Comparing and authenticating on anatomical aspects of *Abrus cantoniensis* and *Abrus mollis* by microscopy

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Submitted: 06-08-2014 Revised: 03-11-2014 Published: 02-02-2015

ABSTRACT

Background: *Abrus cantoniensis* is popularly used as traditional Chinese medicine and a cool tea in South of China. However, due to diminishing source of *A. cantoniensis*, it is usually interchanged or adulterated with other species of *Abrus* genus because of the limited knowledge in identification and differentiation. Especially, *Abrus mollis* is widely mixed on herbal markets and pharmaceutical preparation. **Objective:** To ensure safety and efficacy, a detailed comparison was undertaken to carry out an anatomical and micro-morphological study of two species of *A. cantoniensis* and *A. mollis*. **Materials and Methods:** Microscopic characteristics of roots, leaves and stems, including transverse sections and the crude drug powder, were observed using a light microscope according to the usual microscopic techniques. **Results:** The basic diagnostic features of *A. cantoniensis* include that stem is extremely thin; xylem vessels of root are radially arranged in 10 or more bundles; pith is hollow in stem, and the palisade tissue is made up of two layers of palisade cells. Furthermore, scanning electron microscopy was used to compare nonglandular hairs and the stomata of the leaflet surface. A table of the key authentication parameters based on the analyzed microscopic characteristics was drawn up. **Conclusion:** The study demonstrated that the microscopy and related techniques provided a systematic method that is convenient, feasible, and can be unambiguously applied to the authentication of the species of *Abrus*.

Access this article online

Website: www.phcogres.com

DOI: 10.4103/0974-8490.150526

Quick Response Code:

Key words: *Abrus cantoniensis, Abrus mollis*, authentication, microscopy

INTRODUCTION

Abrus cantoniensis Hance (Family: Leguminosae), commonly known as "Jigucao" in Chinese, has been used as a cool tea in South of China and South East Asia. Nowadays, it was distributed in tropical and subtropical area.[1] However, due to the absence of the resource, *Abrus mollis* Hance, originated from *Abrus* genus of Leguminosae, has been mixed to use as "Jigucao" in herbal markets in China.[2] Furthermore, its taxonomy presents some difficulties beginning with a controversial delimitation of species and intraspecific taxa.[3] In previous study, little systematical micro-morphological characters were considered.

The whole plant of "Jigucao" was used in traditional Chinese medicine. It was firstly recorded in "Linnan caiyaolu".[4] and used for clearing heat and detoxicating, invigorate the circulation of blood, comfortable liver analgesia. Furthermore, it was widely used in the treatment of hepatic and gall diseases and processes the perfect efficiency.[5] Recently, *Abrus* herba has got scientific attention due to its significant biological activities, such as hepatoprotective, anti-oxidant activity, anti-inflammatory, analgesia, and antifungal effects.[6,7] In addition, its effect on inhibition of surface antigen of the hepatitis B virus (HBsAg) and the HBeAg in serum and as immunostimulant has also been reported.[5,8]

According to the Chinese Pharmacopoeia 2010, "Jigucao" refers only to the herb of *A. cantoniensis*.[9] During herbal drug market survey, it was observed that *A. mollis,* known as "Maojigucao" in Chinese, were being sold under the trade name "Jigucao" in mixed form.[10] The two species were interchanged or adulterated with each other and their identification poses considerable difficulties due to the similarity in their appearance. Furthermore, pharmacological studies demonstrated that *A. cantoniensis* can inhibit the auricular tumefaction causes

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by dimethylbenzene but *A. mollis* have no the efficiency.[8] The toxicity cases occasionally happen in the application of "Jigucao" in recent years.[11,12] Therefore, to distinguish the two species of *Abrus* is absolutely necessary.

