© 2015 Journal of Pharmacy & Pharmacognosy Research, 3 (3), 59-68
ISSN 0719-4250
http://jppres.com/jppres

Original Article | Artículo Original

Pharmacognostical identification of *Alchemilla japonica* Nakai et Hara

[Identificación farmacognóstica de *Alchemilla japonica* Nakai et Hara]

Yun Zhu, Ningjing Zhang, Peng Li*

School of Pharmacy, Shihezi University/Key Laboratory of Phytomedicine Resources & Modernization of TCM, Shihezi Xinjiang 832002, PR China.

*E-mail: whitecloud2002001@163.com

Abstract

Context: Alchemilla japonica is a therapeutically important medicinal plant, which is widely used in traditional medicine external application for injuries as well as orally for acute diarrhea, dysmenorrhea, and menorrhagia, among others. However, there is not a correct identification of this species and is of prime importance differentiate it from commonly available adulterants or substitutes, in fresh, dried or powdered state. There is only a small number of data of pharmacological standards for identification and authentication of *A. japonica*.

Aims: To characterize morpho-anatomically the roots, leaves and stems of *Alchemilla japonica* Nakai et Hara (Rosaceae), explore and establish the micromorphology and quality control method for this plant.

Methods: Pharmacognostic and phytochemical investigations were conducted in terms of macroscopic, microscopic and preliminary phytochemical parameters.

Results: The transverse of root showed presence of polygonal parenchyma cells and obvious triangular intercellular space. The stele of the root was tetrarch. There was trichome in the epidermis cells of stem transverse section, but its cortex was wider than others. Xylem cells arranged in continuously cyclization. The phloem was more than ten bundles. There were many trichomes on the folicolous and palisade tissue contained cluster crystal of calcium oxalate, bundle sheath forms ring closed. The diagnostic feature of powder was cluster type of crystals of calcium oxalate. Trichomes were also observed. The main diagnostic feature of powder was Pente-like thickening cell walls of the subsidiary cells.

Conclusions: The leaves, roots, and stem of *A. japonica* can be differentiated by macro and microscopic characters. Various pharmacognostic characters that observed in this study can help in identification and standardization of this species.

Keywords: *Alchemilla japonica*; macro-morphology; micromorphology; pharmacognostical identification.

Resumen

Contexto: Alchemilla japonica es una planta medicinal, terapéuticamente importante, que se utiliza ampliamente en la medicina tradicional por aplicación externa en lesiones, así como por vía oral para la diarrea aguda, dismenorrea y menorragia, entre otras. Sin embargo, no hay una correcta identificación de la especie y es de primordial importancia diferenciar esta de adulterantes comúnmente disponibles o sustitutos, en estado fresco, seco o en polvo. Sólo hay un pequeño número de datos de patrones farmacológicos para la identificación y autenticación de *A. japonica*.

Objetivos: Caracterizar desde el punto de vista morfo-anatómico las raíces, hojas y tallos de *Alchemilla japonica* Nakai et Hara (Rosaceae), analizar y establecer la micromorfología y el método de control de calidad para esta planta.

Métodos: Las investigaciones farmacognósticas y fitoquímicas fueron conducidas en términos de parámetros macroscópicos, microscópicos y fitoquímica preliminar.

Resultados: El corte transversal de la raíz mostró células poligonales del parénquima y espacio intercelular triangular evidente. La estela de la raíz fue tetrarca. Hubo tricomas en las células de la epidermis de la sección transversal del tallo, pero su corteza fue más ancha que otras. Las células del xilema dispuestas en ciclación continua. El floema contenía más de diez haces. Presencia de muchos tricomas en el folículo y el tejido en empalizada contenía racimos de cristales de oxalato de calcio, las formas de la vaina del haz de anillo cerrado. La característica de diagnóstico de polvo fue tipo racimo de cristales de oxalato de calcio. También se observaron tricomas. La principal característica del polvo fue engrosamiento de las paredes de las células auxiliares como pentámeros.

