



Ethno-medicinal study of *Artemisia ordosica* Krasch. (traditional Chinese/Mongolian medicine) extracts for the treatment of allergic rhinitis and nasosinusitis

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ABSTRACT

Ethnopharmacological relevance: *Artemisia ordosica* Krasch. (AOK) has been used for rheumatic arthritis, cold headache, sore throat, etc. in traditional Chinese/Mongolian medicine and is used for nasosinusitis by local Mongolian “barefoot” doctors. Up to now, their mechanisms are still unclear.

Aim: To evaluate the *in vivo* anti-inflammatory and allergic rhinitis (AR) alleviating effect as well as *in vitro* antimicrobial activities of AOK extracts to verify its ethno-medicinal claims.

Materials and methods: Crude extracts (methanol/95%-ethanol/ethyl acetate) of AOK root/stem/leaf and fractions (petroleum ether/ethyl acetate/n-butanol/aqueous) of AOK root extract were prepared. Xylene-induced ear swelling model in mouse and ovalbumin (OVA)-induced AR model in guinea pig were established. Ear swelling degrees of mice were measured. The numbers of rubbing movement and sneezes of guinea pigs were counted to evaluate the symptoms of AR. The serum levels of histamine, INF- γ , IL-2/4/10, and VCAM-1 were measured by ELISA assay. The histological changes of nasal mucosa were investigated by light microscope after H&E staining. Antimicrobial activities of AOK extracts were also tested. LC-MS/MS analysis was performed to characterize the constituents of active extract and molecular docking was conducted to predict the biological mechanism.

Results: In ear-swelling model, extract (100.00 mg/kg) from the ethyl acetate layer of 95% ethanol (100.00 mg/kg) showed better swelling inhibition in mice than positive control (dexamethasone, 191.91 mg/kg). In AR model, extract from the ethyl acetate layer of 95% ethanol significantly alleviated the AR symptoms in guinea pigs, decreased the serum levels of histamine, INF- γ , IL-2/4/10, and VCAM-1, and reduced the infiltration of eosinophil in nasal mucosa. For *Staphylococcus aureus*, the ethyl acetate extract of AOK stem showed the highest inhibition (MIC = 1.25 mg/mL), for *Escherichia coli*, n-butanol layer of 95% ethanol extract of AOK root showed the highest inhibition (MIC = 15.00 mg/mL), for *Candida glabrata*, 95%-ethyl acetate extract of AOK leaf showed the best inhibition (MIC = 0.064 mg/mL), while ethyl acetate and n-butanol layers showed similar inhibition on MRSA (MIC = 7.50 mg/mL). LC-MS/MS characterization showed that dicaffeoylquinic acids account for more than 30% of ethyl acetate layer of AOK extract. Dicaffeoylquinic acids bind with histamine-1 receptor with high affinities and interesting modes.

Conclusions: Extracts from AOK had interesting anti-inflammatory activity in mice, alleviating effect against OVA-induced AR in guinea pigs, and antimicrobial activities *in vitro*, which support the ethno-medicinal use of it. The main constituents in ethyl acetate layer of AOK root extract are dicaffeoylquinic acids and could bind with histamine-1 receptor well. These findings highlighted the importance of natural product chemistry study of AOK.

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1. Introduction

Artemisia ordosica Krasch. (AOK, Compositae family, *Artemisia* genus), also known as *Ordos Artemisia*, *Oil Artemisia*, *Hara Sibag* (Mongolian name), is a main and representative plant in the northwest of China especially the Inner Mongolian district. Recent years, AOK was more extensively distributed by aircraft seeding for sand-fixation purpose (Zhang et al., 2003a; Wang et al., 2002). However, the allergic rhinitis (AR) and asthma cases caused by its pollen keep on increasing. The whole plant of AOK has been used in folk Chinese/Mongolian medicine. It was recorded in "*Chinese Materia Medica*" that AOK was beneficial to dehumidification, detoxicating and detumescence, attending the rheumatoid arthritis, cold headache, sore throat, carbuncle, and swollen boil (Editorial Committee of the Administration Bureau of Traditional Chinese Medicine, 1999). It was also recorded in "*Inner Mongolian Herbal Medicines*" and "*Medicinal Plants in the Desert Region of China*" that AOK root was beneficial for the treatment of nasal bleeding, etc. (Health Bureau of Revolutionary Committee of Inner Mongolia Autonomous Region, 1972; Chinese Academy of Sciences et al., 1973). In addition, AOK was used as anti-phlogistic, hemostatic, wind-expelling, and heat-clearing drug in traditional Mongolian medicine, and was clinically used for nasosinusitis by Mongolian "barefoot" doctors (Xiao et al., 2016). Moreover, preliminary phytochemistry study had revealed the main constituents of the whole AOK plant as essential oils, flavonoids, terpenoids, etc. (Zhao et al., 2005; Zhang et al., 2006; Qi et al., 2013). However, scientific evidence is still relatively few and is in a great need to verify its ethno-medicinal claims.

AR is a common and debilitating disease, characterized by inflammation of the nasal mucosa with hypersensitivity resulting from seasonal or perennial responses to specific environmental allergens. Environmental and lifestyle changes have resulted in a rapid increase in AR prevalence (Turner et al., 1988; von Mutius et al., 1994). Clinical symptoms, such as nasal itching, nasal congestion, sneezing and clear nasal discharge flow, significantly impede patient quality of life and work performance (Lee and Arriola, 1999). Moreover, AR usually leads to chronic nasosinusitis which often accompany with bacterial infection. The therapeutic principles for AR are to avoid allergens/triggering factors, and symptomatic treatment using different drugs, such as antihistamines, decongestants, cromolyn sodium, leukotriene modifiers, nasal glucocorticoids, nasal atropine and allergen immunotherapy (also known as allergy shots), to reduce an individual's sensitivity to the allergen (Quraishi et al., 2004). However, the current treatment for AR is still not ideal as none of these treatments is sufficiently efficacious for nasal obstruction when given as monotherapy (Nguyen and Casale, 2011). Recently, with a growing global interest in natural drugs, the search for appropriate anti-allergic agents has focused on traditional medicinal plants used in oriental clinics (Li and Srivastava, 2006). The inflammatory response in the nasal mucosa includes mast cell-mediated allergic responses and a late phase reaction characterized by inflammatory recruitment of secondary effector cells, such as eosinophils, basophils, and T cells expressing T-helper (Th) 2 cytokines including interleukin (IL)-4 which is a key factor for immunoglobulin (Ig)-E synthesis (Pawankar et al., 2011). Therefore, these cells are considered as major targets for basic and therapeutic research, and manipulation of mediator released from airway epithelial cells is the main therapeutic target for treating AR.

In this study, crude extracts (methanol/95%-ethanol/ethyl acetate) and fractions (petroleum ether/ethyl acetate/n-butanol/aqueous) of AOK root/stem/leaf were prepared. Xylene-induced ear swelling in mouse model was applied to observe the basal anti-inflammatory activity of AOK considering the main indications of its ethno-use. Then, ovalbumin (OVA)-induced AR in guinea pig model were established to further investigate the AR-alleviating effect of AOK, rubbing movement and sneezes of guinea pigs were inspected to evaluate the AR symptoms, the serum levels of cytokines were measured by ELISA assay, and histological changes of nasal mucosa were investigated. Moreover,

antimicrobial activities *in vitro* were also tested considering the folk use of AOK for the treatment of nasosinusitis. Finally, LC-MS/MS analysis was performed to characterize the constituents in the active extract and molecular docking was conducted to predict the biological mechanisms. These results would support the ethno-medicinal claims of this plant resource and provide scientific basis for further natural product chemistry study.

2. Materials and methods

2.1. Preparation of AOK extracts

Whole plants of AOK were collected in Oct 2017 from the Kangbashi district, Ordos, China. After identified, a voucher specimen was deposited in the Ordos School of Clinical Medicine, Inner Mongolia Medical University. Plant material was dried at 20 °C, powered, and stored in paper containers at ambient temperature.

For *in vivo* anti-inflammatory and anti-AR activity tests, 6 kg of dried and crushed powder of AOK root was put into a round bottom flask, and 6000 mL of 95% ethanol was added. After ultrasonically extracted for 3 times with each time of 30 min, the extract was filtered and concentrated under vacuum, and partitioned with PE, EA, and BA successively, then the solvent was recovered and the fractionation endpoint was judged by TLC. All crude extract and fraction powders were stored in refrigerator for the following studies.

For antimicrobial activity test, 10 g of dried and crushed powder of root, stem, and leaf of AOK were put into three round bottom flasks separately, and 100 mL of methanol, 95%-ethanol or PE were added, respectively. After ultrasonically extracted for 3 times with each time of 30 min. The extracts were filtered and concentrated under vacuum using a rotary evaporator. Meanwhile, the extract residue was measured and the extract rates were calculated. In addition, half of the 95% ethanol extract of AOK root was dispensed in the DW, and was partitioned with PE, EA, and BA successively, solvent was recovered and the fractionation endpoint was judged by TLC.

