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Original Article

Anatomy and microscopic characteristics of *Picris japonica*

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ABSTRACT

Picris japonica Thunb., Asteraceae, is an herbal medicine used to dispel heat, reduce swelling and alleviate pain in traditional Mongolian medicine. Its dried whole plant is mainly used to treat flu and mammary abscesses. Given the potential applications of such an herb, detailed pharmacognostic research on *P. japonica* is needed. This study attempted to fill this need by producing permanent and semi-permanent slides of different organs (root, stem, leaf, pollen grain, fruit and powder of the whole plant) using safranine staining, safranine-fast green double staining and common methods. Furthermore, several featured microscopic structures of *P. japonica* are described herein. The results obtained provide us with valuable information for botanical quality control and species identification and enable us to detect adulterations in commercial samples of *Picris* or in laboratory samples.

Introduction

Picris japonica Thunb., Asteraceae, is a perennial herb, which can be found in the Chinese provinces of Heilongjiang, Jilin, Liaoning, Inner Mongolia, Hebei, Shanxi, Shaanxi, Gansu, and Shandong, and also in Japan and Russia. The predictable habitats of *Picris* include biomes in boreal and temperate regions of Europe, Asia and Australia as well as biotopes in the alpine level of high mountains in the Mediterranean region and the (sub)tropical zone of the Arabian Peninsula and Africa (Slovák et al., 2018, p. 186).

The whole plant has been used medicinally, with effect on dispelling heat, reducing swelling and alleviating pain in traditional Mongolian medicine; additionally, the whole plant in dried form is mainly used to treat flu and mammary abscesses (Flora of China, 1997). There are about forty species in the genus *Picris* present in Europe, Asian and north Africa, five of which can be found in China. It has been reported that terpenoids could be isolated from the root of *Picris hieracioides* subsp. *japonica* (Kenji et al., 1989a,b) and four novel terpenoid glycosides were extracted from the whole plant of *P. hieracioides* (Taketo et al., 1990). In screening research involving 116 species of wild plants, Liu et al. (1998) showed that an extract of ethyl acetate from the whole plant of *P. japonica* suppressed the activities of over 70% of cancer cells. The information on the morphological features of *Picris* for the purpose of identifying the species in commercial samples of drugs to perform the botanical quality control of this species is scarce (Wulan and Gao, 2008). The objectives of our work were to provide the basis for the microscopic identification of *P. japonica* microscopic and the detection of adulterations in the commercial and laboratory samples of *Picris*.

Materials and methods

Plant materials

All materials of *Picris japonica* Thunb., Asteraceae, used in this experiment were collected from Loufan county in the Shanxi province of China (latitude 37°51′–38°13′ N and longitude 111°51′–112°02′ E). The permanent slides were stored at the Museum of Chinese Medical Specimens at Shanxi Medical University under the number 080804MCM.

Chemicals

Safranine T was purchased from China Medicine Shanghai Chemical Reagent Company. Paraffin (fusion point: 56–58 °C) and neutral gum were purchased from Shanghai Specimen Model Factory (China). Fast Green FCF was obtained from Amresco (America). Gelatin was purchased from Sinopharm Chemical Reagent Company (China).

FAA fixation solution: formalin (40% methyl aldehyde) (5 ml), glacial acetic acid (5 ml), ethanol (50% or 70%) (90 ml). Ethanol 50%

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instead of 70% was used for delicate materials to prevent material shrinkage. Safranin solution: safranin T (2.5 g), 95% ethanol (25 ml), distilled water (225 ml), aniline (10 ml), shaken well, stored in a brown bottle, filtered before using. Fast green solution: Fast Green FCF (1 g), 95% ethanol (40 ml), aniline (10 ml), shaken well, filtered, used on the same day after preparation.

Methods

Permanent and semi-permanent slides were created with different organs (root, stem, leaf, fruit and powder of the whole plant), safranine staining and Safranine-Fast green double staining were used (Kang, 2003; Gao et al., 2003; Zhang and Jin, 2005).

The steps of paraffin production and the basic process of operation are as follows (Zhang and Jin, 2005): acquiring materials, fixing, washing, dehydration, clearing, paraffin, embedding, slicing, adhesion, dewaxing, dyeing, transparency, and sealing.