Conclusiones: Las hojas, raíces y tallo de *A. japonica* pueden ser diferenciadas por caracteres macro y microscópicos. Varios caracteres farmacognósticos observados en este estudio pueden ayudar en la identificación y estandarización de esta especie.

Palabras Clave: *Alchemilla japonica*; identificación farmacognóstica; macromorfología; micromorfología.

ARTICLE INFO

Received | Recibido: December 8, 2014.
Received in revised form | Recibido en forma corregida: May 13, 2015.
Accepted | Aceptado: May 30, 2015.
Available Online | Publicado en Línea: June 11, 2015.
Declaration of interests | Declaración de Intereses: The authors declare no conflict of interest.
Funding | Financiación: This work was sponsored by the 12th Five-year Grand support of Ministry of Science and Technology of the People's Republic of China (2012BAI30B02).

INTRODUCTION

The use of herbal medicinal products and supplements, principally for primary health care, has increased tremendously over the past three decades with not less than 80% of people worldwide (Ekor, 2013). Recently, considerable attention has been given to utilize eco-friendly and bio-friendly plant-based products for prevention and cure of different human diseases. The Western population is looking for natural remedies, which are safe and effective, considering the adverse effects of synthetic drugs. It is documented that most of the World's population has taken in traditional medicine, particularly plant drug for the primary health care.

Alchemilla (Rosaceae) is a genus of herbaceous perennial plants, with the vulgar name "lady's mantle". *Alchemilla* species are used in traditional medicine topically for wounds as well as orally for acute diarrhoea, dysmenorrhea, and menorrhagia, among others (Bisset et al., 1994; Trendafilova et al., 2012).

There are three species of *Alchemilla* in China and mainly in the northwest. Among them, *Alchemilla japonica* Nakai et Hara mainly distributed in Inner Mongolia, Shaanxi, Gansu, Qinghai, Xinjiang, Sichuan and other provinces, grown at an altitude of 2500-3500 m alpine grasslands. Japan also has an important distribution of this plant (Editorial Board Xinjiang Flora, 1995; The Flora of China Editorial Committee of Flora of China, 1985). It was also contained in Medicinal Flora of the Kazakhs. The whole plant is medicinal, bitter and cool, for detoxification, useful in blood circulation to regulate menstruation, also it has anti-inflammatory effect. It is used to treat menoxenia, menopause syndrome, conjunctivitis, enteritis, diarrhea, skin ulcers embolism (Wang et al., 2010). Though some plants from the same genus have been widely used to cure aphthous stomatitis and other diseases (Shrivastava and John, 2006; Türk et al., 2011).

The type of reproduction of most *Alchemilla* plants is apomixis except a few species, so seed multiplication can maintain its characteristics, the smaller differences between species, and the more difficult for classification (Feng et al., 2005).

On the other hand, there are little data on the phytochemical and pharmacological standards for identification and authentication of *A. japonica* (Ayaz et al., 1999; Fraissea et al., 2000; Shrivastava and John, 2006; Falchero et al., 2009; Türk et al., 2011).

Hence, in the present research pharmacognostic and preliminary phytochemical investigations have been carried out on *A. japonica* for the standardization and quality assurance purposes of the promising Kazakhs ethno-medicinal plant.

MATERIAL AND METHODS

This section was carried out according with Xu (1986) and Manzano et al. (2014).

Plant material

Fresh specimens of *Alchemilla japonica* Nakai et Hara were collected from the natural habitat in Manasi region of Xinjiang in June, 2013. The plant material was authenticated by Professor Pin Yan (College of Life Science, Shihezi University). Voucher specimens (NO.2013071201-06) were preserved in School of Pharmacy of Shihezi University. The harvested plants were dried in shadow at room temperature (temperature 30-40°C), ground into powdered form and stored in airtight containers.

Macroscopic analysis

The plant was studied for morphological characters including size, shape, color, odor, taste, and extra features. The macro-morphological feature of the root, leaf and stem was observed under magnifying lens (10x).