2.2. Chemicals and reagents

Dexamethasone acetate was purchased from Tianjin Lisheng Pharmaceutical Co., Ltd. (20150104, Tianjin, China); Loratadine capsules were obtained from Hainan Huluwa Pharmaceutical Group Co. (H20050628, Haikou, China); Guinea pig histamine, INF- γ , IL-2, IL-4, IL-10, and VCAM-1 ELISA kits were bought from BD Pharmingen (San Diego, USA); Xylene, OVA, and the other chemicals were obtained from Sigma-Aldrich Co. (St. Louis, USA); Standard microbial strains *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Candida glabrata* (ATCC 15126) were purchased from the National Center for Clinical Laboratories of China, while Methicillin-resistant *Staphylococcus aureus* (MRSA) was purified from clinic. Nutrient agar was bought from Hangzhou microorganism reagent Co. (130530, Hangzhou, China); Mueller-Hinton broth was obtained from Hangzhou microorganism reagent Co. (130509, Hangzhou, China); Blood agar plate was purchased from Tianjin Jinzhang technology and development Co. (20121211, Tianjin, China).

2.3. Animals and treatments

Kunming (KM) strain mice (6 weeks, weighing 20 ± 2 g) were purchased from the Experimental Animal Centre of Inner Mongolia Medical University (Huhhot, China). Male Hartley guinea pigs (250 ± 20 g) were obtained from Beijing Vital River Laboratory Animal Technology Co. (SCXK-2016-0011, Beijing, China). Animals were housed in a room at a temperature of 21–23 °C, a relative humidity of 30–70%, 12 h light/dark cycle, and had free access to food and water. All experimental procedures carried out in this study were performed in accordance with the Guidelines for the Care and Use of

Laboratory Animals of Inner Mongolia Medical University. This study was approved by the National and International Ethical Committee.

2.4. Anti-inflammatory assay in xylene-induced ear swelling model

For anti-inflammatory assay, mice were randomly divided into seven groups ($n=10$ per group) as follows: blank control group (Control, treated with normal saline 6.60 mL/kg), positive control group (Positive, treated with dexamethasone acetate 191.91 mg/kg), crude extract group (Crude, treated with 95% ethanol extract of AOK root, 100.00 mg/kg), PE group (treated with petroleum ether layer of crude AOK root extract, 100.00 mg/kg), EA group (treated with ethyl acetate layer of crude AOK root extract, 100.00 mg/kg), BA group (treated with *n*-butyl alcohol layer of crude AOK root extract, 100.00 mg/kg), DW group (treated with distilled water layer of crude AOK root extract, 100.00 mg/kg).

Drugs were administrated orally for 5 consecutive days. 30 min after the last dosing, 100 μ L of xylene was applied to both anterior and posterior surfaces of the right ear for another 1 h. After scarification, a round piece (diameter of 6 mm) of ear was cut and the wet weight was measured. Difference of weight between left and right ear was calculated as swelling degree. Inhibition ratio was calculated as: 1-swelling degree of treated group mouse/swelling degree of control group mouse. Statistical comparisons were carried out.

2.5. OVA-induced AR modelling in Guinea pigs and animal groups

Sensitization of animals to OVA was performed according to the method previously described (Boskabady and Ziaei, 2003; Boskabady and Adel-Kardan, 1999). As shown in Fig. 1, briefly, 0.3 mg of OVA and 30 mg of $\text{Al}(\text{OH})_3$ were mixed as a suspension in 1 mL of saline. Guinea pigs were sensitized with an intraperitoneal injection of 1 mL of suspension every two days. Two weeks later, they were locally treated by 50 μ L of OVA solution (2 mg OVA dissolved in 1 mL saline) by instilling into the bilateral nasal cavities using a micropipette for 7 days. Meanwhile, BC (blank control) group animals were treated with normal saline instead of OVA and $\text{Al}(\text{OH})_3$. After the last treatment of OVA, the AR symptoms of all animals were evaluated for 30 min according to the scoring criteria (including frequency of sneezing, nose-rubbing, and the extent of rhinorrhea) shown in Table 1 which was slightly modified based on the method of Zhao et al. (Zhao et al., 1993; Zhang et al., 2005). The animals whose total score was more than 5 were considered as successful models.

Successfully modelled animals were divided into six groups ($n=8$ per group) as follows: OVA (OVA-induced AR control, administered with normal saline) group, PC (positive control, administered with loratadine) group, PE (administered with petroleum ether layer extract) group, EA (administered with ethyl acetate layer extract) group, BA (administered with *n*-butyl alcohol layer extract) group, DW

Table 1
Scoring criteria of AR symptoms.

Score	Sneezing	Nose-rubbing	Rhinorrhea
1	1–3	1–4	At nostril
2	4–10	> 10	Discharge from nose
3	> 10	/	Drop from nose

(administered with distilled water layer extract) group. From days 21–30, tested group animals were intragastrically administered (1 time/day for 10 days) with AOK extracts (61.25 mg/kg/d), positive control was given loratadine (46.5 mg/kg/d), while BC group and OVA group animals were given with normal saline. The frequency of sneezing and nose-rubbing were counted for 30 min.

2.6. Evaluation of serum histamine, IL-2/4/10, IFN- γ , and VCAM-1 of AR Guinea pigs

At the end of the *in vivo* anti-allergic rhinitis study, the AR guinea pigs were anesthetized with 10% chloral hydrate. The blood was collected from abdominal aorta and placed at room temperature for 2 h. The samples were then centrifuged at 3500 g at 4 °C for 10 min, and the supernatant was collected and immediately stored at –20 °C until analyzed. Finally, serum histamine, IL-2, IL-4, IL-10, IFN- γ , and VCAM-1 were measured by enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's procedures. The concentrations of serum histamine, IL-2, IL-4, IL-10, IFN- γ , and VCAM-1 were calculated out by standard curve plots for the standard solutions in the kits.

2.7. Histological investigation of nasal mucosa tissues of AR Guinea pigs

The AR guinea pigs were sacrificed after the blood collection. The nasal tissues were separated from the skin, muscle, and soft tissue, and were immersed in freshly prepared 4% paraformaldehyde for 2 days. Then the tissues were decalcified in 15% nitric acid solution for 3 days. After that, the tissue were immersed in freshly prepared 4% paraformaldehyde at 4 °C for another 24 h, and was then processed for dehydration through graded alcohol and embedded in paraffin. Serial sections (4–6 μ m) were cut and affiliated onto 3-aminopropyl-ethoxy silane (APES) pretreated glass plate. After dried at 65 °C for 1 h, the slides were stored at 37 °C in oven. After deparaffinization, the sections were stained with H&E, Periodic Acid Schiff (PAS) for goblet cells and Giemsa for eosinophils. Sections were examined under a light microscope (Leica Microsystems GmbH, Wetzlar, Germany).

2.8. Antimicrobial assay

Nutrient agar was treated with super-heat (121 °C) and high

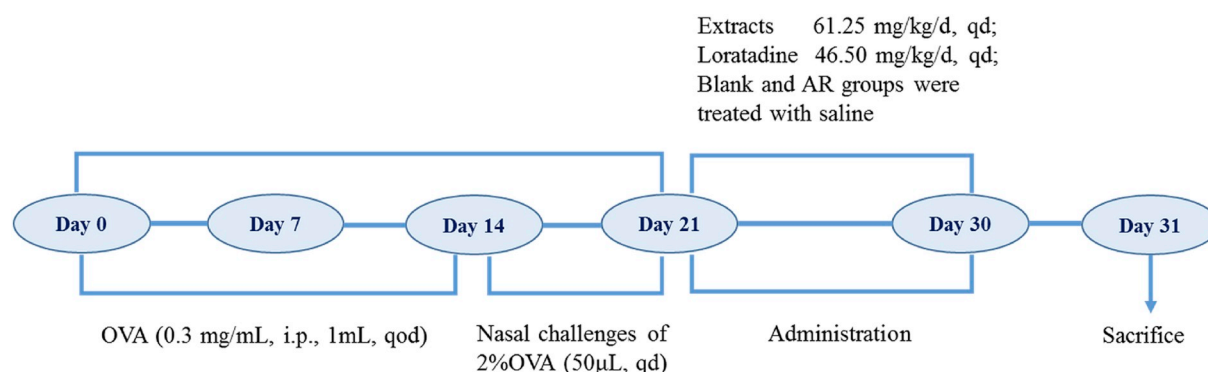


Fig. 1. The experimental protocol. AR animals were sensitized with intraperitoneal OVA mixing solution injection, and challenged with OVA by instilling into the bilateral nasal cavities. Treated groups were intragastrically given loratadine or AOK extracts from day 21 to day 30. Control groups were given normal saline.

pressure (15 Pa) for 15 min. Then the temperature was decreased to 60 °C and agar was poured into culture dish and the medium was plated. *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Candida glabrata* (ATCC 15126), and clinical MRSA were selected as testing microorganisms to evaluate the antimicrobial activity of the samples. Tested strains were placed onto nutrient agar plate and put into the incubator (35 °C, 24 h) for later use. Morphologically similar microbial colony on agar plate was selected and microbial concentration was adjusted with physiological saline, then diluted with nutrient broth to 10⁷ CFU/mL. The microbial suspension was inoculated in 15 min for later use.