Slide of whole-plant powder (Kang, 2003; Lei et al., 2011): the whole plants (including root, stem, leaves, flowers and fruit) were crushed by the grinder, pass through a 50 mesh screen. Then the lab technician picked a little powder with toothpick, placed it in the center of glass, added 1–2 drops of a chloral hydrate test solution, stirred gently with anatomic needle, and added a cover glass after heat permeation.

$$\%\ \text{of stomatal indices} = \frac{\text{Stomatal number per unit area}}{\text{Stomatal number per unit area} + \text{Epidermal cell number of same area}} \times 100\%$$

The stomata number and epidermal cells number were obtained from the same visual field under a 100× objective lens.

$$\text{Stomatal density} = \frac{\text{Total number of stomata}}{\text{area}}$$

The stomata number and area were obtained from the same visual filed under a 10× objective lens.

Stomata index and density: determined 100 results, calculated the average result, choose the minimum and the maximum value.

Length measurement method: determined 100 results and selected the minimum and the maximum value.

An Olympus microscope, a Nikon microscope (Eclipse E200) and a Canon camera (Canon Power Shot A95) were used to observe, describe, and measure the specimens and take photos. For images, an eraser was used to eliminate the impurities, followed by background subtraction and brightness/contrast adjustment, using Photoshop software.

Results

Anatomy and microscopic characteristics of Picris japonica

Root anatomy

The following was observed in the cross section of the root: the epidermis consists of a layer of cells, the suberification of the ecto-tangential wall was apparent, the periderm includes several layers of cork cells at the lateral end of the old root, the cortex was narrow, the phloem was broad with scattered laticifer groups, the cambium layer was looping, the xylem was about 1/2 or more of the root radius with xylem vessels was scattered singly or radially arranged with several together, and the ray was multi-seriate and constituted by at least two layers of parenchyma cells. (Fig. 1A)

The radial root direction being longitudinal with a second, tangential longitudinal section demonstrates that the laticifer scattered in the phloem was articulate laticifer (Fig. 1B). Reticulate vessels and scalariform vessels were predominant.

Stem anatomy

The following was observed from the cross section of the stem: stems have edges with a regular distribution of glandular trichomes on the ridge edge with a common number of ridges being at least 10–20 (Fig. 2A). A layer of epidermal cells was present and approximately square, and the outer surface was covered with cuticle. There were glandular trichomes and non-glandular trichomes on the epidermis. One type of the glandular trichomes, uniseriate filiform simple trichomes (Krak and Mráz, 2008), were often found on the ridge edge displaying a uniseriate foot with 4–5-isodiametric cells and a globose unicellular head (Fig. 2E). The total length was between 20 and 72 μm. The other kind of the glandular trichomes were multiseriate glandular trichomes, which have a pluriserial foot, longitudinally elongated cells arranged in 2–3 layers, and a globular pluricellular head (Fig. 2D). Non-glandular trichomes have a long pluriseriate foot, with a bifurcated head composed of two hook-shaped cells (Figs. 3F, 6A). The total length is 124–1230 μm. The cortex was composed of a series of parenchyma cells. Additionally, on the medial side of the cortex of on the ridge edge, a collenchyma was observed. Endodermis was evidenced by cells that were arranged in an orderly fashion (Fig. 2A, B). The vascular bundles were collateral bundles arranged into intermittent rings. Cambium was evidenced. Pericyclic fibers were developed in old stems and arranged into intermittent rings (Fig. 2B).

Different types of pipes, such as spiral vessel, annular vessel, bordered pit vessel and reticulated vessel, were observed from the longitudinal section of stem (Fig. 2C). The marrow cells were rectangular and arranged in neat rows.

Leaves anatomy (transverse section)

The upper and lower epidermises were both composed of a row of rectangular cells with thick radial walls and cuticles. They are anphistomatic leaves. The epidermal glandular trichomes and stomata are distributed on both epidermises. There were two kinds of glandular trichomes. One of them has an ellipse head composed of multiseriate cells; its handle consists of 1–4 long and narrow cells, arranged in a single row. Some of the heads have one or two bulges that were composed of multiseriate cells (Fig. 3C). The others have a single-celled head with a stem-like handle consisting of 1–5 cells (Figs. 2E, 3B-1, 3D). The non-glandular trichome observed in the leaf corresponds to the same type found in the stem (Fig. 3F).

The mesophyll is dorsiventral, showing in the adaxial face, and manifesting palisade of 1–2 rows of cells (Fig. 3B-1 one row cells, Fig. 3B-2 two rows cells). The cells of the palisade tissue were closely arranged, slightly long cylinders appearing all over the lamina, except in the main vein with the vascular bundle which is surrounded by the parenchyma (Fig. 3B-1, B-2). Irregular spongy tissue cells with loose arrangement have big gap between them (Fig. 3B-1, B-2). Small vascular bundles can be seen in the mesophyll tissue. The vascular tissue in the central main vein is characterized by 1–5 bundles arranged in an arc of the ectophloic type, alternating between big and small (Fig. 3A).