Microscopic analysis

Dried herbs were grinded to coarse powder and packed in suitable container for microscopic identification. Using chloral hydrate and diluted glycerin as clearing agents make powder section. Chloral hydrate (prepared according to procedures described in Appendix 100, Pharmacopoeia of the People's Republic of China (2010). Diluted glycerin, prepared according to procedures described in Appendix 102, Pharmacopoeia of the

People's Republic of China, (Chinese Pharmacopoeia Commission, 2010).

The samples were cut and immediately fixed in formalin 5 mL + acetic acid 5 mL + 70% ethyl alcohol (90 mL). After fixing the samples for 24 hours, they were dehydrated and clarified successively in graded series of ethanol and dimethylbenzene. Posteriorly, the specimens were infiltrated with paraffin wax (melting point 58-60°C) and casted into paraffin blocks. Rotary microtome (YD-1508B) was used to section the paraffin embedded specimens. Each section thickness was 10-12 µm. The dewaxed sections were stained with fast green and safranin.

Photomicrographs of the transverse section (stem, root and leaf) and powder section were taken with the help of Biomicroscopy Primo Star (Zeiss Group, Germany) with 10x and 40x microscope objective lens, and CX21 biomicroscopy unit (Olympus, Nikon D750 digital camera, Matrox Inspector, Matrox Electronic Systems Ltd.).

Physical and chemical identification

The 95% ethanol extract of the herb was scanned at 200 to 400 nm wavelength with the help of ultraviolet spectrophotometer (UV- 2401 spectrophotometer, Shimadzu Corporation). This technique was used to detect the possible presence of flavonoids, alkaloids and glycosides.

RESULTS

Original plant identification

Alchemilla japonica is a perennial herb of 10-13 cm high common. The rhizomes were hypertrophic and lignified. The stems were solitary or tufted, erect or oblique, densely covered with long white pilose. The cauline leaves were 2-3 cm long and 3-7 cm wide. They were round or subcordate and with relatively long petioles, deeply cordate, with 7-9 shallow lobes at the apex, margin serrulate, sparsely pilose on both surfaces, along the veins was dense. The petioles were 3-10 cm length and villous distributed densely. There were membranous stipules and villous densely outside. Cauline leaves were small and its petioles were short or nearly sessile. Stipule margin were serrate and connate at the base, with sparsely pilose on surfaces. Corymbose cymes were yellow-green and more closely; the diameter of flowers were 3-4 cm width. The pedicels were 2-3 cm in length and glabrous foliage or nearly glabrous. The outside of calyx were pilose sparsely. Sepals were long lanceolate and pilose sparsely on the surface. Triangular sepals were 1-1.5 mm in length and pilose sparsely in outside, it is slightly longer than wider in the epicalyx. Stamens were long approximately half of sepals. Linear styles were slightly longer than the stamens. The achenes were oval and about 1.5 mm in length, the shape was acute slightly at the apex and all wrapped in a membranous receptacle (Fig. 1A).

Macroscopical identification

The morphological appearance of plants were shrinkage and crimp. The rhizomes were woody and flourished. The tap-roots were brown and inconspicuous. The fibrous roots were yellowish brown and slender. Stem were yellow-green, curved and slender, 16-28 cm long and diameter about 1 mm. This has vertical edges and white piloses on surface. The stems were crisp and easy to break. Basal leaf petioles green or purple, leaves shrivel, yellow-green surface, flattened, heart-shaped round, coat, gray surface of leaf green, smooth, little coat. Flowers light yellow, cyme, brittle, easily broken, section yellowish white. The herbs' flavor was light and mild (*qi mild*) (Fig. 1B).

Microscopy identification

Transverse section of A. japonica *leaves (Fig. 2):*

a) Epidermal cells, one layer, tightly packed, round the outside wall thickening, occasionally irregular calcium oxalate crystals, there were non-glandular hairs, 4-5 cells around its base, often prominent in the epidermis.
b) Palisade tissue cells, containing scattered cluster crystals, up to 25 µm in diameter.
c) Spongy tissue cell trait was irregular.
d) Bundle sheath cells into the ring, cell wall thickening.

e) Lower epidermis was significantly less than the epidermal cells, with non-glandular hairs.

Transverse section of the stem of A. japonica.

