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# Authentication and Differentiation of Two Easily Confusable Chinese Materia Medica: Herba Solani Lyrati and Herba Aristolochiae Mollissimae

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## ABSTRACT

As people now use Chinese herbs worldwide, authentication of Chinese herbs is becoming a critical, international issue because mistakes can cause illness and even death. Confusion in the names is one aspect of this problem; counterfeiting is another. One example of the former, which has had serious medical consequences, involves the common name "Bai Mao Teng". This name has been used for both Herba Solani Lyrati (*Solanum lyratum* Thunb.) and Herba Aristolochiae Mollissimae (*Aristolochia mollissima* Hance). Apparently, these herbs belong to two different families but look similar. While *S. lyratum* is not harmful, *A. mollissima* contains a toxic substance, aristolochic acid I (AA-I), that can cause kidney failure and cancer of the urinary tract when taken inappropriately. This paper describes a systematic study of one specific example of species confusion, namely the case of *S. lyratum* and *A. mollissima*. To differentiate the two species, authentication was performed based on four criteria: taxonomy, morphology, microscopic characteristics, and chemical analysis. The methods used clearly distinguish the two species, providing specific criteria by which samples of these herbs can be identified.

Key words: aristolochic acid, Herba Solani Lyrati, *Solanum lyratum* Thunb., Herba Aristolochiae Mollissimae, *Aristolochia mollissima* Hance, Bai Mao Teng

## INTRODUCTION

A case of poisoning recently reported in Hong Kong was traced back to confusion over a name in an herbal prescription and ultimately led to the ban on a large number of herbs. A 60 year-old man was diagnosed with kidney failure and cancer of the urinary tract. He had been taking an herbal prescription that called for "Bai Mao Teng" Investigations by the Department of Health revealed that the patient was mistakenly given Herba Aristolochiae Mollissimae (its main action is to relieve rheumatic condition, promote blood circulation, remove obstruction of collaterals, and alleviate pain.) instead of Herba Solani Lyrati (its main action is to remove damp-heat, remove toxic materials, and promote the subsidence of swelling.). Herba Aristolochiae Mollissimae is known to contain poisonous aristolochic acid (AA)(1). Having traced the source of the herbs, it was found that Herba Aristolochiae Mollissimae was erroneously substituted for Herba Solani Lyrati at the wholesale level. It was also found that there is recurring confusion with regard to the names Herba Solani Lyrati, Herba Aristolochiae Mollissimae and Bai Mao Teng. In light of this, the Department of Health called for a suspension of the use of these three Chinese herbs. Subsequently the Department also prohibited importation and sale of all Chinese herbs containing AA(2).

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According to related research(3-7), prolonged and excessive use of herbs containing AA can cause kidney failure and cancer of the urinary tract.

While Herba Aristolochiae Mollissimae and Herba Solani Lyrati have the same common name, Bai Mao Teng, there are many important differences between them, including their origins, morphologies, chemical components and pharmacological activities.

This paper addresses research on the differences between Herba Aristolochiae Mollissimae and Herba Solani Lyrati collected in Hong Kong. Their taxonomies, morphologies, microscopic characteristics and chemical components (particularly AA content) were analysed and compared.

## MATERIALS AND METHODS

I. *Materials*

(I) *Plant Materials*

Herba Aristolochiae Mollissimae is the dried herb of *Aristolochia mollissima* Hance of Fam. Aristolochiaceae (Figure 1A). Herba Solani Lyrati is the dried herb of *Solanum lyratum* Thunb. of Fam. Solanaceae (Figure 1B).

The following 14 authenticated samples were purchased from herb shops in Hong Kong: Herba Solani Lyrati (BY0313-01, BY0317-01, BY0317-02, BY0323-01,

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**Figure 1.** The original plants of Herba Aristolochiae Mollissimae and Herba Solani Lyrati. (A) *Aristolochia mollissima* Hance; (B) *Solanum lyratum* Thunb.

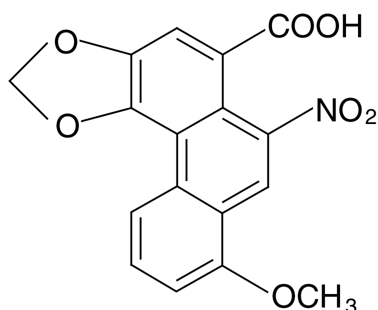


**Figure 2.** The structure of aristolochic acid I.

BY0323-02, BY0324-01, BY0327-01, BY0327-02, BY0327-03 and BY0406-01) and Herba Aristolochiae Mollissimae (XGF0311-01, XGF0311-02, XGF0323-01 and XGF0323-02). Voucher specimens of all samples are deposited at the Hong Kong Baptist University BOC (HK) Chinese Medicines Centre.