The liquid tube double dilution method was applied to do the antimicrobial test of samples. The extracts were dissolved in normal saline and a moderate amount of tween 80 was added to improve the solubility. 100 µL of the dissolved samples were diluted with 1 mL of Mueller-Hinton broth, filtrated, and doubly diluted respectively with 1 mL of Mueller-Hinton broth to obtain different concentration gradients, and were named as 1–6. The 7th tube was added 1 mL of broth as positive control and the 8th was added same as negative control. A 0.5-McFarland standard suspension of *S. aureus* (*E. coli*, *C. glabrata*, or MRSA) in Mueller-Hinton broth was prepared and diluted 1:200 in broth. 50 µL of the above diluted microbial suspension was pipetted into 1–7 tube and was mixed well. The 1–8 tubes were incubated at the temperature of 35 °C for 20 h. The turbidity of the broth in each tube was investigated. When the control tube met the requirement, the lowest concentration of drug that the human eye cannot detect was recorded as the MIC.

2.9. HPLC analysis and HPLC-ESI-MS analysis

The samples of EA layer extract prepared as described in 2.1 were analyzed with a SCIEX Exion LC™ 20 AD system (AB SCIEX, Foster City, CA, USA) consisting of two pumps, a column oven, and photo-diode array (PDA) detector over 70 min period. SCIEX OS software was used for instrument control and data acquisition during the analysis. The separation was performed using a Phenomenex Kinetex C₁₈ column (100 × 2.1 mm, 2.6 µm, Torrance, CA, USA). The mobile phase was 0.02% formic acid and 2 mM ammonium formate in water (A) and methanol in acetonitrile (1:1; v/v; B). The separation was achieved by a linear gradient of 10–95% B over 70 min, followed by isocratic elution of 95% B for 5 min (shown in Table 2). The flow rate was 0.3 mL/min and the column temperature was maintained at 40 °C. The UV–vis spectra was recorded from 190 to 400 nm with selective wavelength monitoring at 254 nm.

MS/MS detection was carried out on an X500R QTOF mass spectrometer equipped with an electrospray ionization (ESI) source (SCIEX, Foster City, CA, USA). The parameters used for ionization source were set as follows: curtain gas (CUR) 35 psi; nebulizer gas (GS 1) 60 psi; auxiliary gas (GS 2) 40 psi; source temperature 600 °C; ion spray voltage (IS) 5500 V for the positive mode analysis and –4500 V for the negative mode analysis. The data acquisition and processing were performed using SCIEX OS software.

2.10. Molecular docking

The structure of human histamine-1 receptor (H1R) complex with first-generation antagonist doxepin was published in 2011 (Shimamura et al., 2011; PDB ID: 3RZE), and was widely used for the molecular docking. H1R-ligand cocrystal (3RZE) was downloaded from the Protein Data Bank (Berman et al., 2000). Docking preparation of receptor H1R was done within the molecular modeling software package Chimera 1.13.1 (National Institutes of Health, Bethesda, MD, USA) (Pettersen et al., 2004). Polar hydrogen and setting grid box parameters (a search grid box was set to cover the whole surface of the H1R protein to collect all possible orientations and conformations of the ligand paired with the protein) were added using MGLTools 1.5.6 (The Scripps

Table 2

The flow program of LC-MS characterization.

Time [min]	Flow [mL/min]	B.Conc [%]	B.Curve
8.00	0.3000	10.0	0
15.00	0.3000	20.0	0
20.00	0.3000	25.0	0
35.00	0.3000	40.0	0
50.00	0.3000	70.0	0
60.00	0.3000	95.0	0
65.00	0.3000	95.0	0
66.00	0.3000	10.0	0
70.00	0.3000	10.0	0

Research Institute, La Jolla, CA, USA) (Sanner, 1999; Morris et al., 2009). Docking calculations were performed using AutoDock Vina 1.1.2 software (The Scripps Research Institute, La Jolla, CA, USA) (Trott and Olson, 2010). Default settings and the Vina scoring function were applied. For ligand preparation, Chem3D Ultra 17.0 software (CambridgeSoft Corporation, Cambridge, MA, USA) was used to convert the 2D structures of candidates into 3D structural data. The analysis and visual investigation of ligand-protein interactions of docking poses were carried out using PyMol v 2.3.3 (The PyMOL Molecular Graphics System, Schrodinger LLC, New York, NY, USA).

2.11. Data analysis

All data were expressed as means ± SD of ten independent experiments. SPSS 13.0 software was used for One-way analysis of variance (ANOVA) followed by Dunnett's test and LSD analysis of variance between groups. All comparisons were made relative to the control group or positive control group, and the significance of any differences was indicated as **p* < 0.05.

3. Results

3.1. Ear-swelling inhibition in mice

Xylene-induced mouse ear swelling is a commonly used experimental model of acute inflammation (Xu and Bian, 2002). Ear swelling was obviously observed at 30 min after the wipe of xylene. It was shown in Fig. 2 that administration of dexamethasone and AOK root extract/fractions inhibited xylene-induced mouse ear swelling compared with the control group (administrated with normal saline). There was good inhibitory activity in the crude extract group, which indicated that 95% ethanol extract of AOK root could be considered as an anti-inflammatory agent. Furthermore, EA fraction (100 mg/kg) of crude AOK

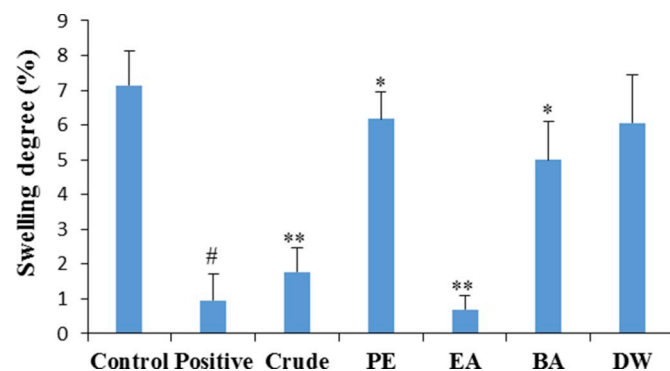


Fig. 2. Effect of 95% ethanol extract and fractions of AOK root on xylene-induced ear swelling degree in mice. Data were shown as means ± SD (n = 10 per group). Compared with Control, #*p* < 0.05; Compared with Positive, **p* < 0.05, ***p* < 0.01. PE, petroleum ether; EA, ethyl acetate; BA, n-butanol alcohol; DW, distilled water.

root extract showed much better inhibitory activity than dexamethasone (192.21 mg/kg), which suggested that the anti-inflammatory constituents of AOK root extract should be in the EA layer. It was shown in Fig. 2 that the inhibition ratio of EA group (90.39%) was higher than dexamethasone group (86.54%), which suggested that the constituents in EA fraction of the 95% ethanol extract of AOK root can dramatically inhibit the mouse ear swelling induced by xylene at the dose of 100.00 mg/kg.

3.2. Behavioral observation of animals

As shown in Fig. 3, compared with control, the numbers of nose-rubbings and sneezing of AR guinea pigs were significantly increased. After 10 days of treatment, compared with negative control, the numbers of nose-rubbing and sneezing in treated groups were reduced. These results suggested that the EA group showed the best activity.

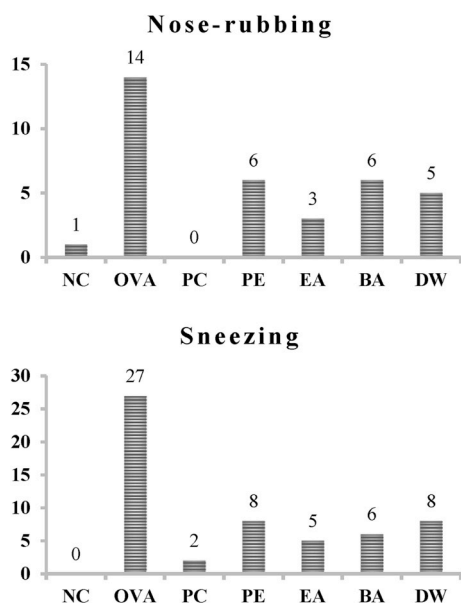


Fig. 3. Behavioral observation of animals treated with AOK root extracts. Left: nose-rubbing; Right: sneezing. The frequency of sneezing and nose-rubbing were counted for 30 min (n=8 per group). NC, normal control; OVA, ovalbumin; PC, positive control; PE, petroleum ether; EA, ethyl acetate; BA, n-butanol alcohol; DW, distilled water.

3.3. Serum levels of histamine, IL-2/4/10, IFN- γ , and VCAM-1

After behavior investigation, guinea pigs were anaesthetized by 10% chloral hydrate, blood samples were taken by cardiac puncture and tested according to the ELISA assay instructions. It was shown in

Table 3, compared with blank control, the levels of histamine, IFN- γ , IL-2, IL-4, IL-10, and VCAM-1 in OVA group guinea pigs significantly increased. Compared with OVA group, the levels of histamine, IFN- γ , IL-2, IL-4, IL-10, and VCAM-1 in EA group guinea pigs significantly decreased, especially histamine, IL-4, and VCAM-1. All in all, the EA layer of AOK root extract significantly reduced the level of serum cytokines and was considered as active fraction.