Stem cross-section round (Fig. 3):

a. Epidermis was a flat rectangle of cells, arranged in neat rows and close, the outer wall thicker, non-glandular hairs on its basal cell uplift.
b. Cortex was wide.
c. Small and flat endodermal cells.
d. Xylem cells arranged in a continuous loop, phloem bundles more than 10, alternate arrangement.
e. Pith large, hollow.

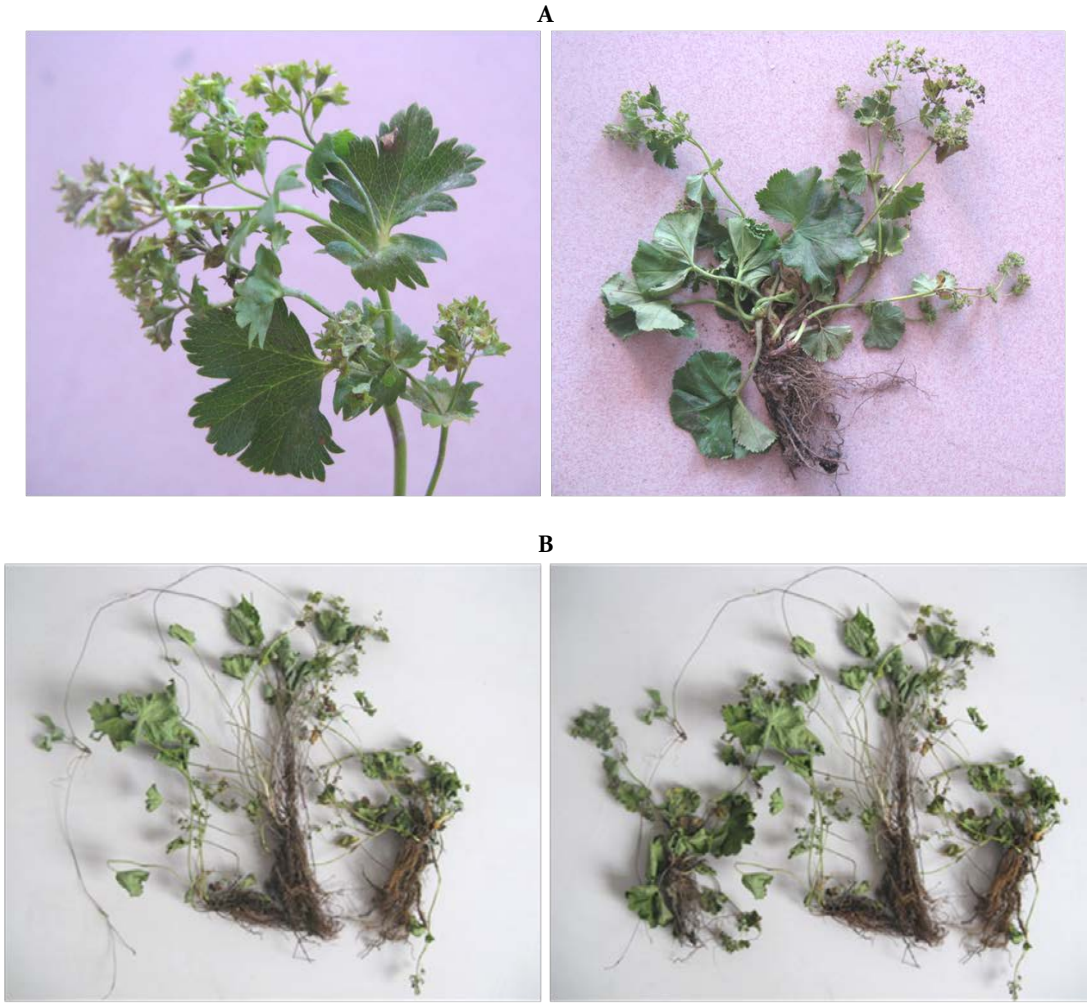

Figure 1. The original plant of *A. japonica*.