(II) *Reagents and Instrumentation*

Microscopic reagents included FAA fixed solution (containing 37~40% formaldehyde, 50% ethanol, acetic acid), ethanol (Uni-chem), xylene, Safranin T (Riedel-dehaën), Fast Green FCF. (Aldrich), Kanada balsam (Serva: feinbiochemica Heidelberg, New York), glycerin, chloral hydrate and paraffin wax (Uni-chem). Chloral hydrate and dilute glycerin were prepared according to the procedures described in Appendices 77 & 79 of the CP (2000 English Edition)[8]. Microscopic instruments included optical microscope SL-BI-S-2, OLYMPUS system biologic microscope C-4KC, Leica Digital Camera, Leica IM50 software and Microtome (Finese-325).

The chemical reference standard of AA-I (Figure 2) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Acetonitrile and acetic acid were purchased from E. Merck (Darmstadt, Germany). Water was obtained from a Milli-Q (Millipore, Bedford, MA, USA) water purification system.

The liquid-chromatography mass spectrometer used was Perkin-Elmer SCIEX API365 LC/MS/MS system. An Alltech C18 column (250 mm × 2.1 mm, 5 μm film thickness) was used at room temperature of 20°C. The voltages of electrospray, orifice and ring were maintained at 5 kV, 30 V and 170 V, respectively. The flow rate of nebulizing gas was kept at 1.04 L/min while the collision energy was 17 eV (lab. scale). Nitrogen was used as the collision gas and its density was maintained at $1.56 \times 10^{15}$ mole/cm$^2$.

II. *Methods*

(I) *Microscopic Examination*

The methods of microscopic identification were based on Appendices 21 and 22 of the CP (2000 English Edition)[8]. The stems of these samples were soaked in FAA fixative, and dehydrated by ethanol of different concentrations, cleared by xylene of different concentrations, embedded in paraffin wax, sliced, stained by safranin and

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fast green solution, and finally mounted in Canada balsam. At the same time, the samples were powdered, and cells examined microscopically using dilute glycerine and chloral hydrate solution as mountants.

(II) *LC-MS Analysis*

Although a variety of analytical methods were available in literatures for the quantification of AA[9-11], the present study adopted a standard analytical protocol from USFDA[12]. A mixture of 5% methanol, 10 mM ammonium acetate and 0.1% formic acid (A) as well as 50% acetonitrile in methanol with 10 mM ammonium acetate and 0.1% formic acid (B) were used as mobile phase in gradient mode. The flow rate was maintained at 200 μL/min, and the injection volume was 25 μL.