3.4. Histological changes of nasal mucosa tissues

Sensitization/challenge of OVA in guinea pigs induced histological changes in the nasal mucosa, such as epithelial disruption with mucosa thickening and infiltration of eosinophils into the nasal mucosa (Fig. 4). The administration of AOK root extracts especially EA fraction at the dose of 61.25 mg/kg/d inhibited the nasal destruction and the infiltration of eosinophils in OVA-sensitized/challenged guinea pigs (Fig. 4E) compared with the OVA control group (Fig. 4B). The inhibitory effect of EA fraction on eosinophil infiltration was similar to that in the loratadine-treated group (Fig. 4C). These data indicated that EA fraction of AOK root extract could prevent nasal destruction by allergic inflammation during AR.

3.5. Antimicrobial activities

For *S. aureus* (Table 4), polar extracts of root, stem, and leaf of AOK showed good inhibitory activities. Compared with methanol and 95% ethanol extracts, EA extracts of root, stem, and leaf of AOK showed good inhibitory activities (with MIC of 2.50, 1.25, and 7.50 mg/mL, respectively). Extracts of AOK stem by methanol, 95% ethanol, and EA showed better inhibitory activities (with MIC of 10.34, 3.75, and 1.25 mg/mL, respectively) compared with root and leaf extracts. For *E. coli* (Table 2), different polar extracts of root, stem, and leaf of AOK showed different inhibitory activities, in which 95% ethanol extract of AOK leaf showed the highest inhibition with MIC of 19.47 mg/mL. For *Candida glabrata*, EA extract of AOK leaf showed the best inhibition (MIC=0.064 mg/mL) while other extracts inhibited with MIC higher than 0.128 mg/mL.

Subsequently, the inhibitory activities of fractions of 95% ethanol extract of AOK root on *S. aureus*, *E. coli*, and MRSA were studied. As it was shown in Table 5, compared with the other fractions, the EA fraction showed the highest inhibition on *S. aureus* (MIC = 3.75 mg/mL), BA fraction showed the highest inhibition on *E. coli* (MIC = 15.00 mg/mL), and both the EA and BA fraction showed inhibitory effects on MRSA (MIC = 7.50 mg/mL). The PE fraction showed no inhibitory activities on the three bacterial strains.

3.6. Characterization of components in EA fraction

The developed analytical method was subsequently applied to the analysis of components in EA fraction, and more than 59 compounds were characterized by matching MS/MS spectrum with the SCIEX TCM

Table 3

The levels of serum cytokine of guinea pigs.

Groups	Histamine	IFN- γ	IL-2	IL-4	IL-10	VCAM-1
BC	22.33 $\pm$ 4.35	32.99 $\pm$ 8.90	56.25 $\pm$ 1.50	2.74 $\pm$ 0.21	62.14 $\pm$ 5.90	80.71 $\pm$ 9.83
OVA	37.45 $\pm$ 5.40**	55.26 $\pm$ 6.10	84.73 $\pm$ 8.50*	4.27 $\pm$ 0.38*	84.83 $\pm$ 4.40**	102.53 $\pm$ 7.23**
PC	23.42 $\pm$ 4.40 ⁺⁺	28.73 $\pm$ 4.10	67.80 $\pm$ 7.10	3.83 $\pm$ 0.28 ⁺	72.50 $\pm$ 5.20 ⁺⁺	79.26 $\pm$ 7.18 ⁺⁺
PE	29.13 $\pm$ 2.90 ⁺	62.87 $\pm$ 7.30	76.22 $\pm$ 9.30	4.08 $\pm$ 0.32	71.26 $\pm$ 11.30 ⁺⁺	93.21 $\pm$ 13.29
EA	17.53 $\pm$ 3.40 ⁺⁺	30.17 $\pm$ 9.00	66.93 $\pm$ 9.50 ⁺	2.39 $\pm$ 0.29	65.88 $\pm$ 4.10 ⁺⁺	79.85 $\pm$ 10.06 ⁺⁺
BA	23.29 $\pm$ 5.70 ⁺⁺	35.62 $\pm$ 6.70	65.73 $\pm$ 5.00 ⁺	4.21 $\pm$ 0.30	62.34 $\pm$ 7.10 ⁺	84.54 $\pm$ 8.60 ⁺⁺
DW	21.39 $\pm$ 6.70 ⁺⁺	47.58 $\pm$ 8.10	72.89 $\pm$ 3.00	3.55 $\pm$ 0.40	58.61 $\pm$ 10.30 ⁺⁺	83.63 $\pm$ 4.52 ⁺⁺

Note: Data were shown as means $\pm$ SD (n=8 per group). *compared with blank control, + compared with AR group, p < 0.01, **/+⁺; p < 0.05, */+. NC, normal control; OVA, ovalbumin; PC, positive control; PE, petroleum ether; EA, ethyl acetate; BA, n-butanol alcohol; DW, distilled water.

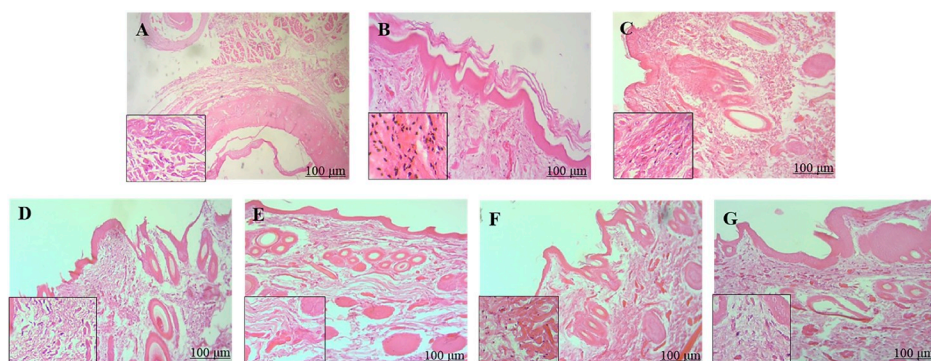


Fig. 4. Effects of AOK extracts on histological changes in the nasal tissues of OVA-induced AR guinea pigs. The histological changes of nasal mucosa were observed by microscope after H&E staining. (BC) normal, blank control; (NC) OVA control, negative control; (PC) OVA + loratadine, (46.50 mg/kg/d); (PE) OVA + petroleum ether fraction (61.25 mg/kg/d); (EE) OVA + ethyl acetate fraction (61.25 mg/kg/d); (BA) OVA + n-butanol alcohol fraction (61.25 mg/kg/d); (DW) OVA + distilled water fraction (61.25 mg/kg/d).

Table 4

Extraction rate and antimicrobial activities of root, stem, and leaf crude extracts of AOK on *Staphylococcus aureus*, *Escherichia coli*, and *Candida glabrata* (MIC, mg/mL).

Crude extracts	Extraction rate	<i>Staphylococcus aureus</i>			<i>Escherichia coli</i>			<i>Candida glabrata</i>		
		root	stem	leaf	root	stem	leaf	root	stem	leaf
Methanol	9.17%	30.78	10.34	30.50	30.14	22.01	36.42	> 0.128	> 0.128	> 0.128
95% Ethanol	10.5%	60.00	3.75	7.50	37.50	71.67	19.47	> 0.128	> 0.128	> 0.128
Ethyl acetate	0.32%	2.50	1.25	7.50	42.66	78.34	59.16	> 0.128	> 0.128	0.064

Table 5

Antibacterial activities of root extract fractions of AOK on *Staphylococcus aureus*, *Escherichia coli*, and MRSA (MIC, mg/mL).

Fractions	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	MRSA
PE	19.40	24.67	11.79
EA	3.75	30.00	7.50
BA	75.00	15.00	7.50
DW	30.00	> 140.10	15.00

HR MS/MS library (shown in Table 6, ranked by Area), in which dicaffeoylquinic acids were found to be main constituents (more than 30%). TOF MS/MS of typical constituents were shown in Fig. 5 which included the library search results.

3.7. Molecular docking

Autodock Vina preferred flexible docking which is crucial for accuracy of docking and the characterization of binding site and mode. In this study, we successfully redocked doxepin into H1R with RMSD of 1.650 Å even without the phosphate ion, which was considered to be important for target-binding of doxepin and olopatadine (Shimamura et al., 2011). After the redock validation, compounds with HPLC area of more than 1.00×10^8 (including caffeic acid and dicaffeoylquinic acids) were selected for molecular docking targeting on H1R.

As shown in Table 7, binding affinities of compounds 2–4 were higher than doxepin (Fig. 6A, -8.2 kcal/mol, 1st generation H1R antagonist) but lower than loratadine (Figs. 6B, -10.1 kcal/mol, 2nd generation H1R antagonist), the best ligand binding affinity was observed for 3,5-dicaffeoylquinic acid, which was calculated to be -9.7 kcal/mol (Fig. 6C), while 3,5-dicaffeoylquinic acid was calculated as -9.6 kcal/mol (Fig. 6D). According to docking simulation, these compounds occupied the H1R ligand-binding pocket in the same manner as doxepin and loratadine (Fig. 6A–D). Because the natural agonist histamine was much smaller than bulky H1R antagonists, some contraction of the binding pocket was likely to accompany ligand-induced H1R activation. Bulky compounds, capable of blocking both

activation-related contraction of the pocket and the Trp 428^{6,48} switch would be very efficient in locking H1R in an inactive conformation, which is considered to be important for the reduction of H1R basal activity by H1R antagonists (Bakker et al., 2001).