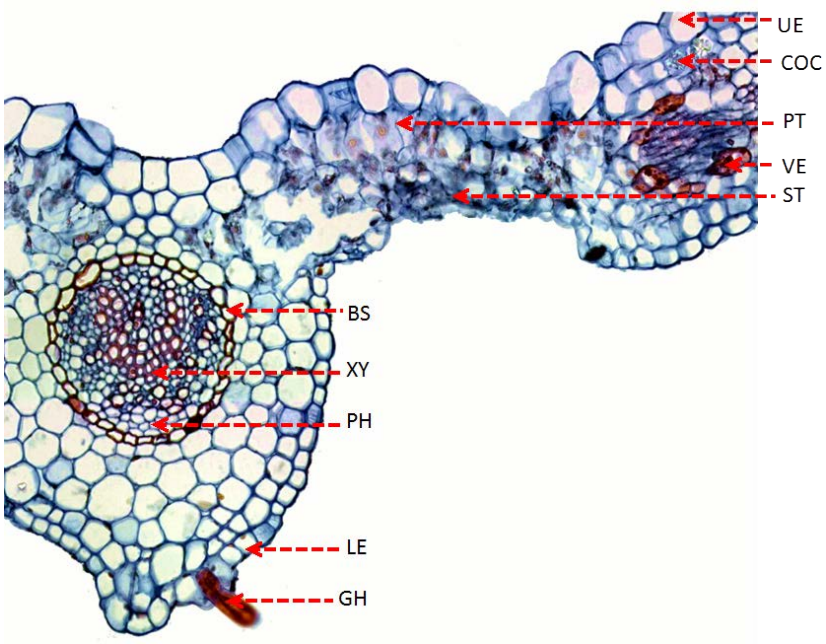

Figure 2. Transverse section of midrib of *A. japonica*.

UE: Upper epidermis, **COC:** Cluster crystal, **PT:** palisade tissue, **VE:** Vessels, **ST:** Spongy tissue, **BS:** Bundle sheath, **XY:** Xylem, **PH:** Phloem, **LE:** Lower epidermal, **GH:** Glandular hair.

Transverse section of the roots of A. japonica.

Quasi-circular shape (Fig. 4):

a) Cork layer of cells 1-2 columns, neat and compact, cork.
b) Cortex wide, thin cell wall, cell loose, slightly more than polygonal parenchyma cells, there were obvious triangular intercellular space, and some flat wrinkled, tightly packed cells into pieces. Near the centre, cells in 5-6 columns, arranged in a ring.
c) Endothelial cells were closed.
d) Tetrarch primary xylem, xylem cells were lignified, visible ducts.

Powder microscopy.

Yellow-green powder:

a) Trichome, unicellular trichome was common, slightly curved. Incomplete, there were segmentations in the middle section, containing the yellow substance, 350-1450 μm long; occasionally multiple arranged radially, non-glandular hairs with lignified cell walls.
b) Stems glandular hairs occasionally, mallet-shaped, containing yellow granular secretions, and it consists of four cells usually, and head of glandular hairs was round or similar round.
c) The anomocytic stoma was about 26 μm long axis, short axis approximately 20 μm. Commonly the stomata have four subsidiary cells; cell wall was curved slightly and thicken which beaded occasionally.
d) Cluster crystals were common, and which outer edges were sharp, the inner edges were blunt relatively. Cluster crystals' diameter was about 8-20 μm and distributed mainly around the veins in the leaf. While its diameter was about 15-40 μm and founded primarily in corolla of flowers.
e) There are the lump calcium oxalate crystal and irregular bulk crystal.
f) Cork cells were yellow or light yellow and which exine was suberification. The surface view of it is oblong or polygonal, and side view was rectangular.

g) Parenchyma cells were polygonal, some of the cell wall turn into moniliform.
h) There were much spiral vessel, bordered pit vessel were uncommon, and some cell wall was lignified.
i) Pollen grains were common, brown, round or oval, about with 1-2 germination pores, 12-79 μm in diameter, surface carved lines showed irregular shape of multilateral (Fig. 5).