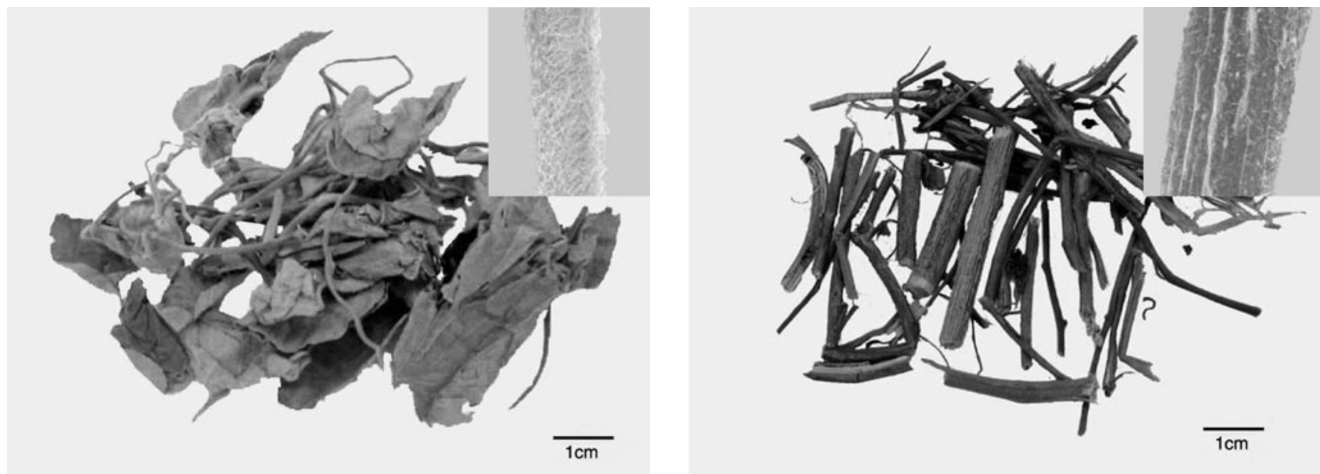


**Figure 3.** The pictures of the medicinal herbs. (A) Herba Aristolochiae Mollissimae; (B) Herba Solani Lyrati (the upper corner is the amplification of the stem).

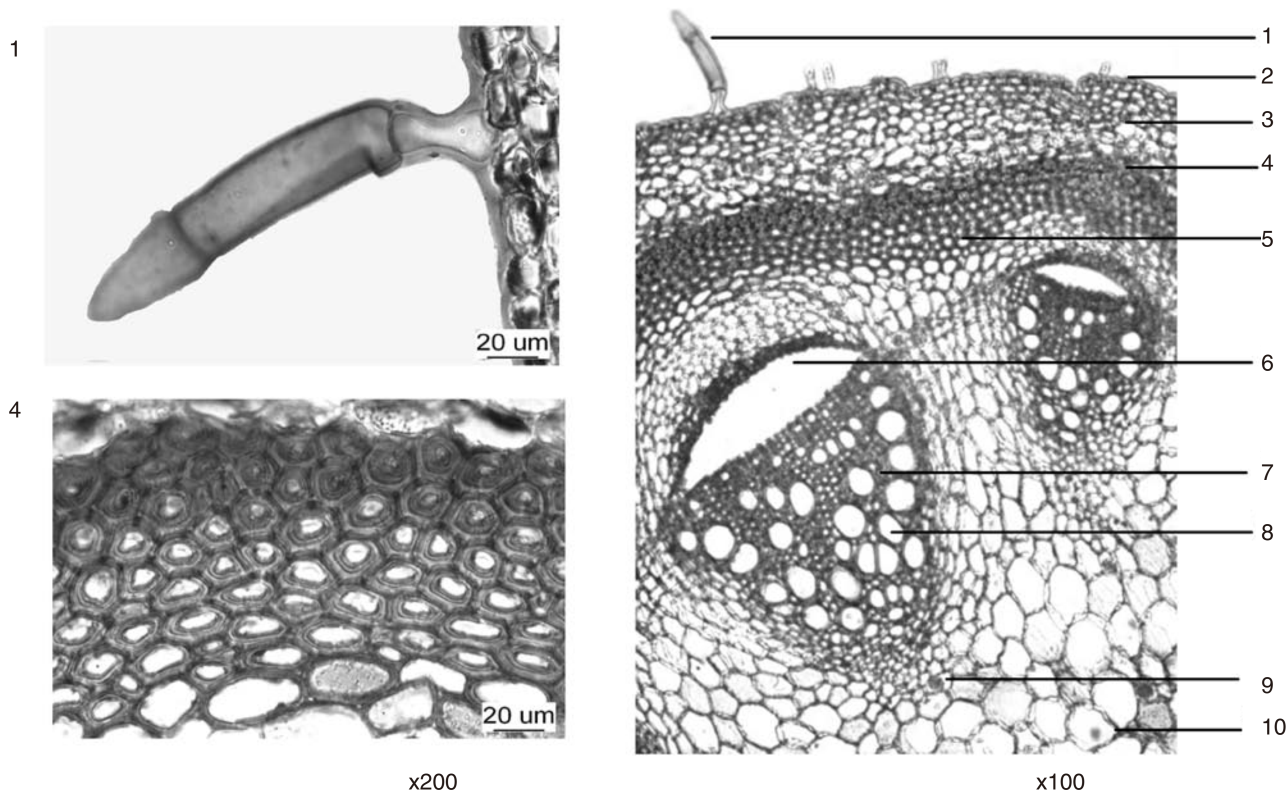


**Figure 4.** Microscopic features of transverse section of Stem Aristolochiae Mollissimae. 1. Non-glandular hair; 2. Epidermis cells; 3. Cortex; 4. Pericyclic fibres; 5. Stone cells; 6. Phloem; 7. Xylem; 8. Vessels; 9. Clusters crystals of calcium oxalate; 10. Pith.
×100, ×200: Magnification of the microscope.