According to Linpin's Rule of 5, compounds with Log P value higher than 5 would be less druggable. Polyphenol structure of compounds 2–4 contributes to the much lower Log P value. However, H1R is located on the cell membrane and does not require the hydrophilic-hydrophobic balance. Therefore, the main constituents of the EA fraction of AOK root extract might be good candidate for the treatment of allergic diseases such as AR.

4. Discussion

AR is caused by IgE-mediated inflammation of airway mucosa with hypersensitivity, and various AR-alleviating drugs including antihistamines, anticholinergics, corticosteroids, and mast cell stabilizers are under clinical use. However, the adverse effects and low clinical efficacy still remain (Mygind, 1993; Bousquet et al., 2001), which motivate people to search for better drugs or agents. Many traditional medicines could improve multi-factorial diseases including AR and are considered a good treatment paradigm (Resnick et al., 2008). In which, AOK is a traditional Chinese/Mongolian medicine documented in several traditional medicinal encyclopedia and is being used to treat inflammatory diseases. In the present study, we confirmed that AOK had a therapeutic effect on inflammatory responses in xylene-induced ear swelling in mouse model. Considering the main components in AOK and their related biological activities as well as the close relationship between inflammation and AR, we further applied OVA-induced AR guinea pig model to assess the anti-allergic effect of AOK extracts.

Among animal models of allergic diseases, such as asthma and rhinitis, the guinea pig model of AR was proven to be a good model for the assessment of anti-allergic drugs, especially those targeting mast cell mediators (Groneberg et al., 2003). Sensitization/challenge of OVA in guinea pigs led to increase in serum levels of histamine and the infiltration of inflammatory cells including eosinophils in the epithelium of the nasal mucosa (Quraishi et al., 2004).

Table 6
Chemical components of AOK EA fraction characterized by LC-MS.

No.	Component Name	Formula	Adduct /Charge	m/z	Mass Error (ppm)	Retention Time	Area
1	Caffeic acid	C ₉ H ₈ O ₄	[M-H]-	179.0345	-2.7	8.37	2.34E+08
2	Isochlorogenic acid B	C ₂₅ H ₂₄ O ₁₂	[M-H]-	515.1186	-1.8	21.72	2.30E+08
3	Dicafeoyl quinic acid	C ₂₅ H ₂₄ O ₁₂	[M-H]-	515.1195	0	23.81	2.03E+08
4	Cynarin	C ₂₅ H ₂₄ O ₁₂	[M-H]-	515.1188	-1.4	22.11	1.81E+08
5	Hydroxycoumarin	C ₉ H ₆ O ₃	[M-H]-	161.0238	-3.7	14.57	5.17E+07
6	Ferulic Acid/Isoferulic acid	C ₁₀ H ₁₀ O ₄	[M-H]-	193.0501	-2.5	16.48	3.27E+07
7	Esculetin/Daphnetin	C ₉ H ₆ O ₄	[M-H]-	177.0189	-2.2	7.84	3.05E+07
8	Protocatechuic Aldehyde	C ₇ H ₆ O ₃	[M-H]-	137.0238	-4.2	4.8	2.96E+07
9	p-Coumaric acid	C ₉ H ₈ O ₃	[M-H]-	163.0396	-2.8	13.92	1.53E+07
10	Quinic acid	C ₇ H ₁₂ O ₆	[M-H]-	191.0556	-2.6	0.88	1.35E+07
11	10-Hydroxy-2-decenoic acid	C ₁₀ H ₁₈ O ₃	[M-H]-	185.1177	-3.1	37.63	1.18E+07
12	Isoscopoletin/Scopoletin/Isoscopoletin	C ₁₀ H ₈ O ₄	[M-H]-	191.0344	-2.9	14.83	1.08E+07
13	Protocatechuic acid	C ₇ H ₆ O ₄	[M-H]-	153.0189	-2.6	3.09	7.66E+06
14	Hyperin Isoquercitrin/Citric acid/L-Malic acid	C ₂₁ H ₂₆ O ₁₂	[M-H]-	463.0878	-0.9	20.07	3.76E+06
15	Fraxetin	C ₁₀ H ₈ O ₅	[M-H]-	207.0296	-1.6	13.17	2.71E+06
16	Amber Acid	C ₈ H ₆ O ₄	[M-H]-	117.0187	-5	1.35	2.64E+06
17	Succinic acid	C ₄ H ₆ O ₄	[M-H]-	117.0187	-5	1.35	2.64E+06
18	Esculin hydrate	C ₁₅ H ₁₆ O ₉	[M-H]-	339.0718	-1	4.65	2.63E+06
19	D-Galactose/D-(+)-Mannose/D-(+)-Glucose	C ₆ H ₁₂ O ₆	[M-H]-	179.0559	-0.9	0.86	1.47E+06
20	Calceoside B	C ₂₃ H ₂₆ O ₁₁	[M-H]-	477.1399	-0.7	19.5	1.33E+06
21	Hydroxygenkwanin/Diosmetin/Glycetein/Acacetin/Genkwanin	C ₁₆ H ₁₂ O ₆	[M-H]-	299.0554	-2.4	39.98	1.33E+06
22	Naringenin	C ₁₅ H ₁₂ O ₅	[M-H]-	271.061	-0.7	31.58	1.20E+06
23	Fumaric acid/Maleic acid	C ₄ H ₄ O ₄	[M-H]-	115.0033	-3	0.92	1.11E+06
24	Emodin	C ₁₅ H ₁₀ O ₅	[M-H]-	269.0449	-2.4	47.55	9.18E+05
25	3-Hydroxy-9,10-Dimethoxypterocarpan	C ₁₇ H ₁₄ O ₅	[M-H]-	299.0922	-0.9	48.04	9.15E+05
26	Pseudoginsenoside-RT5	C ₃₆ H ₆₂ O ₁₀	[M-H]-	653.4243	-4.2	61.07	8.54E+05
27	Mannitol/Sorbitol	C ₆ H ₁₄ O ₆	[M-H]-	181.0716	-0.9	0.84	6.31E+05
28	Uridine	C ₉ H ₁₂ N ₂ O ₆	[M-H]-	243.0619	-1.4	1.24	5.98E+05
29	Adenosine	C ₁₀ H ₁₃ N ₅ O ₄	[M-H]-	266.0892	-1.2	1.4	3.93E+05
30	Echinocystic acid	C ₃₀ H ₄₈ O ₄	[M-H]-	471.3476	-0.9	53.2	3.64E+05
31	Oleanolic acid	C ₃₀ H ₄₈ O ₃	[M-H]-	455.3515	-3.4	63.2	2.53E+05
32	Chrysophanol	C ₁₅ H ₁₀ O ₄	[M-H]-	253.0501	-2	44.89	2.43E+05
33	L(+)-Arginine	C ₆ H ₁₄ N ₄ O ₂	[M-H]-	173.1041	-1.6	0.8	2.02E+05
34	Hydroxytyrosol	C ₈ H ₁₀ O ₃	[M-H]-	153.0554	-2	10.45	1.60E+05
35	Rutin	C ₂₇ H ₃₀ O ₁₆	[M-H]-	609.1464	0.5	19.89	1.52E+05
36	Gallic acid	C ₇ H ₆ O ₅	[M-H]-	169.0139	-2.2	1.68	1.35E+05
37	Guanosine	C ₁₀ H ₁₃ N ₅ O ₅	[M-H]-	282.0841	-1.1	1.28	7.19E+04
38	Curdione	C ₁₅ H ₂₄ O ₂	[M+H]+	237.1843	-2.5	44.57	1.32E+07
39	Dimethoxycoumarin	C ₁₁ H ₁₀ O ₄	[M+H]+	207.065	-1	21.58	7.41E+06
40	Parthenolide	C ₁₅ H ₂₀ O ₃	[M+H]+	249.1482	-1.1	26.96	3.77E+06
41	Proline	C ₅ H ₉ NO ₂	[M+H]+	116.0705	-1	1.48	2.84E+06
42	Anise oil	C ₁₀ H ₁₈ O ₂	[M+H]+	149.096	-0.6	18.23	1.89E+06
43	Atractylenolide I/Dehydrocostus lactone	C ₁₅ H ₁₈ O ₂	[M+H]+	231.1376	-1.4	43.14	1.53E+06
44	6-Methylcoumarin	C ₁₀ H ₈ O ₂	[M+H]+	161.0594	-1.7	39.59	1.37E+06
45	Artemisinin	C ₁₅ H ₂₂ O ₅	[M+H]+	283.1537	-1	28.04	1.27E+06
46	Quercetin/Morin hydrate	C ₁₅ H ₁₀ O ₇	[M+H]+	303.0498	-0.5	20.07	1.18E+06
47	Adenine	C ₅ H ₅ N ₅	[M+H]+	136.0618	0.2	1.08	1.16E+06
48	Syringin/Syringa vulgaris + NH ₃	C ₁₇ H ₂₄ O ₉ .NH ₃	[M+H]+	390.1761	0.7	8.44	1.08E+06
49	Nicotinamide	C ₆ H ₆ N ₂ O	[M+H]+	123.0552	-0.6	1.28	1.07E+06
50	Pulegone/Camphor	C ₁₀ H ₁₆ O	[M+H]+	153.1272	-1.1	21.82	1.07E+06
51	Patchouli alcohol (loss H ₂ O)	C ₁₅ H ₂₄	[M+H]+	205.1946	-2.2	53.3	9.66E+05
52	Griffonilide	C ₈ H ₈ O ₄	[M+H]+	169.0494	-0.6	2.52	8.43E+05
53	Syringaldehyde	C ₉ H ₁₀ O ₄	[M+H]+	183.065	-0.9	14.58	7.79E+05
54	Aurantio-Obtusin	C ₁₇ H ₁₄ O ₇	[M+H]+	331.081	-0.8	36.42	3.72E+05
55	Isorhamnetin	C ₁₆ H ₁₂ O ₇	[M+H]+	317.0653	-0.8	23.9	3.61E+05
56	Linderane	C ₁₅ H ₁₆ O ₄	[M+H]+	261.1119	-0.9	20.95	3.18E+05
57	Methyl linoleate	C ₁₉ H ₃₄ O ₂	[M+H]+	295.2626	-1.8	58.17	2.86E+05
58	Nicotinic acid	C ₆ H ₅ NO ₂	[M+H]+	124.0391	-2.1	1.25	8.89E+04
59	Cordycepin	C ₁₀ H ₁₃ N ₅ O ₃	[M+H]+	252.1091	-0.1	1.47	3.28E+04