Based on the modern phytochemical and pharmacological studies, the compounds responsible for the biological activities of *Abrus* may be a series of steroidal alkaloids and flavonoids.[13-15] One of the most important alkaloid is abrine, which can potentiate the humoral immune response of the host. Therefore, abrine was used as a marker to control the quality of *A. cantoniensis* and *A. mollis*.[16] However, phytochemical identification and analysis for botanic drugs were based on the detection of one or more chemical markers in the botanic drugs. Obviously, this strategy cannot distinguish effectively between *A. cantoniensis* and its kindred plants, which may contain similar constituents in the same family or genus.[17,18] Furthermore, some compounds other than the chemical markers in these kindred plants could be of potential danger for health of human beings. Besides, this method is also invalid for identification of the adulterants spiked with some chemical markers.

However, microscopic authentication is a facile, inexpensive, and objective method to solve the problem, which has been recorded in Chinese Pharmacopoeia, American Herbal Pharmacopoeia and Japanese Pharmacopoeia. It is also frequently reported in literatures for authentication of Chinese materia medica (CMM), traditional Tibetan medicine, and Chinese patent medicine.[19-23]

Despite microscopic characteristics can often be useful in distinguishing the various species from the same family or genus, few works on the anatomical structure of *A. cantoniensis* and *A. mollis* were reported. Therefore, a systematic and detailed microscopic method was performed for the authentication of the two herbs. Subsequently, the representative and unique microscopic features were characterized and recorded by digital color photography.

MATERIALS AND METHODS

Both *A. cantoniensis* and *A. mollis* were collected in 2011 [Table 1], and authenticated by Hao Zhang (West China School of Pharmacy, Sichuan University, Chengdu, P. R. China). Voucher specimens were deposited in Southwest University for Nationalities. The dried samples were used in the study.

All the transverse sections of the materials were prepared using a Leica 3325R microtome (Leica Instruments, Nussloch, Germany). An imaging system consisting of a Carl Zeiss Axioplan two imaging optical microscope (Carl Zeiss, Oberkochen, Germany) was used for photographs acquisition. Scanning electron microscope (SEM) (Philips XL30 Esem-FEG, Netherland).

Federal Aviation Administration (FAA 50) (Formalin, glacial acetic acid, and 50% ethanol in the ratio of 5:5:90 parts, respectively) and a gradual ethanol series (from 30% to 95%) were prepared for the specimen fixation and dehydration, respectively. Safranin and fast green solution were used for staining. Chloral hydrate and diluted glycerin were prepared based on the procedures described in Appendix XV B of the Pharmacopoeia of the People's Republic of China. Dimethylbenzene, xylene, paraffin wax, and Canadian balsam used in this study were all chemical grade.

The morphological identification is similar to the previously reported methods,[20] color digital photographs were taken with the Nikon digital camera D90.

The dried materials were firstly softened by boiling and then cut into appropriate sizes and fixed in FAA. Mid-regions of stem and leaf, the most mature region of the root available were taken to do the transverse section. Samples of root, stem and leaf were passed through the gradual ethanol series and xylene, embedded using paraffin, and sectioned on a microtome in slices 10–15 μm thick. Tissues were dewaxed using xylene and stained with safranin and fast green solution. Finally, the slice was mounted in Canada balsam before observation. Five samples per species were studied to reveal the key authentication tissue characters. The crude drug was powdered and passed through a 250 μm sieve. Ten different slides from the same powder were observed and studied to reveal the key authentication parameters. The values of various cells and tissues were obtained by

Table 1: Source of materials of the two *Abrus* species

Taxon	Batches	Locality	Elevation (m)	Date	Voucher
Abrus cantoniensis	AC-001	Nanning, Guangxi	350	August, 2011	J12081601
	AC-002	Hengxian, Guangxi	200	August, 2011	J12081602
	AC-003	Fushan, Guangdong	180	August, 2011	J12081801
Abrus mollis	AM-001	Yulin, Guangxi	200	August, 2011	M12081701
	AM-002	Yulin, Guangxi	200	August, 2011	M12081702
	AM-003	Wuzhou, Guangxi	550	August, 2011	M12081703

taking at least 20 measurements for each batch per species. All representative microscopic features were recorded by microscopic digital imaging system.