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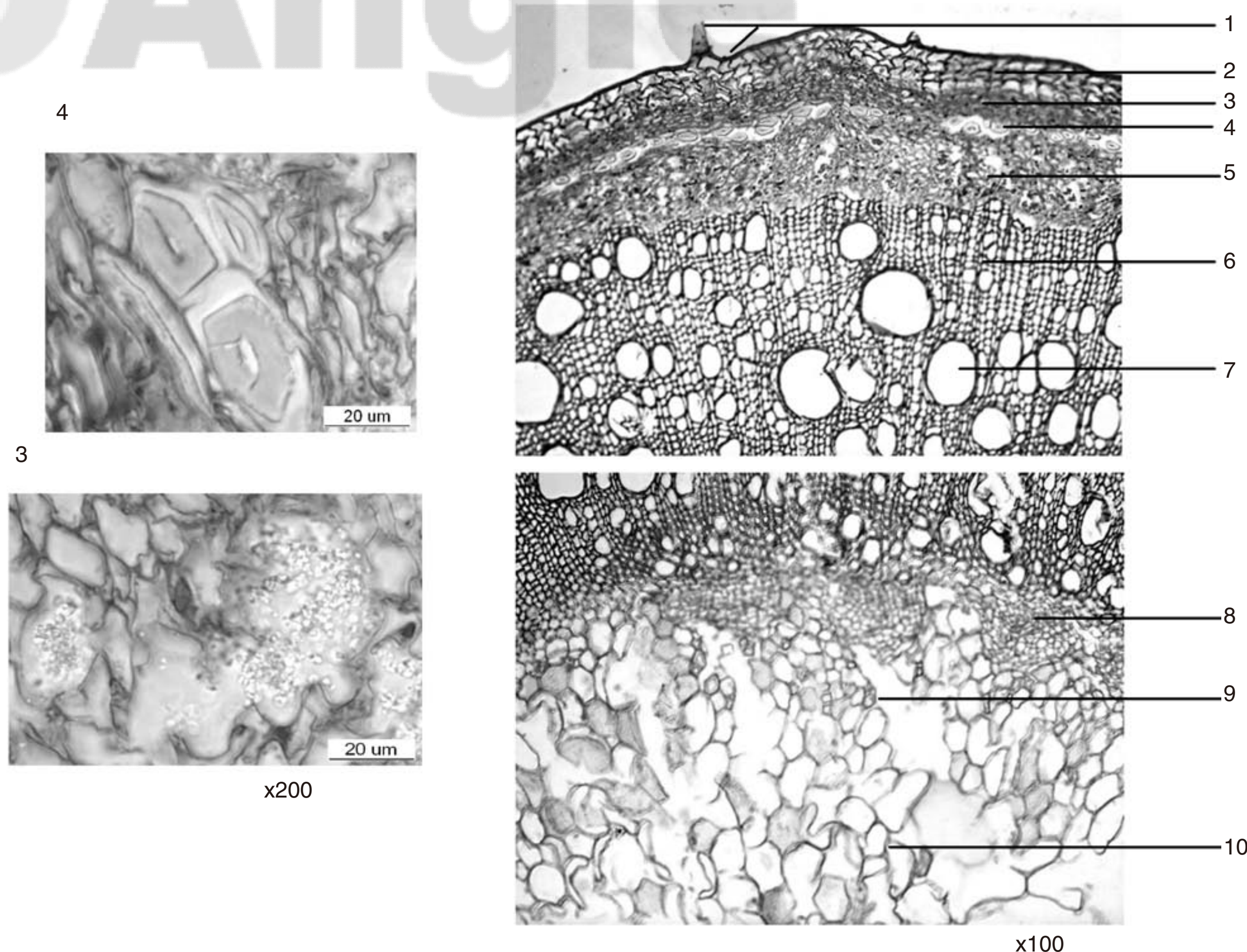


**Figure 5.** Microscopic features of transverse section of Stem Solani Lyrati. 1. Epidermis cells and non-glandular hair; 2. Phellem; 3. Cortex; 4. Pericyclic fibres; 5. Phloem; 6. Xylem; 7. Vessels; 8. Fibres; 9. Pith. ×100, ×200: Magnification of the microscope.