Nasal symptoms of AR are mediated by histamine and inflammatory mediators which are secreted from activated mast cells, eosinophils and basophils (Shinmei et al., 2009; Martynova et al., 2005). Mast cell-derived mediators collectively induced acute-phase clinical symptoms by enhancing vascular leakage, bronchospasm and activation of nociceptive neurons linked to parasympathetic reflex (Zhang et al., 2003b). Specific IgE produced from B cells mediated by IL-4 is a critical step in the allergic cascade (Pawankar et al., 2011; Quraishi et al., 2004),

VCAM-1 play an important role in eosinophilic inflammation in allergic rhinoconjunctivitis. IL-4 induced the expression of VCAM-1, leading to the VCAM-1-mediated adhesion of eosinophils to activated endothelium. Upon allergen challenge, eosinophils were recruited into several organs, such as lungs, nose skin and eyes, and biological fluids, induced the release of oxygen radicals, newly generated mediators, such as platelet-activating factor, etc. (Tomassini et al., 1996). In this study, EA fraction of AOK root extract significantly inhibited the release

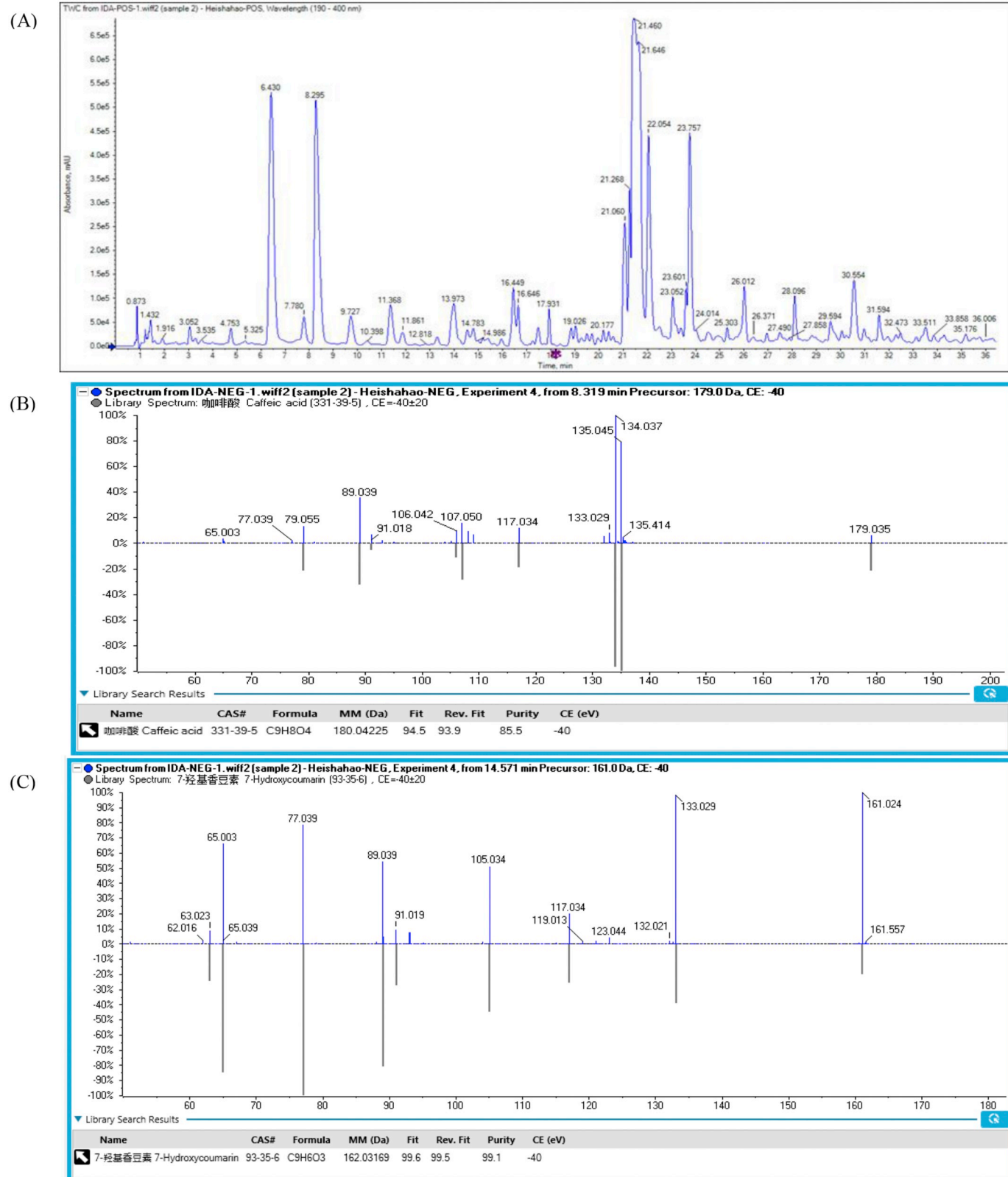


Fig. 5. (A) HPLC chromatogram of EA fraction of AOK root extract; (B) TOF MS/MS spectrum for caffeic acid on negative mode; (C) TOF MS/MS spectrum for 7-hydroxycoumarin on negative mode; (D) TOF MS/MS spectrum for 6,7-dimethoxycoumarin on positive mode; (E) TOF MS/MS spectrum for patchouli alcohol on positive mode.

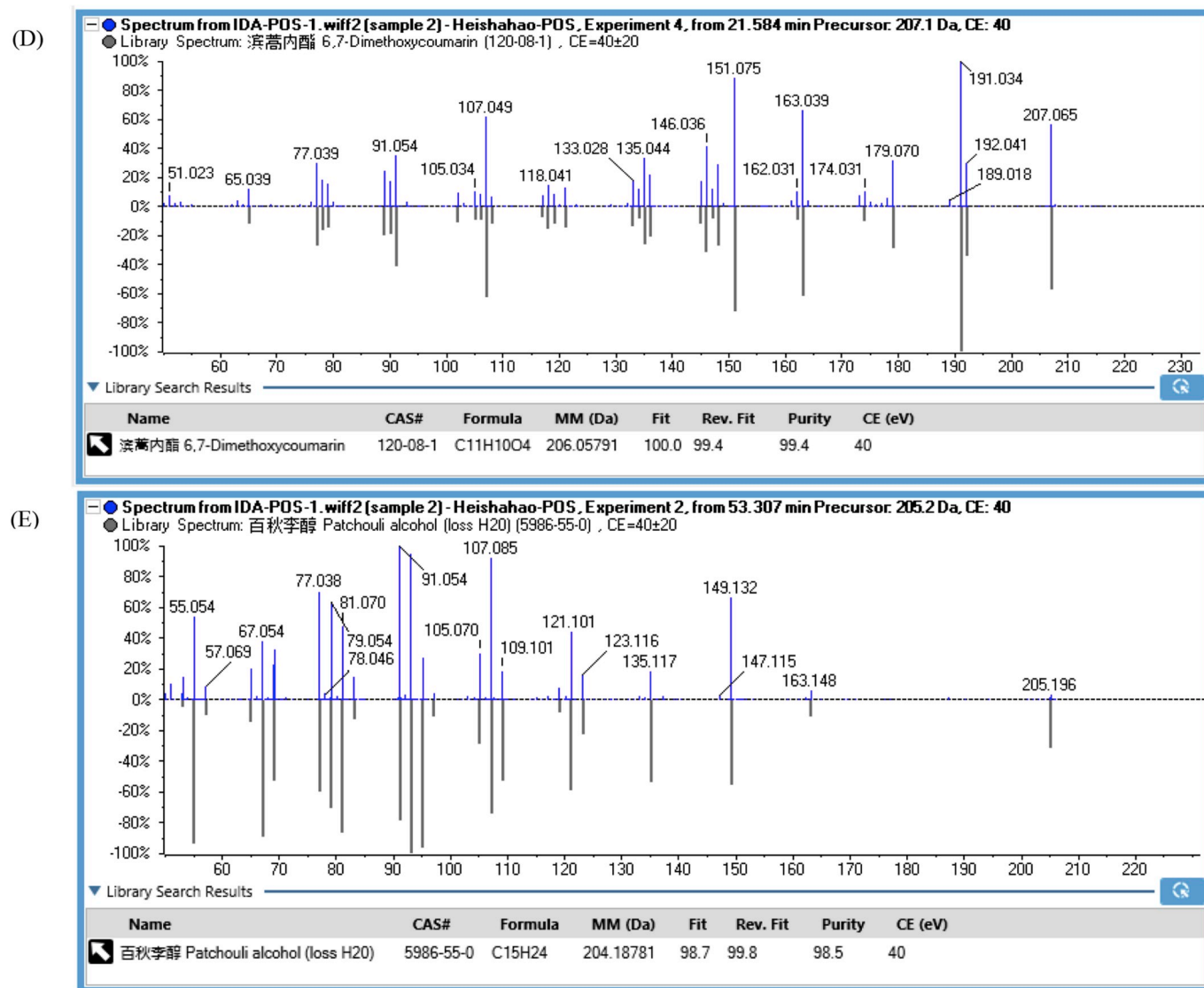


Fig. 5. (continued)