Leaf fragments (about 5 mm × 5 mm) were mounted on aluminum stubs, then coated with gold to an approximate thickness of 10 nm. The leaf fragments were examined under a SEM (Philips XL30 Esem-FEG) at 30 kV and were photographed.

RESULTS

Macroscopic characters

Abrus cantoniensis: It is a climbing lianas, about 1–2 m long. Root grayish-brown to puce, tapering gradually to the end, fibrous root is few and not developed. There are 3–7 branches near the rhizome. The surface is grayish-brown, coarse and with fine longitudinal wrinkles. Stem is fasciculate, extremely thin, about 1 mm in diameter. The surface is smooth and grayish-brown to puce, with sparsely and shortly pubescence. It has pinnately alternate compound leaf, 6–11 pairs of leaflets and almost fall off when dried, short petiole, quadrature circle or sub-rectangle, 0.5–1.5 cm long and 0.3–0.6 cm wide, truncate at the apex, with a small tip, and entire. The upper epidermis of the leaflets is sparsely pubescent, and the lower epidermis is densely strigose. The veins of the two sides are protruding. The odor is slightly fragrant, and the taste is slightly bitter.

Abrus mollis

It is also a climbing lianas, root is significant and lateral roots are numerous and developed. There are 1–2 branches or no branches near the rhizome. The stem is bigger than that of *A. cantoniensis*, about 2 mm in diameter. The surface is coarse, twigs are yellowish-green, with densely velutinous. The leaflets 9–16 pairs, 1.0–2.5 cm long and 0.5–1.0 cm wide, and the upper epidermis are densely puberulous, the lower epidermis is white pilose. The veins of the two sides are not obvious. The morphological features of the crude drugs of the two species are shown in Figure 1 and Table 2.

Microscopic characters

Root anatomy

The transverse section of the root of *A. cantoniensis* is almost circular in outline. The cork consists of 3–6 layers of brown cells. Cortex relatively narrows. Stone cells and prisms of calcium oxalate were interruptedly arranged in a ring under the cortex. Phloem fibers occasionally visible, phloem narrow, about 10 layers of parenchyma cells and the outside layers have bigger cells with gaps. Xylem broad, forming a continuous ring of lignified tissue, including vessels with spiral and reticulated thickenings. Vessels are radially arranged in 10 or more bundles. Xylem ray 10 or more, with 3–8 rows of parenchyma cells. The center is occupied by primary xylem with sparse vessels, solitary or in groups of 2–3 [Figure 2].

The transverse section of the root of *A. mollis* is mostly similar except that xylem vessels are radially arranged into

