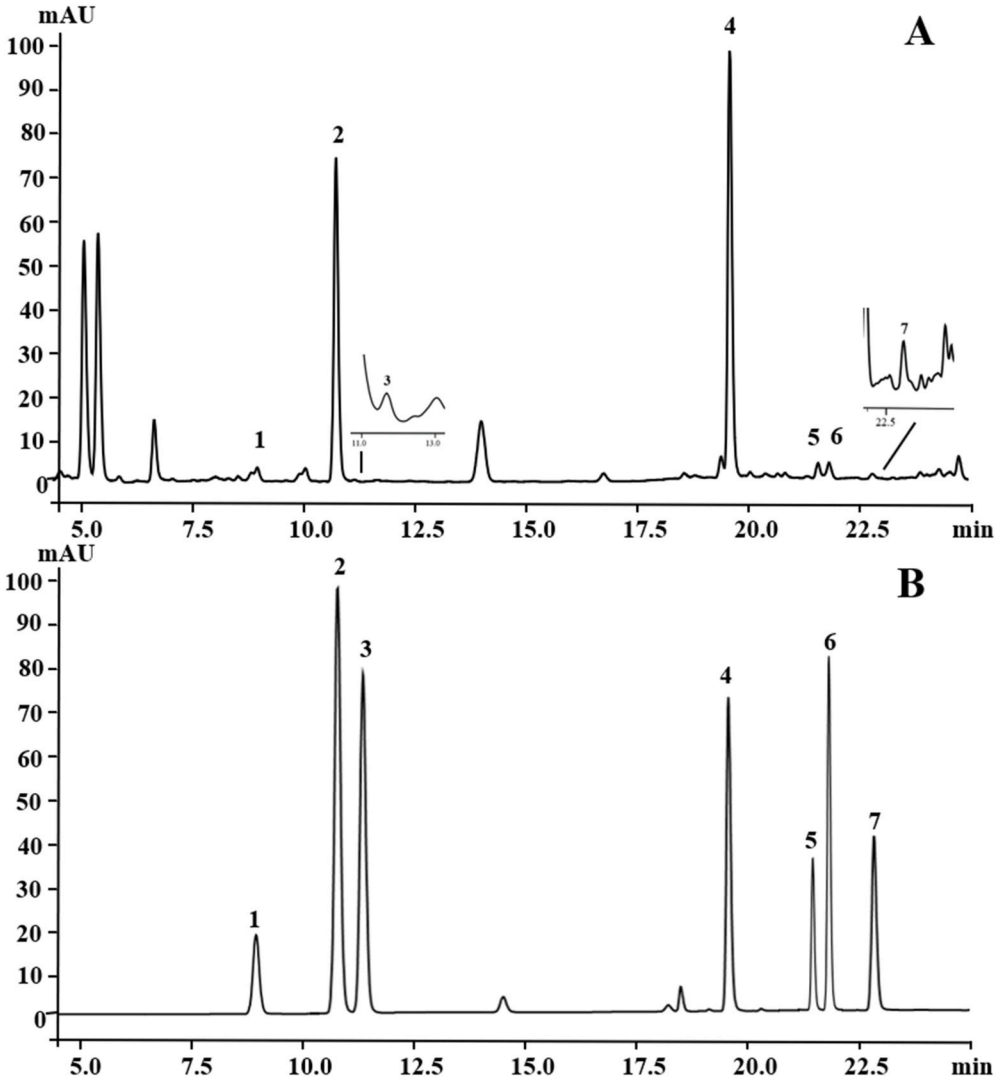

Fig. 3. HPLC chromatograms of *A. dahurica leaves* (A) and reference substances mixture (B).

Table 3
The regression equations, correlation coefficient (R^2), linear ranges, limit of detection (LOD) and limit of quantification (LOQ) of the seven quantified coumarins.

Compounds	Regression equation	R^2	Linear ranges (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)	Contents (μg/g) (mean ± SD, n = 5)
Xanthotoxol	$y = 2.42 \times 10^7x$-6557.70	0.9979	0.43–85	0.05	0.17	39.70 ± 28.34
Oxypeucedanin hydrate	$y = 2.82 \times 10^7x$+4533.68	0.9985	1.44–155	0.05	0.10	498.38 ± 165.81
Byakangelicin	$y = 1.21 \times 10^7x$-3750.76	0.9993	0.60–120	0.08	0.24	3.54 ± 3.27
Oxypeucedanin	$y = 9.06 \times 10^6x$+17829.67	0.9996	2.00–400	0.06	0.18	1475.58 ± 701.24
Imperatorin	$y = 2.33 \times 10^7x$-2060.24	0.9997	0.55–220	0.09	0.28	15.90 ± 8.01
Phellopterin	$y = 2.42 \times 10^7x$-8632.49	0.9995	1.28–255	0.13	0.32	27.58 ± 9.29
Isoimperatorin	$y = 4.76 \times 10^7x$-2168.48	0.9998	0.33–260	0.07	0.16	9.68 ± 10.64

imperatorin and isoimperatorin were important quality indexes of *A. dahurica* root [1]. *A. dahurica* leaves also contained these two components. Oxypeucedanin, the highest coumarin component in *A. dahurica* leaves, was reported to be able to enhance the anti-breast cancer effect of doxorubicin by inhibiting *P*-Sugar protein [25,26]. It could also reverse the drug resistance of doxorubicin in breast cancer treatment [27].