Leaves anatomy (permanent specimen of epidermis of leaves)

The anticlinal walls of upper epithelial cells were slightly straight; the anticlinal walls of the lower epidermal cells were wavy. The superficial cutin texture was radial (Fig. 3E). There were more glandular trichomes and non-glandular trichomes on the upper and lower epidermis, and the non-glandular trichome observed in the leaf corresponds to the same type found in the stem.

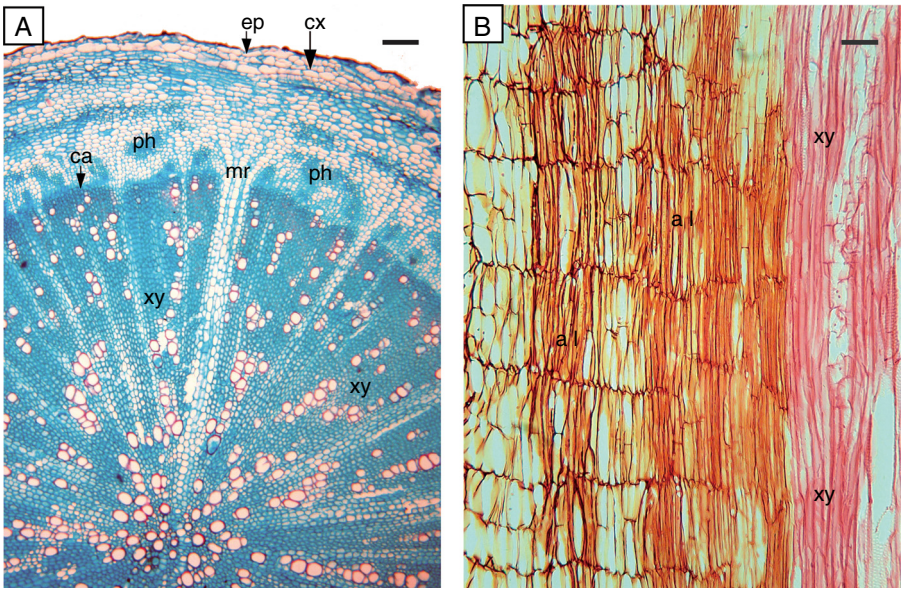

Fig. 1. Photomicrographs of root of *Picris japonica*. (A) Cross section; (B) longitudinal section, showing laticifer. *Abbreviations*: al, articulate laticifer; cx, cortex; ca, cambium; ep, epidermis; mr, medullary ray; ph, phloem; and xy, xylem. Scale bar = 200 μm (A) and 50 μm (B).