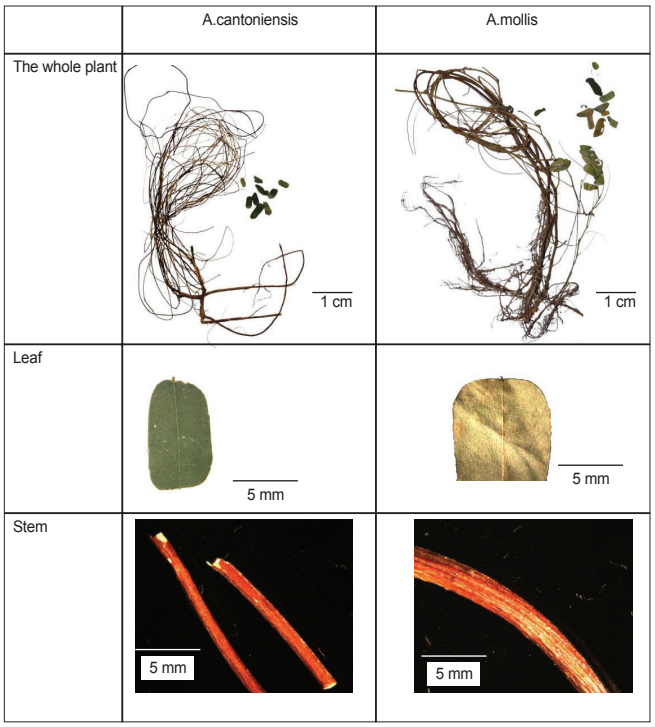

Figure 1: The morphological features of the two species of *Abrus*

Table 2: Comparison of the macroscopic characters of the two *Abrus* species

Key authentication and comparison parameters	*Abrus cantoniensis*	*Abrus mollis*
Root	Main root is significant and fibrous root is few, not developed 3-7 branches near the rhizome	Main root is significant and fibrous roots are numerous and developed 1-2 branches or no branches near the rhizome
Stem	Extremely thin, about 1 mm in diameter. The surface is smooth, with sparsely and shortly pubescence	About 2 mm in diameter. The surface is coarse, with densely velutinous
Leaf	6-11 pairs of leaflets The veins of the two sides are protruding 0.5-1.5 cm long and 0.3-0.6 cm wide	9-16 pairs of leaflets The veins of the two sides are not obvious 1.0-2.5 cm long and 0.5-1.0 cm wide

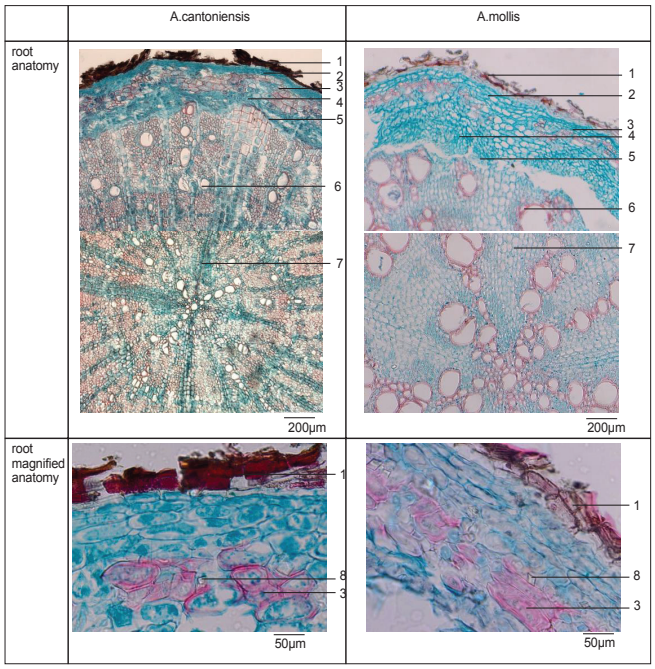

Figure 2: The transverse section illustration of the root of the two species of *Abrus* (1: Cork, 2: Cortex, 3: Stone cells, 4: Phloem, 5: Cambium, 6: Xylem, 7: Ray, 8: Pith, 9: Prisms of calcium oxalate, 10: Phloem fibers)

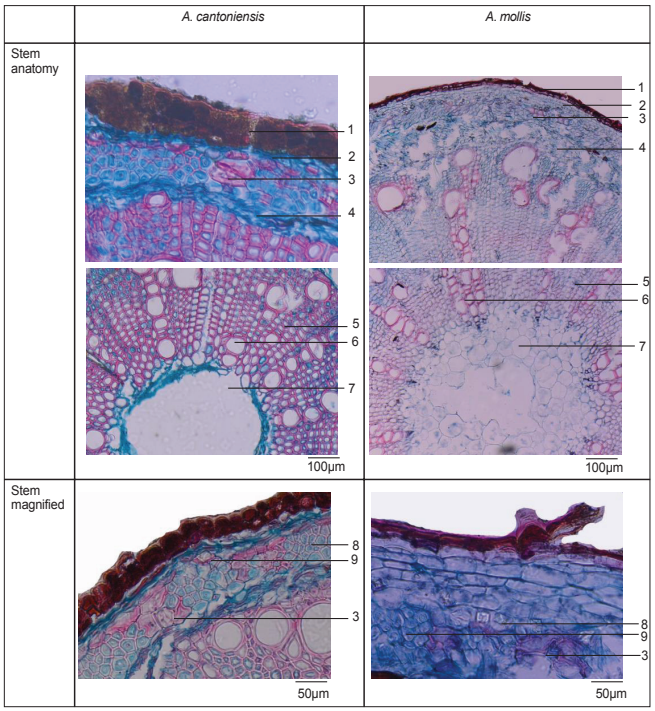

Figure 3: The transverse section illustration of the stem of the two species of *Abrus* (1: Cork, 2: Cortex, 3: Pholem, 4: Xylem, 5: Pith, 6: Pholem fibers, 7: Prisms of calcium oxalate, 8: Stone cells)