Confirmation of aristolochic acid I: AA-I was confirmed by the presence of a chromatographic peak at the retention time with corresponding ion ratio with respect to the standard AA-I.

Detection limit: Based on repeated injections of a low-content AA-I standard, the detection limit was 0.01 µg/mL.

Methanol extract of herbs: Samples of herbal materials were pulverized to fine powder. Each finely powdered sample (about 0.5 g) was extracted consecutively with 20 mL, 10 mL, and 10 mL of methanol and sonicated for 60 min at 40°C. The extracts were combined and centrifuged at 3000 rpm for 5 min. The supernatant was transferred into a 50-mL volumetric flask. Two milliliter of 10% formic acid/water solution was added, and then made up to volume with methanol. Methanol extract (0.5 mL) was then transferred into another 10-mL volumetric flask and made up to volume with methanol. The solution was filtered through a 0.45 µm filter membrane and the filtrate was ready as the test solution.

Water extract of herbs: Each finely powdered sample (about 0.2 g) was soaked in 10 mL of water for 20 min and then boiled for 40 min. After centrifugation at 3000 rpm for 5 min, the supernatant was transferred into a 50-mL volumetric flask and adjusted to volume with water. Five milliliter of water solution was extracted by 5 mL of ethyl acetate; the organic layer was then dried under a stream of air. The residue was reconstituted in methanol and the volume was adjusted to 10 mL in a volumetric flask. The solution was filtered through a 0.45 µm filter membrane and the filtrate was ready as the test solution.

## RESULTS

### I. *Macroscopic Identification*

#### (I) *Herba Aristolochiae Mollissimae (Figure 3A)*

Typically, the crude drug comprises many intact leaves, with few stems. Stems are tough, hard, slightly flexible; faint green, 1~2 mm in diameter, with woolly white hair. Leaves are mostly intact and faint green, cordate or oval in shape; both sides with woolly white hair. Odour is faint, fragrant. Taste is bitter.

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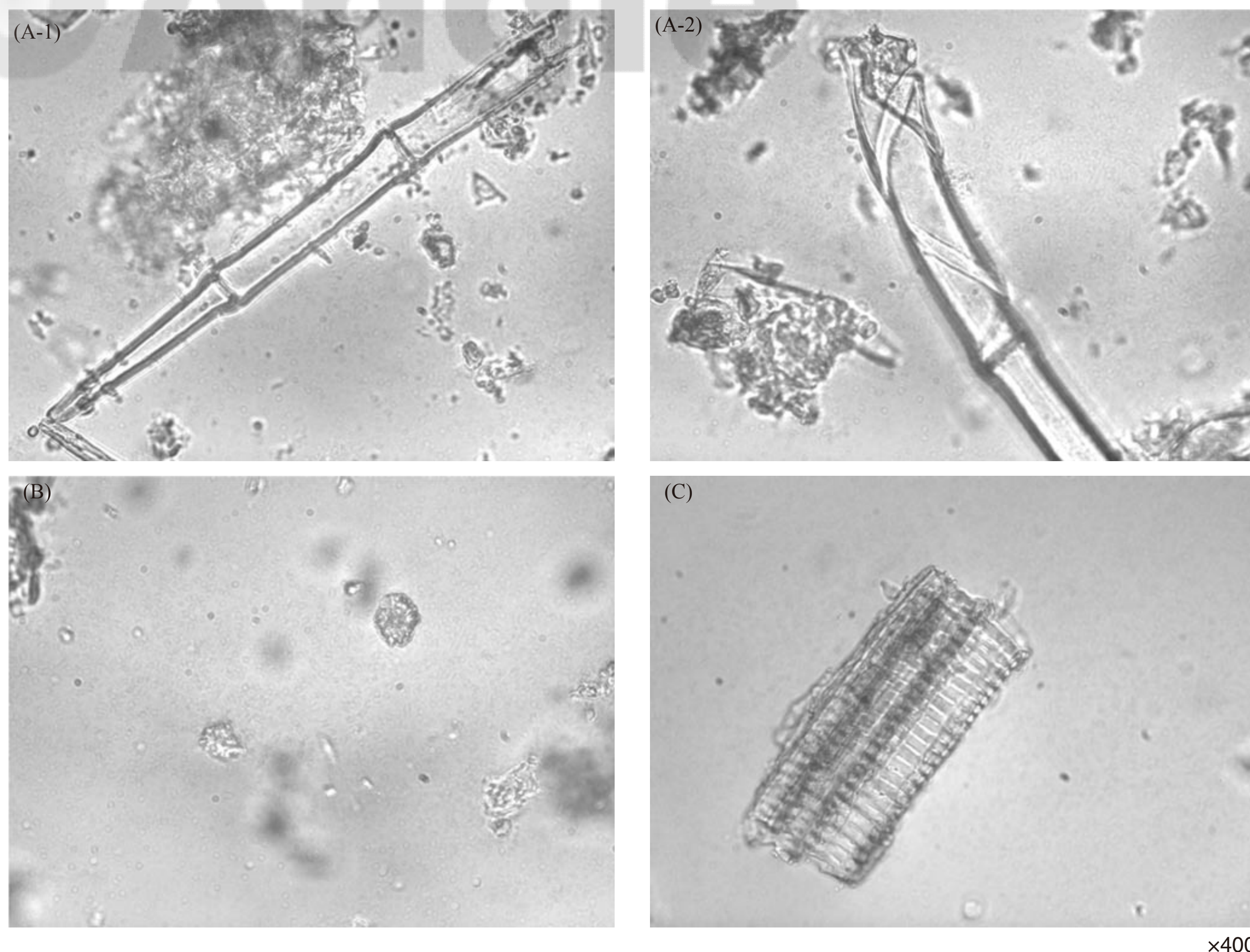


