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Antioxidant Activity and Irritation Test of Extracts Obtained from *Angelica dahurica*

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Abstract

In this study, we assessed the free radical scavenging and xanthine oxidase inhibitory activities of extracts isolated from the dried roots and stems (including leaves) of *Angelica dahurica*. The irritation response from these extracts was also assessed to determine potential cosmetic use. Both sources of *A. dahurica* extracts exhibited radical scavenging properties to different extents. The free radical scavenging potency (EC_{50}) of the stems (including leaves) of *A. dahurica* was 243.33 μg/mL, which is significantly lower ($p<0.01$) than that observed of the roots (1,161.79 μg/mL). The inhibition values (EC_{50}) of xanthine oxidase were as follows: 435.19 μg/mL (roots) and 434.66 μg/mL (stems). We noted no significant differences between the two plant parts with regard to ability to inhibit xanthine oxidase activity. After the application of *A. dahurica* extracts to rabbits for skin and eye irritation tests, no negative effects were observed; therefore, the extracts are considered to be non-irritating to the skin and eye.

Key words: *Angelica dahurica*, antioxidant, irritation, free radical scavenging, xanthine oxidase inhibition

INTRODUCTION

Many plants have been extensively utilized as biological materials for food and natural products, and chemicals from plants have been isolated in many searches for new compounds for the treatment of diseases, as well as for functional foods and cosmetics (1). As a source for a variety of cosmetics, the plant products are often formulated as hot water extracts, displaying some pharmacological activity and no toxicity.

A. dahurica is a perennial herb that grows to a height of 2.5 m. The plant is characterized by a hollow stem, large three-branched leaves, and umbels bearing many white flower heads. The plant grows wild in thickets located in China, Japan, Korea, and Russia. The roots have been identified as containing a sweat-inducing agent, which is capable of countering harmful external influences on the skin, including cold, heat, dampness, and dryness (2). *A. dahurica* and its extracts have also been proven to be effective in the treatment of skin ailment (acne, psoriasis), erythema, headache, toothache, sinusitis, colds, flu and excessive leukorrhea (2-4). According to the results of a recent study, extracts of this plant can protect against dexamethasone-induced disorders, and have also been shown to exhibit liver-protective activity, antimicrobial activity (5,6), anti-inflammatory activity, and antimutagenic activity (7). Over twenty coumarins and furanocoumarins, including coumarin, scopoletin, psoralen, xanthotoxin, bergapten, and imperatorin have been isolated from *A. dahurica* (8-10).

In this study, we evaluated the free radical scavenging activity and xanthine oxidase inhibitory activity of the dried roots and stems (including leaves) of *A. dahurica*, as well as its potential irritability to the skin and eyes.

MATERIALS AND METHODS

Extract preparation

The dried roots and stem parts (including leaves) of *A. dahurica* (100 g) were cut into small pieces and successfully extracted with 1,000 mL of 70% methanol-water solution (MeOH-H_2O). The organic solvent was concentrated to yield the MeOH-H_2O extracts.

Assays for DPPH radical scavenging activity

The DPPH radical scavenging activity was measured by the method of Yang et al. (11), with slight modification. The MeOH-H_2O extracts were initially dissolved in 1 mL of deionized water (DDW) followed by dilution with DDW to prepare trial samples of different concentrations. The free radical scavenging activities of the roots and stems (including leaves) of *A. dahurica* were measured as follows. The reaction mixture contained 1 mL of 0.5 mM DPPH-ethanol solution, 0.9 mL of 10 mM acetate buffer (pH 5.6) and 0.1 mL of either test sample at different concentrations or deionized water (control). The mixture was allowed to react at room temperature for 20 minutes, and the absorbance values were evaluated at 517 nm and converted into percentage antioxidant activity, and that of the samples was expressed as the percentage decrease in absorbance as compared

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with the controls. The experiments were conducted in triplicate.

Xanthine oxidase inhibition assays

Xanthine oxidase activity was evaluated on the basis of uric acid formation from xanthine (12). The MeOH-H_2O extracts were initially dissolved in 1 mL of methanol (95%) followed by dilution with 0.1 mM phosphate buffer (pH 7.4) in order to prepare trial samples of different concentrations. The xanthine oxidase inhibition activities of the roots and stems (including leaves) of *A. dahurica* were measured as follows. The reaction mixtures containing 100 μL of xanthine water solution (1.286 mM), 40 μL of xanthine oxidase solution (0.0741 units/mL), 100 μL of sample, and phosphate buffer for adjustment of the final volume to 2 mL. The inhibition of xanthine oxidase activity was evaluated via the measurement of the formation of uric acid from xanthine with a spectrophotometer at 295 nm for 3 min. Reaction mixture without sample was measured as a control, and ascorbic acid was utilized as a standard material. Xanthine oxidase inhibition was calculated using the following equation: Inhibition (%)=(absorbance of control − absorbance of sample)/ absorbance of control × 100%. The experiment was conducted in triplicate.