six bundles and the diameter of vessels is bigger. Xylem rays 6, with 3–6 rows of parenchyma cells [Figure 2].

Stem anatomy

The transverse section of the stem of *A. cantoniensis* is almost circular in outline and the center is hollow. The cork consists of 2–3 layers of cells. Cortex is very narrow, with 2–4 layers of compressed cells. Phloem fibers are visible. Stone cells and prisms of calcium oxalate were occasionally arranged among the fibres. Phloem narrow. Xylem relatively broad, forming a continuous ring of lignified tissue. Xylem vessels bundles 9, surrounded with many xylem fibers. Xylem ray radially arranged and extended from pith to cambium. The central zone is the pith that is almost hollow and composed of 1–2 parenchyma cell layers.

However, Cortex relatively border, with 4–6 rows of oblong cells for the transverse section of the stem of *A. mollis*. The phloem fibers are numerous and integrate with bundles. Xylem vessels bundles are more than 10. The central zone is the pith that is composed of parenchyma cells. Sometimes the parenchyma cells break and become a small hollow [Figure 3].

Leaf anatomy

The upper epidermis of *A. cantoniensis* composed of one layer of cells, subsquare or rectangular. Prisms of calcium oxalate are occasionally visible under the epidermis. Palisade tissue consists of two layers of palisade cells, arranged densely. Spongy tissue cell is sub-rounded and arranged loosely. Vascular bundles collateral in type. The prisms of calcium oxalate are visible in the parenchyma cell of the upper and lower sides of the midrib. Xylem vessels radially arranged. The phloem is narrow, consisting of 3–6 layers of parenchyma cells. Fibres are visible around vascular bundles, arranged densely, and slightly lignified. Lower epidermis composed of one layer of cells, irregular, smaller than that of the upper epidermis.

However, for the transverse section of the leaf of *A. mollis*, upper epidermis composed of one layer of cells, subsquare or subrounded, with some nonglandular hairs. Palisade tissue consists of one layer of palisade cells. Upper epidermis of the midrib is slightly protruding, and lower epidermis is "U" shape. Prisms of calcium oxalate and fibers of the outside of the midrib are less than that of *A. cantoniensis* [Figure 4].

Powders of the whole plant: *A. cantoniensis*, the color of the powder is grayish-green, Leaf epidermis curve, stomata anomocytictype in type, with two elliptical guard cell and 2–4 subsidiary cells. Cork cells brown rectangular, elliptical or irregular. Prisms of calcium oxalate numerous, 6–43 μm in diameter, polychromatic under the polarized microscope. Nonglandular hairs consist of a single cell, apex gradually acuminate, and with fine warty protuberance on the surface. Vessels are mainly bordered-pitted vessels, 10–53 μm in diameter. Fibers are single or in a bundle, 8–36 μm

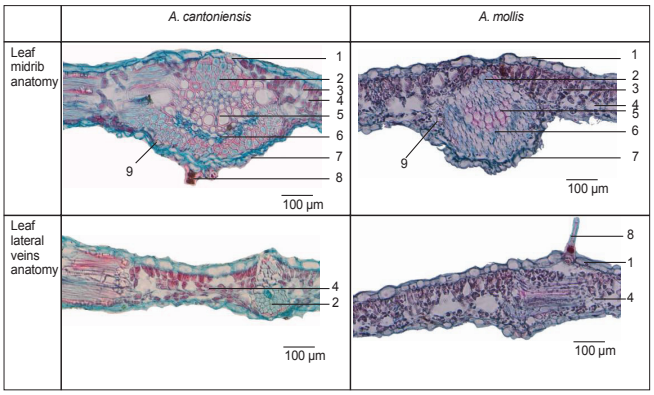

Figure 4: The transverse section illustration of the dried leaflet of the two species of *Abrus* (1: Upper epidermis, 2: Fibers, 3: Palisade tissue, 4: Spongy tissue, 5: Xylem, 6: Phloem, 7: Lower epidermis, 8: Nonglandular hair, 9: Prisms of calcium oxalate)