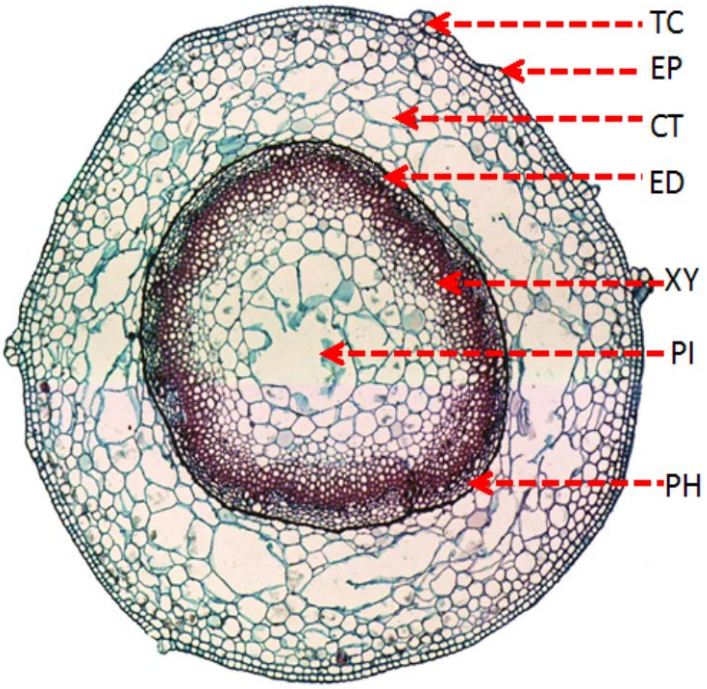

Figure 3. Transverse section of the stem of *A. japonica.*

TC: Trichome, **EP:** Epidermis, **CT:** Cortex, **ED:** Endodermis, **XY:** Xylem, **PI:** Pith, **PH:** Phloem.

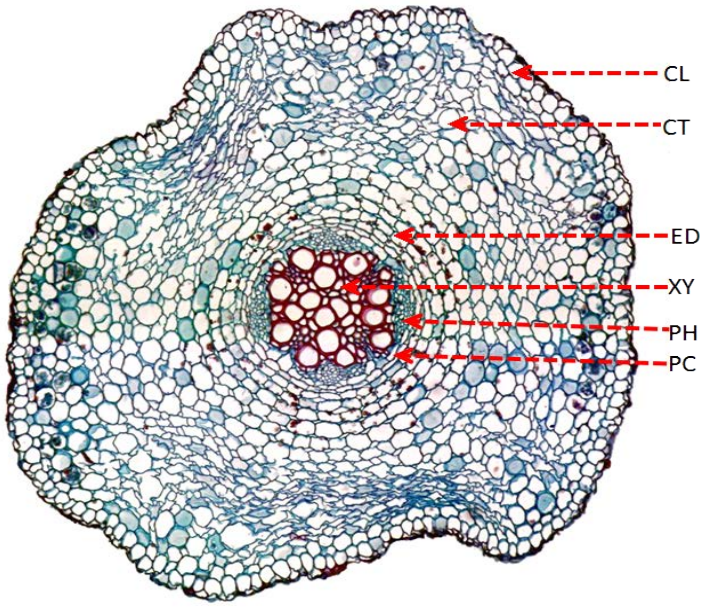

Figure 4. Transverse section of the root of *A. japonica.*

CL: Cork layer, **CT:** Cortex, ED: Endodermis, **XY:** Xylem, **PH:** Phloem, **PC:** Pericycle.