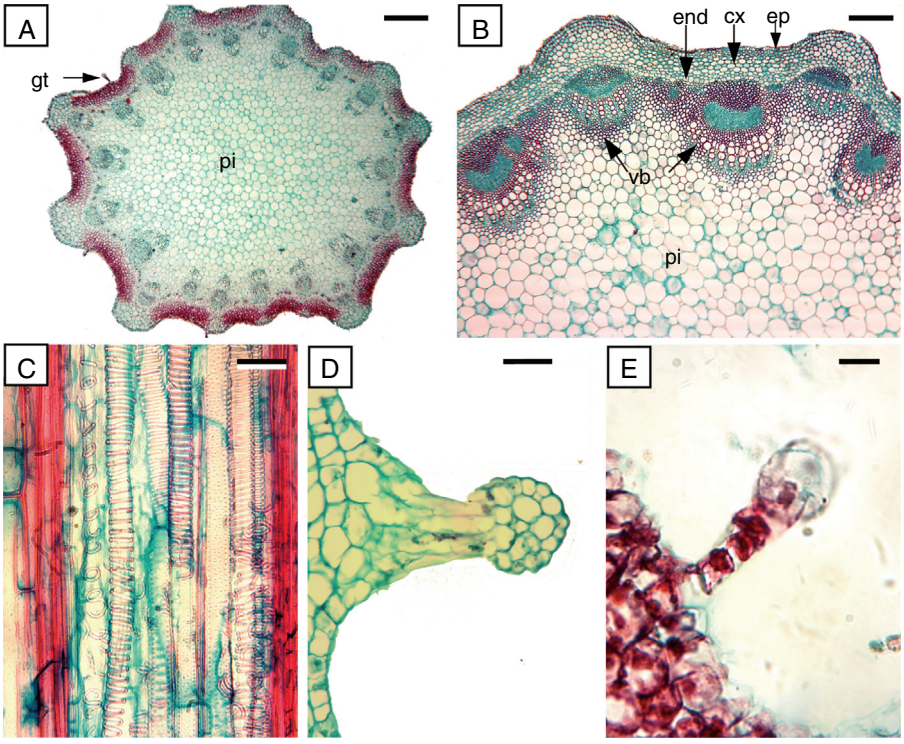

Fig. 2. Photomicrographs of stem of *Picris japonica*. (A) Cross section of young stem; (B) cross section of old stem; (C) longitudinal section of old stem, showing the vessels; (D) multi-seriate glandular trichome; (E) uni-seriate filiform simple trichome. *Abbreviations*: cx, cortex; ep, epidermis; end, endodermis; gt, glandular trichome; pi, pith; and vb, vascular bundle. Scale bar = 200 μm (A, B), 50 μm (C, D), and 10 μm (E).

The top of most of the non-glandular trichomes have two bifurcated hooks (Fig. 3F) although three bifurcated hooks or hooks without bifurcation are seen occasionally. Most of the glandular trichomes were composed of 3–6 single row cells; the head of which were single, large-scale cells (Fig. 2E). The stomata were of an anisocytic type or an anomocytic type with 4–6 subsidiary cells of anomocytic type or 7–8 subsidiary cells being seen occasionally (Fig. 3E). The stomata index of upper leaf epidermis was 6.50–15.6–25.0, stomata density was 75–132–225, the stomata index of the lower leaf epidermis was 15.8–23.5–29.4, stomata density was 200–294–425.

Pollen sample

The pollen grain was canary yellow, nearly round-shaped, with a diameter from 24 μm to 53 μm, and had three apertures (Wang and Gao, 2011). The pollen exine with thorn-like ornamentation formed the polygon grid ornamentation. The pollen grain had different shapes in different development stages and when observed under different viewing surfaces (Fig. 4A–I). Hexagonal polygon grid ornamentation was common, with a round cavity that was in the middle, and its periphery had six big gear-like spikes.