**Figure 6.** Microscopic features of powder of Herba Aristolochiae Mollissimae. (A) Non-glandular hair; (B) Clusters crystals of calcium oxalate; (C) Annular vessels. ×400: Magnification of the microscope.

(II) *Herba Solani Lyrati (Figure 3B)*

Typically, the crude drug comprises primarily stems, with few broken leaves. Stems are tough, but brittle and easily broken, with a fibrous fracture; yellow- to brown-greenish, 1~7 mm in diameter, with grey-white hair (not woolly) if any (The main stem commonly has little or no hair.). Leaves are mostly broken and dark green, panduriform or ovate in shape and have hair. Odour is faint. Taste is bitter.

II. *Microscopic Identification*

Identification of crude drugs/medicinal plants with microscopic technique requires small amount of sample. Therefore, the place and season of collection usually do not affect the result.

(I) *Transverse Section*

1. Herba Aristolochiae Mollissimae (Figure 4)

Stem: Epidermal cells are in a single layer. The outside cell wall is thick, covered with non-glandular hair. Cortex cells are orbicular or oblong in shape. Pericyclic fibres are arranged in an intermittent ring, with stone cells visible inside. Stone cells scattered singly or in groups, square or ellipsoid in shape. Cell walls are slightly thickened with distinct pits. Vascular bundles are of the collateral type. Phloem is composed of collapsed cells and clefts. Six to eight vascular bundles are scattered radially; the size of each bundle is different. Inter-cambium is distinct. Cortical parenchymatous cells, xylary rays and pith all contain clusters of calcium oxalate crystals, 15~40 μm in diameter.

2. Herba Solani Lyrati (Figure 5)

Stem: Epidermal cells are in a single layer, covered with non-glandular or glandular hairs. Two to four layers of cork cells are under the epidermis. Cortex cells mostly collapsed. Pericyclic fibres are arranged in an intermittent ring. Vascular bundles are of the bicollateral type. Phloem cells mostly collapsed. Cambium forms a ring. Xylem is