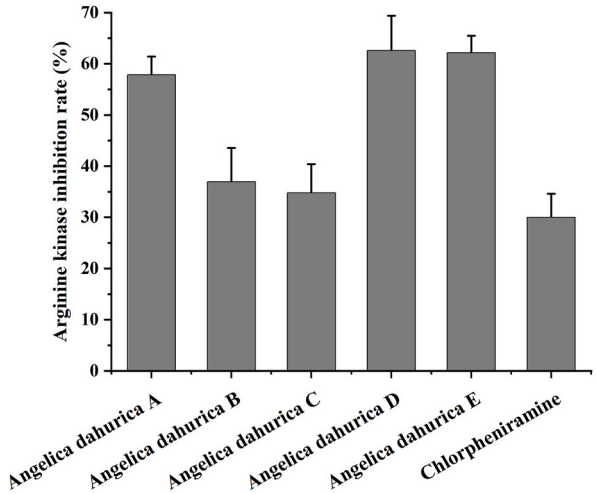

Fig. 4. AK inhibition rates of *A. dahurica leaves* and chlorpheniramine (n = 3).

Table 4
List of components with the grey relational coefficients more than 0.8.

Evaluation items	Coefficients
L-Aspartic acid	0.92
Arabinitol	0.912
Phellopterin	0.911
L-Alanine	0.901
Glycine	0.898
Xylose	0.884
Ethanolamine	0.881
Galactose oxime	0.88
D-Glucose	0.878
L-Threonine	0.878
D-Galactose	0.875
L-Serine	0.875
D-Gluconic acid	0.875
Galactose oxime	0.873
D-Fructose	0.872
1-Hexadecanol	0.868
D-Threitol	0.866
L-Proline	0.864
L-Valine	0.863
Myo-Inositol	0.858
4-Aminobutanoic acid	0.855
Malic acid	0.854
2-Butenedioic acid, (Z)-	0.848
Oxalic acid	0.848
Erythritol	0.842
Ribitol	0.841
Xanthotoxol	0.838
Citric acid	0.828
Oxypeucedanin	0.827
2-Butenedioic acid, (E)-	0.826
Glyceric acid	0.81
Lactic acid	0.807
Byakangelicin	0.803
Shikimic acid	0.802

5.4. Characterization of potential AK inhibitors in A. dahurica leaves

AK has become a hot target for developing new highly selective insecticides because it exists only in invertebrates [28,29]. AK also reported to be a main allergen in seafood [9,10]. Thus, the characterization of AK inhibitors would be helpful for mammal-friendly insecticide or anti-allergy drug development. Cu^{2+} and some flavonoids such as myricetin, baicalin, quercetin, kaempferol and

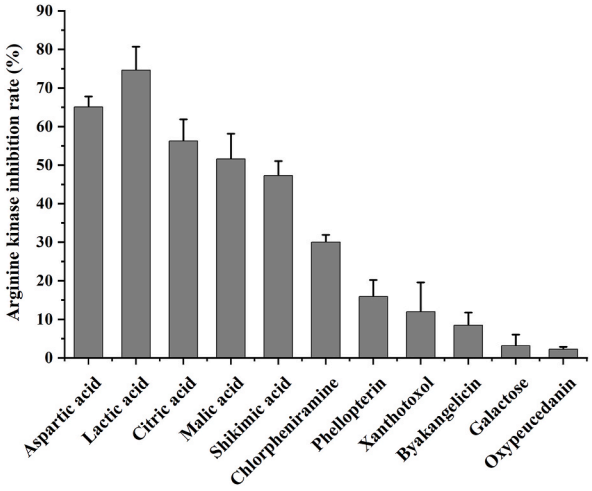

Fig. 5. AK inhibition rates of 10 compounds comparing with chlorpheniramine at 2 mM (n = 3).

berberine showed inhibitory effects on AK [30,31]. Molecular interaction studies were applied for evaluation of the mechanisms of inhibition on AK. Some molecules might interact with AK which leading to its exposure of hydrophobic groups [31,32]. Such spacial structure alteration deactivated AK. Some arginine analogues could inhibit the activity of AK by competitively binding to the enzyme or interfering with intermediates [33]. In the present study, correlation analysis between chemical composition and AK inhibition activity was applied for screening potential AK inhibitors. Comparing with chlorpheniramine, which is a commercial anti-allergic medicine, some components from *A. dahurica* leaves such as aspartic acid, lactic acid, citric acid, malic acid, shikimic acid showed better inhibitory effects on AK. These compounds could be candidates for further developing of pesticides or anti-allergic drugs.