Primary skin irritation study in rabbits

Four adult rabbits (male) of the New Zealand strain, weighing 1.50 to 2.05 kg, were selected for each of the trial samples. Prior to dosing, the application sites were prepared by clipping the hair from the saddle areas of the rabbits. Two abraded areas located diagonally on the skin of each rabbit were prepared via the cutting of minor epidermal incisions with a hypodermic needle. MeOH-H_2O extracts of roots and stems (including leaves) of *A. dahurica* were dissolved in DDW in order to prepare sample solutions of 50 mg/mL. The samples were then applied in a quantity of 0.5 mL under a 2-square-centimeter surgical gauze patch on an area of intact skin and an area of abraded skin on each of the rabbits, and 0.5 mL DDW was also applied under gauze patches on other areas of the skin, serving as a control. After the application of the patches, the trunks of each rabbit were wrapped with bandages. The animals were restrained for 24 hr. At the end of the exposure period, the patches were removed and the reactions were immediately scored.

Ocular irritation study in rabbit

Four adult rabbits (male) of the New Zealand strain, weighing 1.50 to 2.05 kg were selected for each of the trial samples. MeOH-H_2O extracts of the roots and stems (including leaves) of *A. dahurica* were dissolved in DDW in order to prepare sample solutions of 50 mg/mL. 0.1 mL of sample solution was applied to the conjunctival sac of the left eye of each of the test rabbits, and 0.1 mL of DDW was applied to the right eye, serving as a control. The upper and lower eyelids were then held together gently for a few seconds, then released. The treated eyes of the two rabbits were flushed with DDW after 30 seconds. Examinations for gross signs of eye irritation were conducted at 1, 2, 3, 5, and 7 days after application.

Statistical analysis

All data were expressed as the means ± SD. The statistical analysis was performed using Statistical Package for the Social Science version 17.0 for Windows (SPSS INC, Chicago, IL, USA). Probability values <0.01 were student's *t*-test, significantly different compared between two trial groups.

RESULTS

Assays for DPPH radical scavenging activity

The DPPH radical scavenging activity of *A. dahurica* was examined at five different concentrations (100, 250, 500, 1,000 and 2,000 μg/mL) as shown in Table 1. All *A. dahurica* extracts evidenced radical scavenging properties to a different extent, and DPPH radical formation was gradually reduced with increases in the concentration of *A. dahurica*. When the concentrations required to inhibit DPPH radical formation by 50% (EC_{50}) were assessed, the free radical scavenging potency of the stems (including leaves) of *A. dahurica* was 243.33 μg/mL, which is significantly lower ($p<0.01$) than that observed in the roots (1,161.79 μg/mL). These results showed that both the dried roots and stems (including leaves) of *A. dahurica* evidence free radical scavenging activity, and that of the stems is more powerful.

Table 1. Free radical scavenging activity of *A. dahurica*

Concentration (μg/mL)	Free radical scavenging activity of *A. dahurica* (%)	
	Roots	Stems (including leaves)
0	0	0
100	3.92 ± 0.41	24.22 ± 0.16
250	9.46 ± 0.13	60.36 ± 2.46
500	19.55 ± 0.59	73.09 ± 4.37
1,000	39.06 ± 0.81	73.13 ± 0.85
2,000	75.31 ± 1.91	74.00 ± 0.25
EC_{50} (μg/mL)	1,161.79 ± 43.23	243.33 ± 24.15**

Values represent the mean ± SD of 3 independent experiments. **$p<0.01$ (Student's *t*-test), significantly different compared between two trial groups. EC_{50} values were determined by linear regression analysis.

Table 2. The inhibition of activity of roots and stems of *A. dahurica* on xanthine oxidase

Concentration (μg/mL)	Inhibition of xanthine oxidase (%)	
	Roots	Stems (including leaves)
0	0	0
25	3.93±1.43	7.87±3.27
50	7.49±4.04	17.53±3.27
125	23.10±8.88	28.98±10.95
250	47.94±6.91	35.03±7.87
500	54.90±9.34	50.50±6.50
750	57.68±6.56	88.31±11.00
EC_{50} (μg/mL)	435.19±145.46	434.66±175.37

Values represent the mean±SD of 3 independent experiments. EC_{50} values were determined by linear regression analysis.