in diameter, and relative thick walls. Surrounding cells occasionally contain prisms of calcium oxalate and form crystal fibers, which is polychromatic under the polarized microscope. The stone cells are elliptical or sub-square, 11–74 μm in diameter, and the wall is slightly thick.

The characteristics occurring in the powders of *A. mollis* are similar to *A. cantoniensis* except that the former has more nonglandular hairs and more phloem fibers [Figure 5].

Scanning electron microscopy characters

The stomata and nonglandular hairs were investigated and their values were obtained by taking at least 20 measurements for each batch per species. The nonglandular hairs are in the lower surface of the leaf, with single cells. The diameter

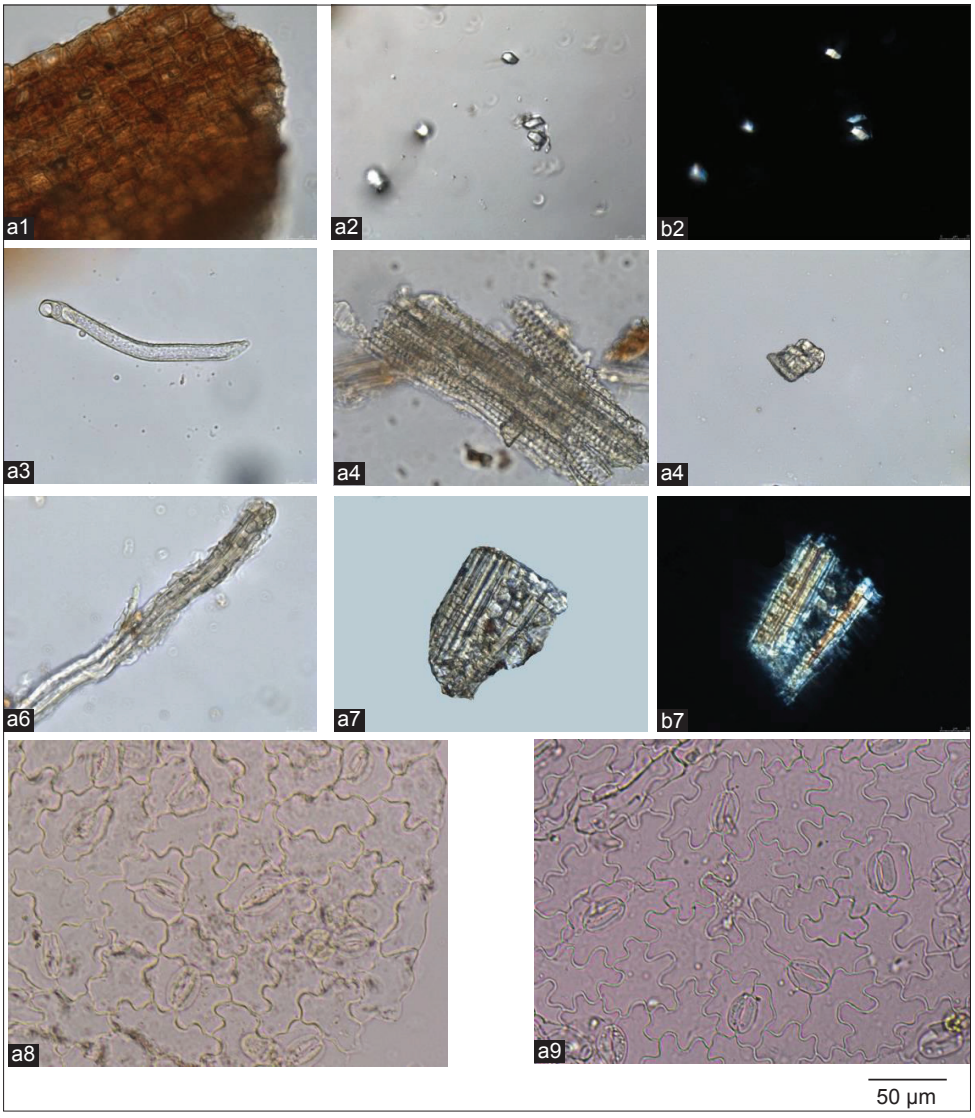

Figure 5: The microscopic features of powder of *Abrus* herba (1: Cork cells, 2: Prisms of calcium oxalate, 3: Nonglandular hair, 4: Stone cell, 5: Bordered pitted vessel, 6: Fibers in bundle, 7: Crystal fibres, 8: Epidermis cell of *Abrus cantoniensis*, 9: Epidermis cell of *A. mollis* (a) under the light microscope, (b) under the polarized microscope)

of nonglandular hair of *A. cantoniensis* (12–25 μm) is bigger than that of *A. mollis* (8–18 μm). However, the density of *A. mollis* (178 U/mm^2) is higher those of *A. cantoniensis* (33 U/mm^2). The sunken stomata of *A. cantoniensis* and *A. mollis* are similar in type and size. The density of the two species is almost the same about 40 U/mm^2. The inner edge of the external wall of the stomata is smooth. Outer stomata rims of the stomata apparatus are single and risen in *A. cantoniensis* but not risen in another species [Figure 6].

DISCUSSION