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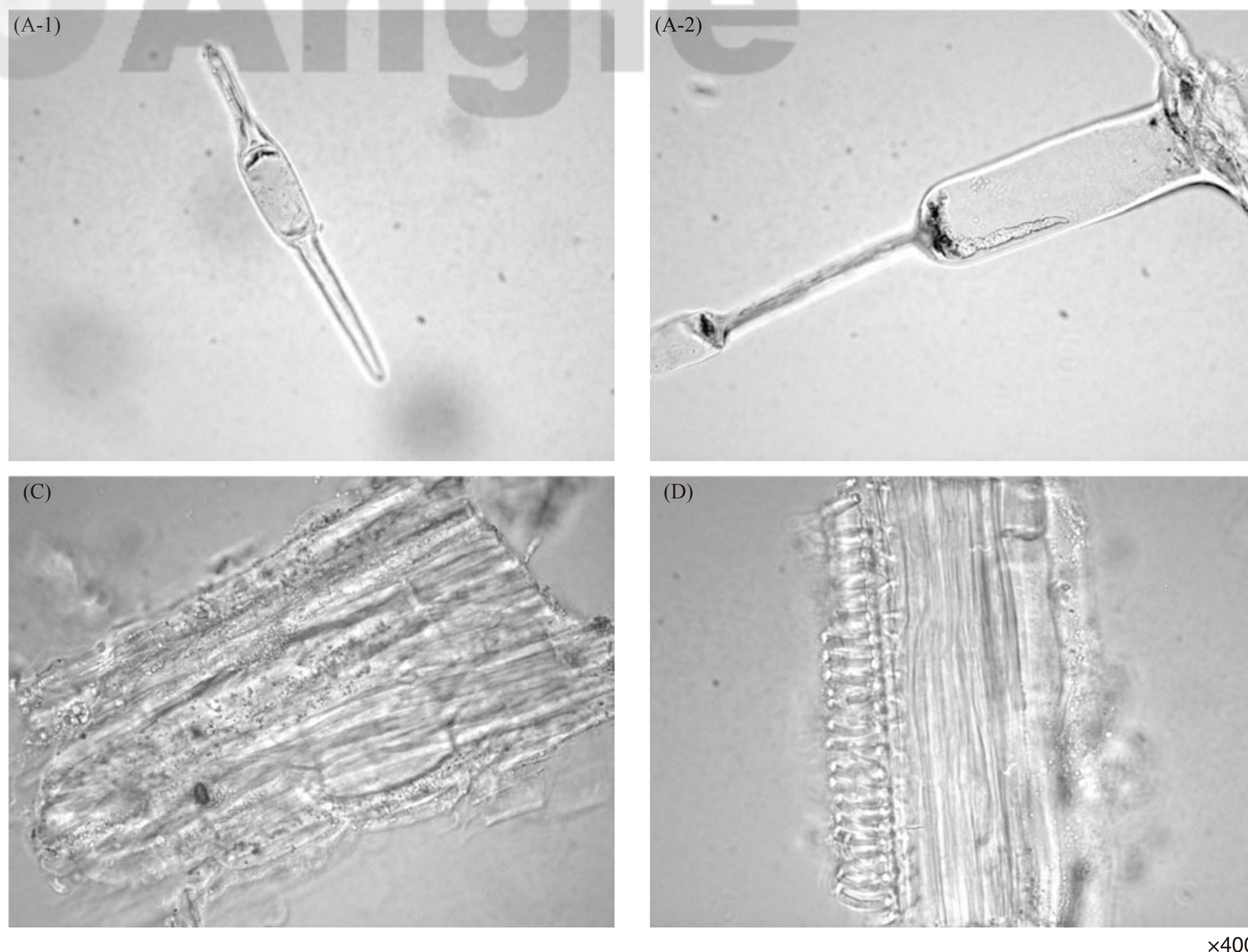


**Figure 7.** Microscopic features of powder of Herba Solani Lyrati. (A) Non-glandular hair; (B) Sandy crystals of calcium oxalate; (C) Spiral vessels. ×400: Magnification of the microscope.

wide, with indistinctive xylary rays. Pith is wide with visible fibres. Parenchymatous cells in the cortex and pith contain sandy crystals of calcium oxalate.

(II) *Powder*

1. Herba Aristolochiae Mollissimae (Figure 6)

Yellowish green color. (1) Non-glandular: many, single or 2~4 cells, 12~35 μm in diameter. Sometimes spiral striation is visible in the lower part. (2) Clusters of calcium oxalate crystals are scattered single or contained in parenchymatous cells, 20~45 μm in diameter. (3) Vessel: mainly bordered pitted vessel and annular vessel.

2. Herba Solani Lyrati (Figure 7)

Yellowish brown color. (1) Cork cell: multiangular, cell wall slightly thickened. (2) Non-glandular: few, 2~4 cells, 20~40 μm in diameter. Some are wrinkled in the middle or at both ends. (3) Sandy crystals of calcium oxalate: scattered singly or contained in parenchymatous cells. (4) Xylary fibre: wall thickened about 6 mm and 16~30 μm in diameter, pit cannals visible. (5) Vessel: mainly bordered pitted vessel and spiral vessel.