Xanthine oxidase inhibition assays

The effects of *A. dahurica* on the inhibition of xanthine oxidase were assessed at six different concentrations, as is shown in Table 2. All *A. dahurica* extracts displayed inhibition activities on xanthine oxidase to different extents, and the formation of uric acid from xanthine declined gradually with increases in the concentration of *A. dahurica*. The dried roots of *A. dahurica* inhibited xanthine oxidase activity by 50% (EC_{50}) at a concentration of 435.19 μg/mL, and that of the stems (including leaves) was 434.66 μg/mL. No significant differences were observed between the two plant parts on the inhibition of xanthine oxidase activity.

Primary skin irritation study in rabbit

All animals survived for the duration of the study, and all evidenced gains in body weight. No overt signs of toxicity were observed in any of the rabbits during the course of the study (Table 3). After the application of *A. dahurica* to rabbits, light yellow staining was observed at the treated skin sites, which did not affect the evaluation of skin responses. The control sites evidenced no response to the control procedure. No edema, erythema, or eschar formation was observed in any of the rabbits (Table 4). Therefore, *A. dahurica* was considered to be a non-irritant to the skin.

Ocular irritation study in rabbit

After the application of the samples to rabbit eyes (ocular membrane), all of the rabbit eyes were normal. No abnormal changes, such as lacrimation, reddening, swelling, or pus formation were observed for up to 7 days after exposure (Table 5). Therefore, *A. dahurica* was considered to be a non-irritant to the eye.

Table 4. Effect of roots and stems of *A. dahurica* on skin irritation in rabbit

Reaction	DDW	*A. dahurica*	
		Roots	Stems (including leaves)
Edema	0	0	0
Erythema	0	0	0
Atonia	0	0	0
Desquamation	0	0	0
Hecrosis	0	0	0
Coriaceousness	0	0	0
Fissuring	0	0	0

DISCUSSION

Free radicals, which are powerful oxidants, are species that harbor unpaired electrons. They are generated in a host of bioorganic redox processes, may induce oxidative damage in various body components (e.g., lipids, proteins, nucleic acids, and saccharides) and may also be involved in processes resulting in the formation of mutations (13). Furthermore, radical reactions appear to perform a significant function in the development of chronic diseases including cancer, hypertension, cardiac infarction, arteriosclerosis, rheumatism, and cataracts (14). Free radicals may also constitute a contributory factor in a progressive decline in immune system function (15). Cooperative defense systems that protect the body against free radical damage include antioxidant nutrients and enzymes (16). One important way that the body is protected against oxidative stress via an increase in antioxidant levels (13). Therefore, the evaluation of the antioxidative properties of some materials, which are candidates for the prevention of oxidative damage, remains a highly active area of research. DPPH is a stable free radical, which is frequently utilized in evaluations of the antioxidant activity of several natural compounds (17). Antioxidants, upon interaction with DPPH, transfer electrons or hydrogen atoms to DPPH, thus neutralizing its free radical character. Xanthine oxidase (XO) is the enzyme responsible for the formation of uric acid from the purines, hypoxanthine and xanthine, and is responsible for the medical condition referred to as gout. Gout

Table 3. Clinical observations

A. dahurica	Dosage (mg/mL)	N	Body weight (kg)		Weight gain (kg)	Signs of toxicity
			Day 0	Day 3		
Roots	50	4	1.78±0.389	1.88±0.389	0.10±0.000	None
Stems	50	4	1.78±0.177	1.80±0.212	0.03±0.035	None

Values represent the mean±SD.

Table 5. Effect of roots and stems of *A. dahurica* on eye irritation in rabbit

Organs	DDW	*A. dahurica*			
		Roots		Stems (including leaves)	
		Washing group	No washing group	Washing group	No washing group
Corneal	0	0	0	0	0
Iris	0	0	0	0	0
Conjunctival	0	0	0	0	0
Edema	0	0	0	0	0

is induced by the deposition of uric acid in the joints, resulting in painful inflammation, with XO inhibition resulting in a remission in gout (18). XO also functions as an important biological source of oxygen-derived free radicals, which contribute to oxidative damage to living tissues that are involved in a variety of pathological processes, including inflammation, atherosclerosis, cancer, and aging (19). XO inhibitors may potentially prove useful for the treatment of gout or other XO-induced diseases (20).

The free radical scavenging and antioxidant activity observed in *A. dahurica* may be associated with certain of its compounds. Piao et al. (21) isolated 11 furanocoumarins from the roots of *A. dahurica*. Two of them, 9-hydroxy-4-methoxypsoralen and alloisoimperatorin, displayed profound radical scavenging properties, whereas the other nine furanocoumarins had only minimal activities.

In the current study, the roots and stems (including leaves) were determined to exert potent antioxidant effects against the DPPH radical and xanthine oxidase.

Additionally, according to the results of the primary skin irritation and ocular irritation tests, *A. dahurica* was determined to be non-irritating to the skin and eye at a concentration of 50 mg/mL.

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