The evidence from previous anatomic studies demonstrated that variations have been existed in the species of *Abrus* genus, including *A. precatorius*, *A. pulchellus* and *A. canescens*.[24,25] Some similar case can be observed in both *A. cantoniensis* and *A. mollis*. The comparative microscopic observations of root, stem and leaflet various species of *Abrus* revealed that many of these anatomical characteristics are homologous. Accordingly, we draw up a generalized description to account for these similarities and differences: The transverse section of root is almost circular in outline. The major difference is the number of xylem vessels bundles. It is also considered as the standard to distinguish from *A. precatorius*, *A. pulchellus* and *A. canescens*. Other variations were observed to be due to the numbers of layers of certain tissues such as cortex, xylem ray and pith cell, which are similar to those reported by Hu.[10] Stem is easy to distinguish *A. cantoniensis* and *A. mollis*. The stem of *A. cantoniensis* is extremely thin; about 1 mm but *A. mollis* is more than 2 mm. The transvers section of stem is almost similar; the major difference is cortex cells. It is compressed flatten cells in the cortex of *A. cantoniensis,* but it composed of oblong cells. Comparing anatomy of

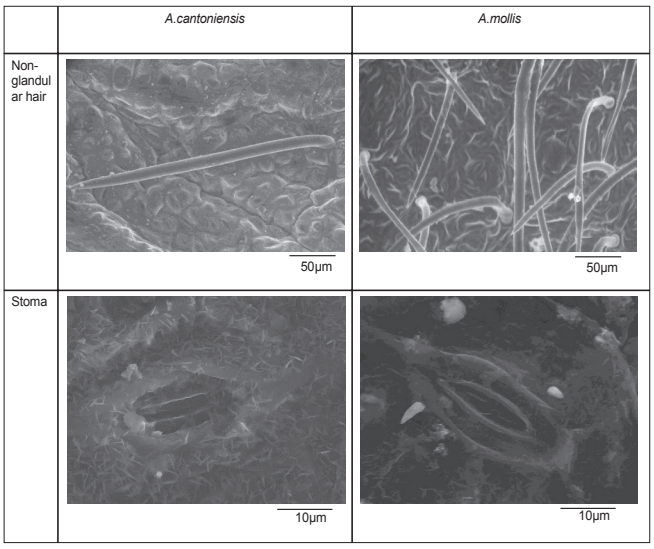

Figure 6: The scanning electron microscope features of nonglandular hair and stomata of leaflets epidermis

leaflet revealed similarity and overlap in certain features. The stomata density and stomata index is of restricted value because it is environmentally influenced for *Abrus* species.[26] However, the numbers of palisade tissue offers some useful parameters for distinguishing the two species.