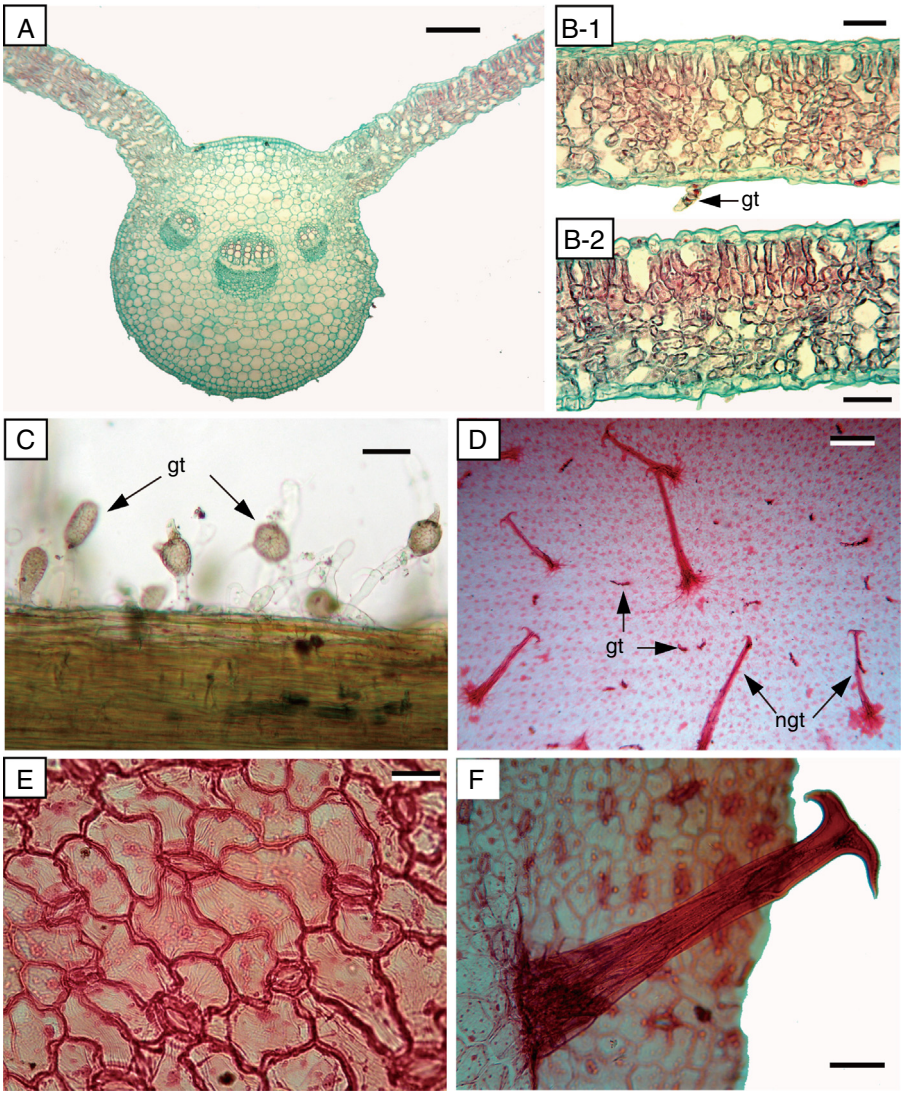

Fig. 3. Photomicrographs of the leaves of *Picris japonica*. (A) Cross-section of leaf, showing the vein; (B-1, B-2) cross-section of leaf, showing mesophyll tissue; (C) a superficial view of the foliar epidermis, showing glandular trichomes; (D) lower epidermis of leaf; (E) lower epidermis of leaf, showing the stomata and subsidiary cells; (F) epidermis of leaf, showing multi-seriate non-glandular trichome with branched apical part. *Abbreviations*: gt, glandular trichome and ngt, non-glandular trichome. Scale bar = 200 μm (A, D), 50 μm (B-1, B-2, C, F), and 20 μm (E).

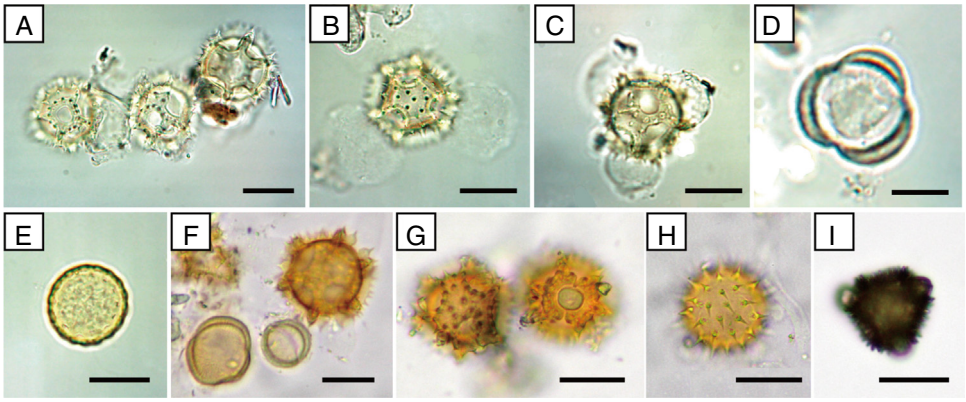

Fig. 4. Photomicrographs of pollen grains of *Picris japonica*. (A–E) Completely permeabilized pollen grains; (F–H) incompletely permeabilized pollen grains; (I) pollen grains not permeabilized with formaldehyde. Scale bar = 20 μm.

Fruit anatomy (transverse section)

The exocarp consisted of a single layer of epidermis covered by a cuticle; the cells of the epicarp with a slightly thick wall had wave-shaped bending, and digitation fragments with free epidermal cells were present constantly (Fig. 5B, C; Wang and Gao, 2011). The mesocarp consisted of multiple layers of cells with a