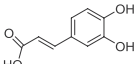
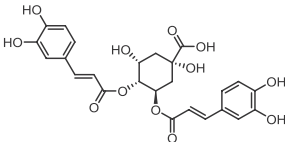
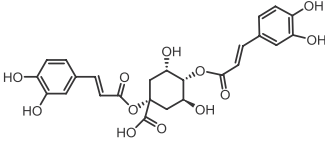
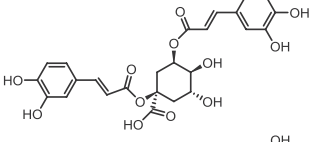
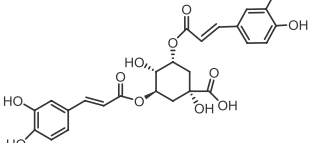
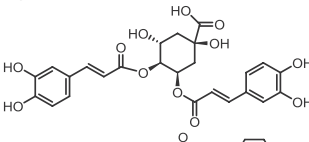
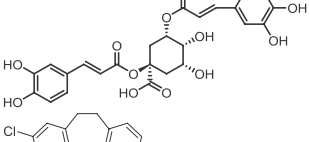
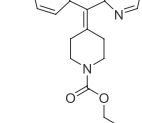
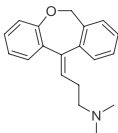
of histamine and the production of IL-4 and VCAM-1 in the serum of OVA-sensitized/challenged guinea pigs in OVA-sensitized/challenged guinea pigs. Meanwhile, EA fraction significantly inhibited the infiltration of eosinophils in the epithelium of the nasal mucosa. These results demonstrated that EA fraction of AOK root extract could reduce allergic reactions and protect guinea pigs against allergen-induced allergic immune responses of AR.

AR usually leads to chronic nasosinusitis which is associated with bacterial infection in many cases. AOK was reported to show antibacterial activity, and was clinically used for nasosinusitis by Mongolian “barefoot” doctors (Xiao et al., 2016). In this study, the antimicrobial activities of AOK extracts were tested. Compared with the other extracts, the EA extract of AOK stem showed the best antibacterial activity to *Staphylococcus aureus*, BA fraction of 95% ethanol extract of AOK root showed the best antibacterial activity to *Escherichia coli* and MRSA, while EA extract of AOK leaf showed the best inhibition to *Candida glabrata*. Extracts from AOK might be complementary or alternative as antibiotic agent in the treatment of nasosinusitis.

Modern research had revealed that there were flavonoids, terpenoids, etc. in AOK, and these natural products often possess various biological activities. However, pharmaceutical especially the biological material basis research of AOK is relatively few. Based on the developed analytical method and TCM HR MS/MS Library database, more than 59 compounds were MS-characterized from the active extract of AOK root, in which dicaffeoylquinic acids were found to be more than 30%. Increasing evidence has shown that dicaffeoylquinic acids have anti-inflammatory, anti-allergic, antimicrobial, and anti-oxidant activities (Zhang et al., 2018). The ability of AOK extract to control the allergic inflammatory response in AR might be a result of the synergistic anti-allergic and anti-inflammatory effects of its main constituents. Nevertheless, further studies should be focused on identifying the active compounds and the mechanisms of the anti-allergic effects.

A good many of biological targets play important roles during AR process, in which, H1R is activated by histamine released from mast cells and is stimulated by various antigens. H1R belongs to the G-protein-coupled-receptor (GPCR) superfamily and is expressed in cells that

Table 7
Molecular docking and binding results targeting on H1R.

Number	Compounds	Molecular structure	Molecular weight	Log P	Affinity (kcal/mol)
1	Caffeic acid		180.16	1.15	−7.2
2	3,4-Dicaffeoylquinic acid (Isochlorogenic acid B)		516.46	0.94	−9.1
3	1,4-Dicaffeoylquinic acid		516.46	0.94	−8.9
	1,5-Dicaffeoylquinic acid		516.46	0.94	−8.3
	3,5-Dicaffeoylquinic acid		516.46	0.94	−9.7
	4,5-Dicaffeoylquinic acid		516.46	0.94	−9.6
4	1,3-Dicaffeoylquinic acid (cynarin)		516.46	0.94	−9.2
2nd generation H1R antagonist	loratadine		382.89	4.13	−10.1
1st generation H1R antagonist	doxepin		279.38	3.72	−8.2

mediates allergies and other pathophysiological diseases. Curcumin (with a π -electron conjugated system in the molecular structure) was found to be immunoregulatory, anti-inflammatory, and anti-allergic (Aggarwal et al., 2007; Antony et al., 1999; Thakare et al., 2013), and molecular docking study had revealed that curcumin bound with H1R with high affinity and interesting hydrogen bonding information (Riza et al., 2019). In this study, considering the similar compound skeleton of dicaffeoylquinic acid and curcumin, we performed molecular docking of dicaffeoylquinic acids targeting on H1R, in which 3,5-dicaffeoylquinic acid showed the highest binding affinity and interesting binding mode. Besides, caffeoylquinic acids were already known to be capable of inhibiting the release of histamine (Wang et al., 2006; Chen et al., 2000). Therefore, dicaffeoylquinic acids, the main constituents in EA fraction of AOK root extract, might be beneficial for allergic diseases through inhibiting the histamine release and antagonizing H1R simultaneously.

5. Conclusions

In the study, we demonstrated that extracts from AOK exhibited *in vivo* potential anti-inflammatory and *in vivo* AR-therapeutic properties by suppressing allergic and inflammatory mediators, such as histamine, IL-4, IFN- γ , etc., inhibiting nasal obstruction and eosinophils infiltration, and exhibited *in vitro* antimicrobial activities. The ability of AOK extracts to control the allergic inflammatory response in AR might be a result of the synergistic anti-allergic and anti-inflammatory effects of the dicaffeoylquinic acids in it. To the best of our knowledge, this is the first report on anti-inflammatory and AR-therapeutic properties of AOK extracts, which provided a scientific evidence and basis for its clinical application. As extracts and fractions are mixtures of different constituents, more studies should be carried out unravelling the constituents and their biological activities.

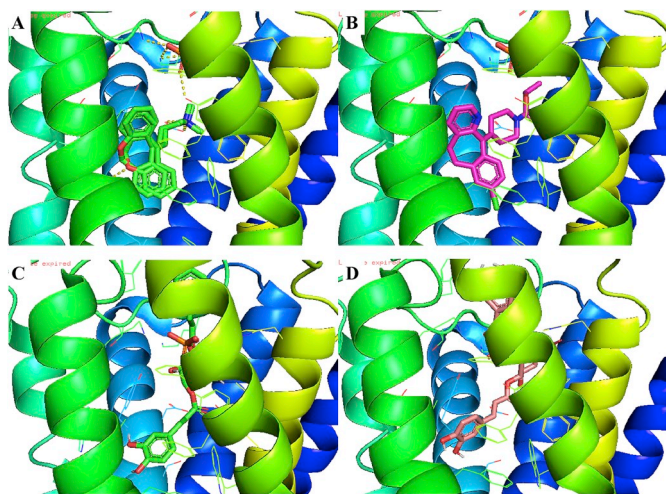


Fig. 6. Ligand binding conformations with H1R. (A) superposition of redocked doxepin with published doxepin-H1R cocrystal 3ZRE (RMSD of 1.650 Å); (B) binding mode of loratadine with H1R ligand binding pocket (binding affinity: 10.1 kcal/mol); (C) binding mode of 3,5-dicaffeoylquinic acid with H1R ligand binding pocket (binding affinity: 9.7 kcal/mol); (D) binding mode of 4,5-dicaffeoylquinic acid with H1R ligand binding pocket (binding affinity: 9.6 kcal/mol).

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jep.2019.112262>.