In previous study, the difference of chemical constituents of whole plant as well as different parts of *A. cantoniensis* and *A. mollis* were studied by Fourier transform infrared (FTIR) with secondary derivative spectra and semi-quantitative analysis. The results demonstrated that the two species contain volatile oil, triterpenes, flavonoids, saponins, polysaccharides and *A. mollis* was higher than *A. cantoniensis*.[27] However, if there is no another species to compare, it is difficult to identify the species by FTIR. Furthermore, it may greatly change for the content of the different location samples. Therefore, it is difficult to distinguish the two species by the chemical methods. Although there is a report about comparison of the morphological difference about *A. cantoniensis* and *A. mollis,* but they are tremendous similar in the morphology of root, stem and leaf.[10] Hence, it is a difficulty in the identification of plant species based on the morphologic features alone. In recent years, the development of more sophisticated chemical or molecular methods to be employed in quality control of CMM has become a trend.[28] However, such methods may be costly enough that they should not be employed when simpler methods could serve. Microscopic identification is the oldest, simplest, and cheapest one of all methods, thus to be preferred when its use is feasible.[29,30]

In Chinese pharmacopeia, only the identification of powder was documented by microscope. So, it is impossible to distinguish the two species due to their similar characters of the powder. Therefore, a systematically comparative method was built by microscopy in this study, which is useful for distinguishing the plants of the same genus.[31,32] Plant organs' transverse section, tissues and crude drug powder of the two species of *Abrus* were systematically observed by microscope. Furthermore, scan electric microscopy was firstly used for the study the stomata and the nonglandular hair. However, different species possess the unique microstructural characteristics in the transverse section. The anatomy and micromorphology of *Abrus* also reflect the high degree of diversity, which can be taken as the identifying standard of *Abrus* plants. The characteristics in Table 3 are a summary of key authentication and comparison parameters of the two species in the present study.

CONCLUSION

Through the comparative study of the anatomy and microscopic feature of the two species, we would like to

Table 3: Key microscopic authentication and comparison parameters of the two species of Abrus

Key microscopic authentication and comparison parameters	*Abrus cantoniensis*	*Abrus mollis*
Root	Xylem vessels are radially arranged in 10 or more bundles. Xylem ray 10 or more, with 3-8 rows of parenchyma cells	Vessels are radially arranged into 6 bundles. Xylem rays 6, with 3-6 rows of parenchyma cells
Stem	Cortex is very narrow, with 2-4 layers of compressed cells Xylem vessels bundles is more than 10	Cortex relatively boarder, with 4-6 rows of oblong cells Xylem vessels bundles is more than 10
Leaf	Palisade tissue consists of 2 layers of palisade cells Fibres visible around the vascular bundles	Palisade tissue consists of 1 layer of palisade cells Fibres were less
Nonglandular hair (SEM)	Few, 12-25 μm in diameter	Many, 8-18 μm in diameter
Stomatal rims (SEM)	Risen	Not risen

SEM=Scanning electron microscope

further study the relationship between anatomical structure and physiological function. Meanwhile, we also hope to lay the foundation for an accurate evaluation of different botanical origins of *Abrus* so as to ensure their safety and efficacy.

ACKNOWLEDGMENTS

The research was supported by the funds of basic research for application of Sichuan province (2014JY0233).

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Cite this article as: Zhang Z, Lu L, Liu Y, Qing L. Comparing and authenticating on anatomical aspects of Abrus cantoniensis and Abrus mollis by microscopy. Phcog Res 2015;7:148-55.

Source of Support: Nil, **Conflict of Interest:** None declared.