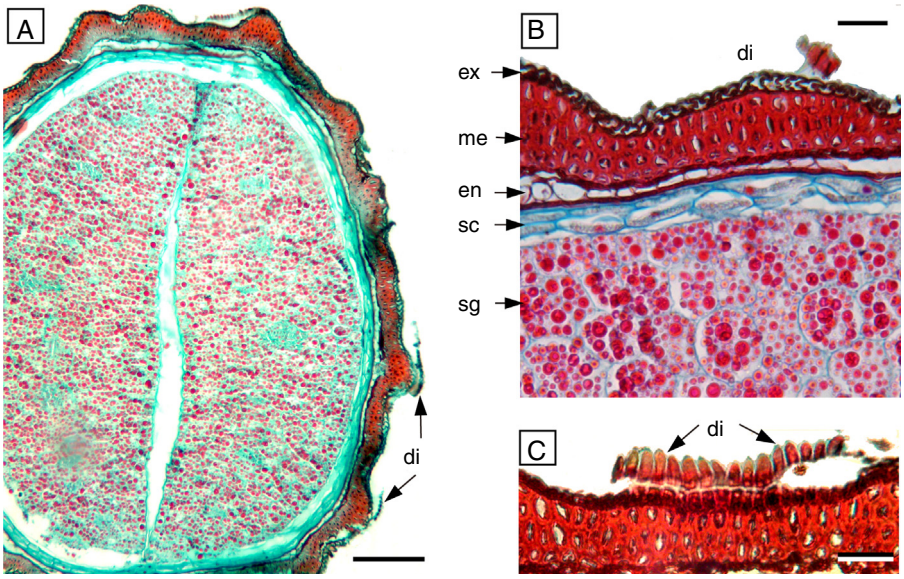

Fig. 5. Photomicrographs of fruit of *Picris japonica*. (A, B) Overall structure of fruit; (C) digitations of fruit. *Abbreviations*: ex, exocarp; me, mesocarp; en, endocarp; sc, seed coat; sg, starch grain; and de, digitations. Scale bar = 100 μm (A) and 20 μm (B, C).

heavily thick wall (Fig. 5B). The endocarp was a layer of oval cells (Fig. 5B). The seed coat was formed by two layers of elongated parenchyma cells (Fig. 5B). There was no endosperm. Cotyledons were round-like or oval parenchyma cells that contained a large amount of aleurone grains (Fig. 5A, B). Several round-like vascular bundles were scattered in the cotyledons, and the vascular bundles were made of multiple smaller tightly arranged round-like or oval parenchyma cells that contained fewer aleurone grains (Fig. 5A, B). The outmost layer cells of the cotyledons contained calcium oxalate crystals.

Powder sample

The multiseriate non-glandular trichome with branched apical section (Fig. 6A) and multicellular head of glandular trichomes were common (Fig. 6B–1, B–2). The pollen grain was observed (Fig. 6C, D). Most of the vessels were the spiral vessel or the bordered pit vessel, as well as the reticulated, scalariform and annular vessels (Fig. 6E–I). The diameter of the spiral vessel was 27–453 μm, and the diameter of the bordered pit vessel was 53–427 μm. There were many broken pappus and columns (Fig. 6J–L). Fibers, fiber bundles and parenchyma cells were common (Fig. 6M, O). Epidermal stem cells were common and purple (Fig. 6N). Subsidiary cells of leaves (Fig. 6P) and calcium oxalate crystal were rare, and prism crystals were more than cluster crystals (Fig. 6Q). Digitations, tracheid and laticifer were rare (Fig. 6R–T). The laticifer was articulate laticifer (Fig. 6T).

Discussion

The root or the whole plant of *P. japonica* has medicinal value with clinical applications (Chinese Materia Medica, 1999). Study of the morphological features of plants is crucial for species identification. The identification of pharmaceutically used species in pharmacopeias traditionally relies, besides phytochemical characterization, on morphology and anatomy. Morphological and anatomical characteristics are thus mandatory for drugs' pharmaceutical identification and purity control. However, few detailed morphological and anatomical studies of the plant organs of *Picirs* have been conducted, and these have only studied parts of the organs: Mature cypselas of *P. hieracioides* have been studied morphologically and anatomically with the help of light microscope (Das and Mukherjee, 2012) and the foliar characters of *Picris echioides* (Cichorieae) have been studied morphologically (Rivera et al., 2017). Previous work (Wulan and Gao, 2008) described characteristics of the stem, leaf and the powder of *P. japonica*. An optical microscope and a scanning electron microscope were used to observe the pollen grain and fruit of *P. japonica* in our previous work (Wang and Gao, 2011). In this paper, detailed morphological and anatomical studies of the root, stem, leaf, pollen grain, and fruit of *P. japonica* were performed, a paraffin section and other conventional methods were used to prepare specimens of the root, stem, leaf and powder of *P. japonica*. Furthermore, we defined the mainly anatomical characteristics of the root, stem, leaf, pollen grain and fruit of this plant, and describe featured microscopic structures for the identification of *P. japonica* such as following: (i) multiseriate non-glandular trichome with two branched apical parts covering the epidermis of the aerial parts of plants (Figs. 2D, 3F). (ii) There are three types of glandular trichomes: one kind, uniseriate filiform simple trichomes (Krak and Mráz, 2008), was often found on the ridge edge with a uniseriate foot, 4–5-isodiametric cells and a globose unicellular head (Fig. 2E); the second kind was multiseriate glandular trichomes with a pluriserial foot and longitudinally elongated cells arranged in 2–3 layers with a globular pluricellular head (Fig. 2D); and the third kind has ellipse head which was composed of multiseriate cells and the handle consisting of 1–4 long, narrow cells, arranged in a single row with some of the heads having one or two bulges that were composed of multiseriate cells (Fig. 3C). (iii) The laticifer of root was articulate laticifer (Fig. 6T). (iv) Most of the vessels were the spiral vessel or the bordered pit vessel, as well as the reticulated, scalariform and annular vessels (Figs. 2C, 6E–I). (v) The pollen exine with thorn-like ornamentation forming the polygon grid ornamentation had three apertures. (vi) Digitations on the exocarp of fruit were special (Figs. 5C, 6R).