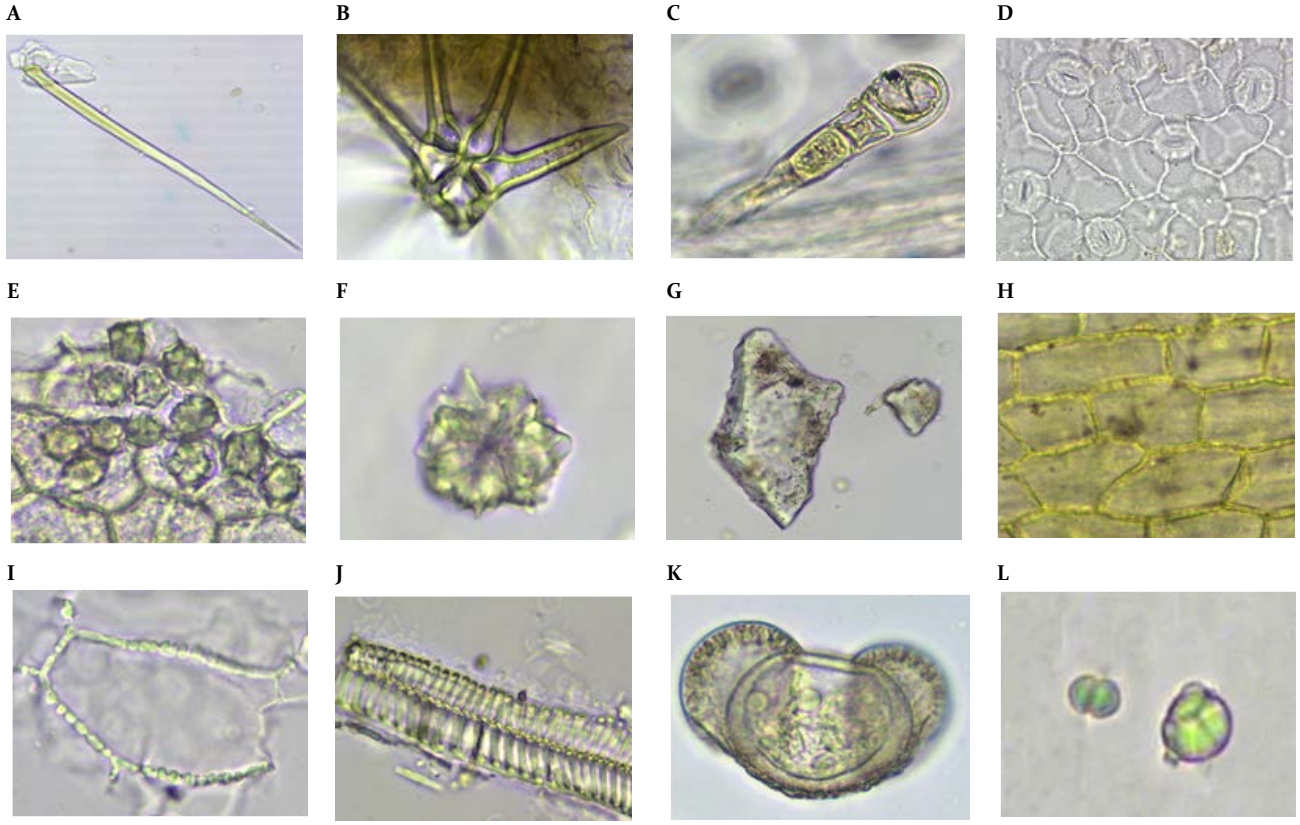

Figure 5. Powder characteristics study of crude drugs of *A. japonica*.

A: unicellular clothing trichome, **B:** Branched clothing trichome, **C:** multicellular stalk and unicellular head glandular trichomes, **D:** anomocytic stoma, **E-F:** clusters of calcium oxalate, G: solitary crystal, **H:** phellem cells arranged densely, **I:** parenchyma cells with moniliform cell wall, **J:** spirally vessels; **K:** pollen grains; **L:** starch grain.

Preliminary phytochemical screening

Preliminary phytochemical screening showed the presence of flavonoids, alkaloids, sterols, tannin, volatile oil, saponins, proteins and amino acids, carbohydrates, reducing sugars, and absence of cyanogenetic glycosides, anthraquinone glycosides, cardiac glycosides, mucilage (Table 1).

The results of the UV spectra of *A. japonica* with 95% ethanol for blank, scanning from 200 to 400 nm wavelength, are shown in Fig. 6. There were two distinct peaks at 208 nm and 281 nm, where the maximum absorption peak was showed at 208 nm.