**Table 1.** Contents of aristolochic acid I (AA-I) in water and methanol extractions of Herba Aristolochiae Mollissimae

| Sample code | Content of AA-I (μg/g) | |
|---|---|---|
| (in duplicate) | Extracted in methanol | Extracted in water |
| XGF 0311-01 | 225 | 79 |
| | 217 | 84 |
| XGF 0311-02 | 303 | 95 |
| | 204 | 94 |
| XGF 0323-01 | 206 | 78 |
| | 198 | 68 |
| XGF 0323-02 | 452 | 169 |
| | 429 | 164 |
| Mean ± S.D. | 279.25 ± 105.07 | 103.88 ± 39.65 |

III. *Chemical Identification: Determination of Aristolochic Acid by LC-MS*

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**Table 2.** A comparison between Herba Aristolochiae Mollissimae and Herba Solani Lyrati

| Name of Chinese medicines | Herba Aristolochiae Mollissimae | Herba Solani Lyrati |
|---|---|---|
| Original plant | *Aristolochia mollissima* Hance | *Solanum lyratum* Thunb. |
| Family | Aristolochiaceae | Solanaceae |
| Macroscopic features | 1. The stem is faint green, 1~2mm in diameter, marked with woolly white hair.<br>2. Leaves are mostly intact, faint green, presenting cordate or oval shape. | 1. The stem is yellowish green to greenish brown, 1~7mm in diameter, marked with grey-whitish hair.<br>2. Leaves are mostly broken, dark green. The intact leaf presents panduriform or ovate shape. |
| Microscopic features | 1. Vascular bundles are collateral type.<br>2. The stone cells can be visible in the interval of the pericyclic fibres.<br>3. The xylary rays are distinct.<br>4. Parenchymatous cells contain clusters crystals of calcium oxalate. | 1. Vascular bundles are bicollateral type.<br>2. There is no stone cell in the pericyclic fibres.<br>3. The xylary rays are indistinct.<br>4. Parenchymatous cells contain sandy crystals of calcium oxalate. |
| Aristolochic acid I | Present | Absent |

The calibration curve was constructed by injecting different concentrations within the range of 0.00~0.15 μg/mL of AA-I. The regression equation of the curve and the coefficient were calculated as follows: y = 216721 × –346.46 ($R^2$ = 0.9971)

An analytical method using LC-MS method was used to determine the contents of AA-I in both Herba Aristolochiae Mollissimae and Herba Solani Lyrati samples. In Herba Aristolochiae Mollissimae, the content of AA-I extracted by methanol varied from 201 to 440 μg/g, generally higher than that of AA-I extracted by water (Table 1). AA-I was not found in Herba Solani Lyrati samples.

## DISCUSSION

While Herba Solani Lyrati and Herba Aristolochiae Mollissimae look similar, they can be differentiated on the basis of macroscopic and microscopic features, as well as chromatographic analysis. Table 2 summarizes the distinguishing features. Based on colour of stem, distribution of hair and appearance of leaf, these two species of herbs can be easily differentiated from each other. In addition, these two species can be distinguished based on the types of vascular bundles, crystals of calcium oxalate, the presence of stone cells in pericyclic fibers and distinctiveness of xylary rays under a microscope. Aristolochic acid, another unequivocal means of differentiation, is present in Herba Aristolochiae Mollissimae but not in the confusable counterpart, Herba Solani Lyrati. Although conventional microscopic analysis cannot detect the presence of aristolochic acid, application of fluorescent microscopy might be an alternative for facile identification of aristocholic acid in Herba Aristolochiae Mollissimae.

Confusion with the common name Bai Mao Teng is potentially deadly due to the toxicity of aristolochic acid. Our experimental results showed that the average content of AA-I obtained by water and methanol extraction was 103.88 μg/g and 279.25 μg/g, respectively. It has been reported that aristolochic acid would have a significant risk factor for urothelial carcinoma, probably associated with the highest risk when the cumulative level exceeds 200 g of crude drug[5]. Therefore, safe and effective use of these two herbs rely on proper authentication of the source material. Such identification is feasible and straightforward using standard microscopic and analytical techniques. Establishing identification details and standards for all Chinese herbs, such as we have done for Bai Mao Teng, will greatly enhance their safe use throughout the world.

## ACKNOWLEDGEMENTS

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