Compared to the foliar characters of *Picris echioides* which have been studied by Rivera et al. (2017), the characters of the midvein we observed were consistent with that of *Picris echioides* in the following aspects: the midvein occupies the central position of the leaf; the epidermis of the midvein was the same as in the lamina and both with thick outer epidermis wall; the vascular tissue generally formed an arch, with parenchyma at both ends of the vascular bundles.

The morphological features of fruit of *P. japonica* were observed in our study. It was observed that the cypsela of *P. japonica* was elliptic in shape, 3–5 mm long, tan brown, with 5 to more than

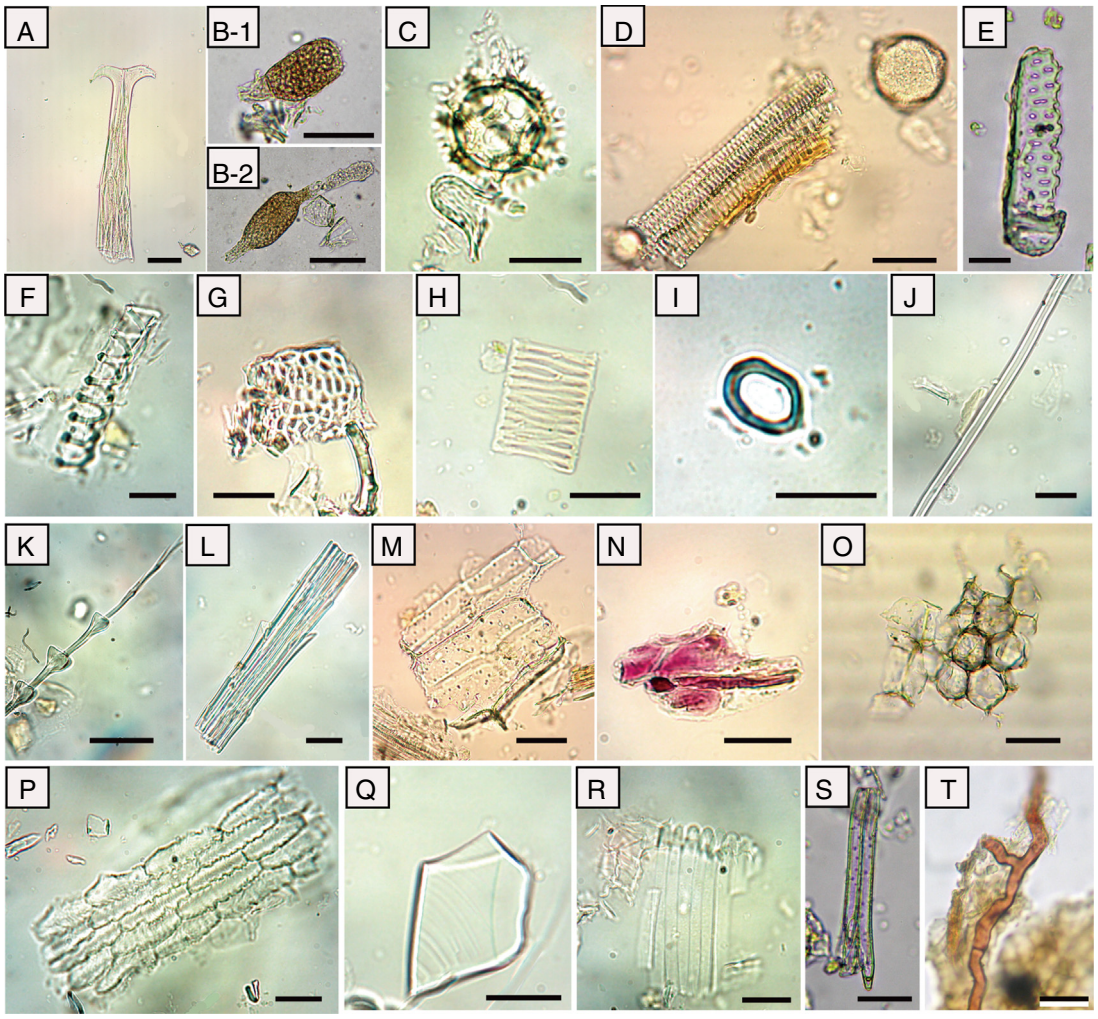

Fig. 6. Photomicrographs of powder of *Picris japonica*. (A) Multiseriate non-glandular trichome with branched apical part; (B-1, B-2) the head of glandular trichome; (C) pollen grain; (D) pollen grain and vessels; (E) bordered pit vessel; (F) spiral vessel; (G) reticulate vessel; (H) scalariform vessel; (I) annular vessel; (J, K) broken pappus; (L) column; (M) fiber; (N) broken epidermis of stem; (O) parenchyma cells; (P) subsidiary cells; (Q) calcium oxalate square crystal; (R) digitations of fruit; (S) tracheid; (T) broken laticifer. Scale bar = 50 μm (A, D, K, M, O, T), and 20 μm (B, C, E–J, L, N, P, Q, R, S).

ten longitudinal ribs, five deep longitudinal furrows, and horizontal ridges (i.e., digitations on the exocarp of fruit, Fig. 5) on or between the ribs. Our findings were confirmed by the studies on the cypsela features performed by Das and Mukherjee (Das and Mukherjee, 2012). *P. hieracioides* and *P. japonica* belongs to the same genus, and we found that some features of fruit of *P. japonica* were similar to that obtained by Das and Mukherjee in the studies on cypselar features of *P. hieracioides*. The cypselar of *P. hieracioides* is black in color, 3–5 mm long, ellipsoid-fusiform shaped, straight, narrowed toward base, truncate at apex, not beaked, with five ribs and furrows and each rib bears a vascular trace, apical part of the muricate cells with dense contents; on the cypselar wall, transversely distinctly muricate was observed (Das and Mukherjee, 2012).

In the present study, we found several differences from the previous study (Wulan and Gao, 2008) of the microscopic structures of *P. japonica*. First, the leaf palisade tissue Wulan and Gao (2008) observed was composed of a row of cells without passing through a vascular bundle. In this paper we observed that the leaf palisade tissue consisted of 1–2 rows of cells passing through the main vein but not through the vascular