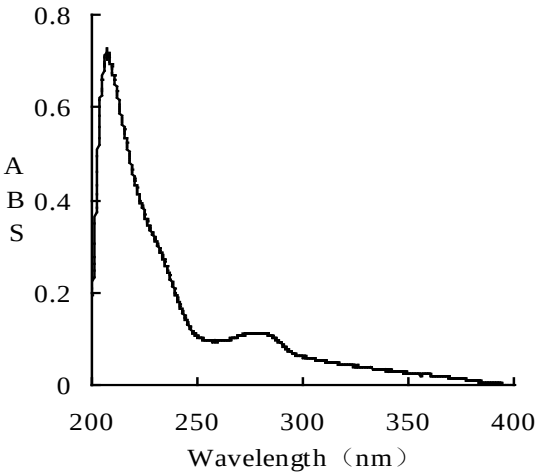

Figure 6. The UV absorption spectrum of *A. japonica*.

Table 1. Phytochemical analysis of *A. japonica*.

Metabolite groups	Name of the test	Ethyl alcohol extract
Flavonoid	HCl-Mg reaction	+
	$AlCl_3$ reaction	+
Glycoside	Molisch reaction	+
Alkaloid	Dragendorff's reagent test	+
	Wagner's reagent test	+
	Hager's reagent test	+
	Bertrand's reagent test	+
Saponin	Liebermann-Burchard test	+
Tannin	Ferric chloride test	+
Color		Yellowish-green

DISCUSSION

For determining the suitability or denunciation of a crude drug the sensory evaluation plays a key role. Organoleptic testing of a crude drug is mainly for qualitative evaluation based on the observation of morphological and sensory profile. In this report, various morphological, microscopical and physicochemical standards have been developed (Periyanayagam et al., 2013). Hence, we have undertaken this study to serve as a tool for developing standards for identification, quality and purity of *Alchemilla japonica* Nakai et Hara.

Several studies suggest that adulteration and misidentification of crude drugs can cause serious health problems to consumers and legal problems for the pharmaceutical industries. It can be conducted via a variety of techniques, namely macro and microscopic identification and chemical analysis, especially description of microscopic botanical aspects to determine definitively the proper species of plant material while it is still in its non-extracted form. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs. These characters are especially important for identification of powdered drugs, because in these cases most of the morphological diagnostic features are lost. Microscopic evaluation is one of the simplest and cheapest methods for the correct identification of the source of the materials (Patel and Zaveri, 2011). The macroscopic and organoleptic characters of the leaf can serve as diagnostic parameters (Singh et al., 2010).

Alchemilla japonica transection root cross-sections structure, vascular cylinder nearly square, trachoma, anomocytic stoma, calcium oxalate crystal, cork cell and thickened and beaded polygonal can be regarded as identification character (Nayak and Patel, 2010).

Phytochemical evaluation and molecular characterization of plants is an important task in medicinal botany and drug discovery (Yashvanth et al., 2011). Preliminary phytochemical screening

showed the presence of flavonoids, alkaloids, sterols, tannin, saponins, volatile oil, protein and amino acids, reducing sugars, carbohydrates, and absence of terpenoids, anthraquinone glycosides.

The signals at 208 and 281 nm in the UV absorption spectrum corroborate the possible presence of flavonoids.

Efforts have been made by the authors to bring out every detail on the macroscopical and microscopical characters of *Alchemilla japonica* Nakai et Hara (Kataria et al., 2013). The study of pharmacognostical features had shown the standards, which will be useful for the detection of its identity and authenticity. Beside the other studies viz-physical evaluation, preliminary phytochemical test add to its quality control and quality assurance for proper identification.

CONCLUSIONS

The present study on the pharmacognostical standardization and evaluation of the *Alchemilla japonica* Nakai et Hara might be useful to supplement information with regard to its identification parameters, which are assumed significant for the acceptability of herbal drugs in the present scenario that lacks regulatory laws to control the quality of herbal drugs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

This work was sponsored by the 12th Five-year Grand support of Ministry of Science and Technology of the People's Republic of China (2012BAI30B02).

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