References

- Aggarwal, B.B., Sundaram, C., Malani, N., Ichikawa, H., 2007. Curcumin: the Indian solid gold. *Adv. Exp. Med. Biol.* 595, 1–75.
- Antony, S., Kuttan, R., Kuttan, G., 1999. Immunomodulatory activity of curcumin. *Immunol. Investig.* 28, 291–303.
- Bakker, R.A., Schoonus, S.B., Smit, M.J., Timmerman, H., Leurs, R., 2001. Histamine H₁-receptor activation of nuclear factor- κ B: roles for G β γ - and G $\alpha_{q/11}$ -subunits in constitutive and agonist-mediated signaling. *Mol. Pharmacol.* 60, 1133–1142.
- Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N., Bourne, P.E., 2000. The protein data bank. *Nucl. Acids Res.* 28, 235–242.
- Boskabady, M.H., Adel-Kardan, S., 1999. Increased muscarinic receptor blockade by atropine in tracheal chains of ovalbumin-sensitized Guinea pigs. *Pharmacology* 58, 300–308.
- Boskabady, M.H., Ziaei, T., 2003. Effect of ascorbic acid on airway responsiveness in ovalbumin sensitized Guinea pigs. *Respirology* 8, 473–478.
- Bousquet, J., Van Cauwenberge, P., Khaltaev, N., 2001. Aria workshop group, World Health Organization. Allergic rhinitis and its impact on asthma. *J. Allergy Clin. Immunol.* 108, S147–S334.
- Chen, S., Gong, J., Liu, F., Mohammed, U., 2000. Naturally occurring polyphenolic antioxidants modulate IgE-mediated mast cell activation. *Immunology* 100, 471–480.
- Department of desert studies, Gansu institute of glacial permafrost desert, Chinese academy of sciences, 1973. Medicinal Plants in the Desert Region of China (Zhongguo Shamo Diqu Yaoyong Zhiwu). Gansu People's Publishing House, Lanzhou.
- Editorial Committee of the Administration Bureau of Traditional Chinese Medicine, 1999. Chinese Materia Medica (Zhonghua Bencao). Shanghai Science & Technology Press, Shanghai.
- Groneberg, D.A., Bielory, L., Fischer, A., Bonini, S., Wahn, U., 2003. Animal models of allergic and inflammatory conjunctivitis. *Allergy* 58, 1101–1113.
- Health Bureau of Revolutionary Committee of Inner Mongolia Autonomous Region, 1972.

- Inner Mongolian Herbal Medicines (Neimenggu Zhongcaoyao). Inner Mongolia People's Publishing House, Huhhot.
- Lee, N.P., Arriola, E.R., 1999. How to treat allergic rhinitis. *West. J. Med.* 171, 31–34.
- Li, X.M., Srivastava, K., 2006. Traditional Chinese medicine for the therapy of allergic disorders. *Curr. Opin. Otolaryngol. Head Neck Surg.* 14, 191–196.
- Martynova, M.G., Bystrova, O.A., Moiseeva, O.M., Evdonin, A.L., Kondratov, K.A., Medvedeva, N.D., 2005. The presence of ANP in rat peritoneal mast cells. *Cell Res.* 15, 811–816.
- Morris, G.M., Huey, R., Lindstrom, W., Sanner, M.F., Bewle, R.K., Goodsell, D.S., Olson, A.J., 2009. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J. Comput. Chem.* 16, 2785–2791.
- Mygind, N., 1993. Glycocorticosteroids and rhinitis. *Allergy* 48, 476–490.
- Nguyen, T.H., Casale, T.B., 2011. Immune modulation for treatment of allergic disease. *Immunol. Rev.* 242, 258–271.
- Pawankar, R., Mpro, S., Ozu, C., Kimura, S., 2011. Overview on the pathomechanisms of allergic rhinitis. *Asia Pac. Allergy* 1, 157–167.
- Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., Ferrin, T.E., 2004. UCSF Chimera-A visualization system for exploratory research and analysis. *J. Comput. Chem.* 13, 1605–1612.
- Qi, J.M., Zhang, X., Lv, Q.Z., 2013. Density functional theory calculations on antioxidant activity of four flavones from herbs of *Artemisia Ordosica*. *Comp. Appl. Chem.* 30, 267–272.
- Quraishi, S.A., Davies, M.J., Craig, T.J., 2004. Inflammatory responses in allergic rhinitis: traditional approaches and novel treatment strategies. *J. Am. Osteopath. Assoc.* 104, S7–S15.
- Resnick, E.S., Bielory, B.P., Bielory, L., 2008. Complementary therapy in allergic rhinitis. *Curr. Allergy Asthma Rep.* 8, 118–125.
- Riza, Y.M., Parves, M.R., Tithi, F.A., Alam, S., 2019. Quantum chemical calculation and binding modes of H1R; a combined study of molecular docking and DFT for suggesting therapeutically potent H1R antagonist. *In Silico Pharmacol.* 7, 1.
- Sanner, M.F., 1999. Python: a programming language for software integration and development. *J. Mol. Graph. Model.* 17, 57–61.
- Shimamura, T., Shiroishi, M., Weyand, S., Tsujimoto, H., Winter, G., Katritch, V., Abagyan, R., Cherezov, V., Liu, W., Han, G.W., Kobayashi, T., Stevens, R.C., Iwata, S., 2011. Structure of the human histamine H₁ receptor complex with doxepin. *Nature* 475, 65–72.
- Shinmei, Y., Yano, H., Kagawa, Y., Izawa, K., Akagi, M., Inoue, T., Kamei, C., 2009. Effect of Brazilian propolis on sneezing and nasal rubbing in experimental allergic rhinitis of mice. *Immunopharmacol. Immunotoxicol.* 31, 688–693.
- Thakare, V.N., Osama, M.M., Naik, S.R., 2013. Therapeutic potential of curcumin in experimentally induced allergic rhinitis in Guinea pigs. *Int. Immunopharmacol.* 17, 18–25.
- Tomassini, M., Magrini, L., De Petrillo, G., Adriani, E., Bonini, S., Balsano, F., Bonini, S., 1996. Serum levels of eosinophil cationic protein in allergic disease and natural allergen exposure. *J. Allergy Clin. Immunol.* 97, 1350–1355.
- Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 31, 455–461.
- Turner, K., Stewart, G., Woolcock, A., Green, W., Alpers, M., 1988. Relationship between mite densities and the prevalence of asthma: comparative studies in two populations in the Eastern Highlands of Papua New Guinea. *Guin. Clin. Exp. Allergy* 18, 331–340.
- von Mutius, E., Martinez, F.D., Fritzsche, C., Nicolai, T., Roell, G., Thiemann, H.-H., 1994. Prevalence of asthma and atopy in two areas of West and East Germany. *Am. J. Respir. Crit. Care Med.* 149, 358–364.
- Wang, L.Q., Chen, S.H., Hao, L.Z., 2002. The study of ecological biological characters & geographical distribution law of *Artemisia ordosica* Krasch. *J. Arid Land Resour. Environ.* 16, 95–98.
- Wang, Y., Wang, N.L., Yao, X.S., Kitagawa, S., 2006. Caffeoylquinic acid derivatives from *Bidens parviflora* and their antihistamine release activities. *Chin. Tradit. Herb. Drugs* 37, 966–970.
- Xiao, B., Bai, J.J., Qi, L., Lu, L.S., Tian, X.R., Yin, J., Su, Y.X., 2016. Research progress on resource distribution, chemical components, and pharmacological activities of *Artemisia ordosica* Krasch. *J. Chin. Pharm.* 13, 1862–1864.
- Xu, S.Y., Bian, R.L., 2002. Experimental Methodology of Pharmacology. People's Medical Publishing House, Beijing.
- Zhang, J., Ma, J.Y., Yao, J., Yang, Y.L., Huang, A.L., Ji, X.H., 2003a. Research on the resource utilization of *Artemisia ordosica*. *Chin. Wild. Plant Resour.* 22, 27–29.
- Zhang, H.Q., Sun, Y., Xu, F., 2003b. Therapeutic effects of interleukin-1 receptor antagonist on allergic rhinitis of Guinea pig. *Acta Pharmacol. Sin.* 24, 251–255.
- Zhang, R., Yu, Y., Du, J.R., Liu, J., Zhang, J., Wei, T., Li, X., Li, J., Long, R., 2005. Influence of Xinqin tablets on Guinea-pig nasal hypersensitivity. *China J. Chin. Mater. Med.* 30, 785–788.
- Zhang, T., Hu, T., Jiang, J., Zhao, J., Zhu, W., 2018. Antioxidant and anti-inflammatory effects of polyphenols extracted from *Ilex latifolia* Thunb. *RSC Adv.* 8, 7134–7141.
- Zhang, W., Zhao, D.B., Li, M.J., Liu, X.H., Wang, H.Q., 2006. Studies on flavonoid constituents from herbs of *Artemisia ordosica* Krasch. *Chin. J. Chin. Mater. Med.* 31, 1959–1961.
- Zhao, D.B., Yang, Y.X., Zhang, W., Liu, X.H., Wang, H.Q., 2005. Studies on flavonoids from herb of *Artemisia ordosica* Krasch. *China J. Chin. Mater. Med.* 30, 1430–1432.
- Zhao, X.J., Dong, Z., Yang, Z.Q., Zhao, D.R., 1993. A nasal allergy model developed in Guinea pig. *Chin. J. Otorhinolaryngology* 28, 17–18.