6. Conclusion

A. dahurica is a medicinal plant with its roots widely used in clinical treatment. The large aboveground part of *A. dahurica* is normally dumped in the field. To avoid resource waste, the chemical composition of *A. dahurica* leaves was profiled by GC-MS and HPLC. In total, 131 compounds were identified and quantified including alkanes, alkenes, aldehydes, alcohols, esters, sugars, organic acids, amino acids and coumarins. The chemical diversity of *A. dahurica* leaves implied its potential development values. The extracts and components of *A. dahurica* leaves exhibited strong inhibitory effects on AK. The characterized potential AK inhibitors would have a broad application prospect for the development of new insecticides or anti-allergic drugs, especially for treatment of seafood allergy.

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CRediT authorship contribution statement

Aitong Yang: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation. **Junyan Zhang:** Writing – review & editing, Validation, Investigation. **Guangying Lv:** Investigation. **Jiabao Chen:** Data curation. **Long Guo:** Writing – review & editing, Resources. **Yan Liu:** Writing – review & editing, Supervision. **Yuguang Zheng:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Lei Wang:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

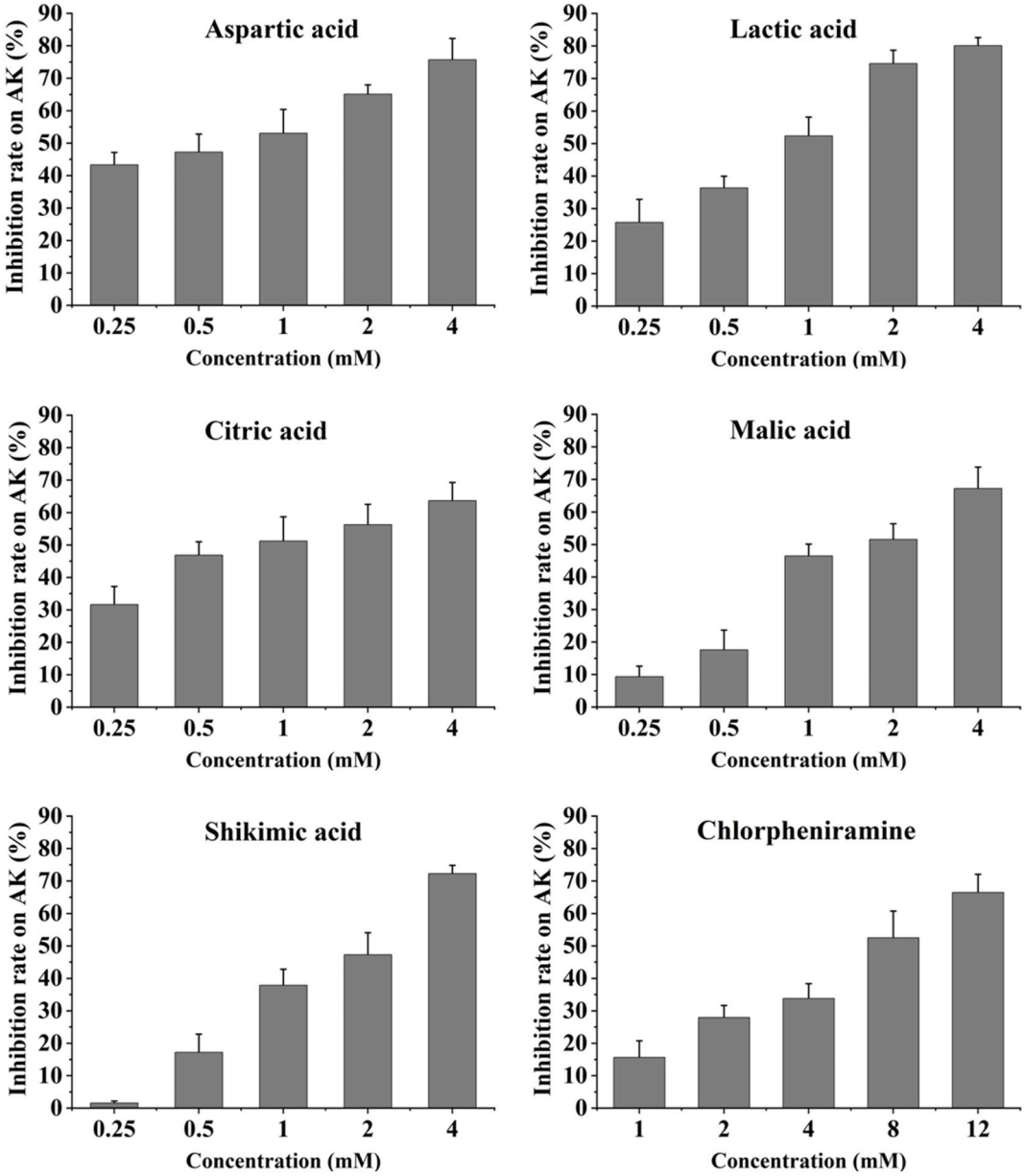

Fig. 6. Inhibition rates of aspartic acid, lactic acid, citric acid, malic acid, shikimic acid and chlorpheniramine on AK.

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