bundle (Fig. 3B-1, B-2). We also determined the number and morphology of the vascular bundle in the main vein of the transverse section of the leaf was 1–5 collateral vascular bundles of various sizes arranged into a half circle. The minor differences in the morphology of leaves may be caused by environmental variation or be due to sampling errors. The occurrence of specific character combinations in each species is due to a very fast evolutionary process involving the growth form, the adaptation to the environment, and the phylogeny of the family (Rivera et al., 2017).

Second, they did not observe the leaf surface. This paper had been observed on the surface of the leaf and get the characters as follows. The anticlinal walls of the lower epidermal cells were wavy and the superficial cutin texture was radial (Fig. 3E). There were more glandular trichomes and non-glandular trichomes on the upper and lower epidermis. The top of most of the non-glandular trichomes have two bifurcated hooks (Fig. 3F) although three bifurcated hooks or hooks without bifurcation are seen occasionally. Most of the glandular trichomes were composed of 3–6 single row cells; the head of which were single, large-scale cells (Fig. 2E). They are anphistomatic leaves. The stomata were of an anisocytic type or an anomocytic type with 4–6 subsidiary cells of anomocytic type or 7–8 subsidiary cells being seen occasionally (Fig. 3E).

Last, specimen of powder: The glandular hairs they observed were T-shaped, equivalent to the broken tops of the multiseriate, non-glandular trichome in our study (Fig. 6A.) In Wulan and Gao's work, the broken non-glandular hairs were believed to be composed of single cells with straight and long thick

wall; in present paper we found they consisted of broken pappus (Fig. 6J, K).

As shown in the literature (Krak and Mráz, 2008), studied of the characters of plant organs (such as trichomes) have been considered an important tool in taxa delimitation in many plant families. The findings we have now obtained will be significantly useful in species identification.

Authors' contributions

YHW contributed in collecting plant samples, conducting the laboratory work, analyzing the data and drafting the paper. YYW contributed to writing the manuscript in English. JG designed the study, collected and identified plant samples, supervised the laboratory work and contributed to the critical reading of the manuscript. All of the authors have read the final manuscript and approved its submission.

Conflicts of interest

The authors declare no conflicts of interest.

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