

Polygoni Avicularis Herba

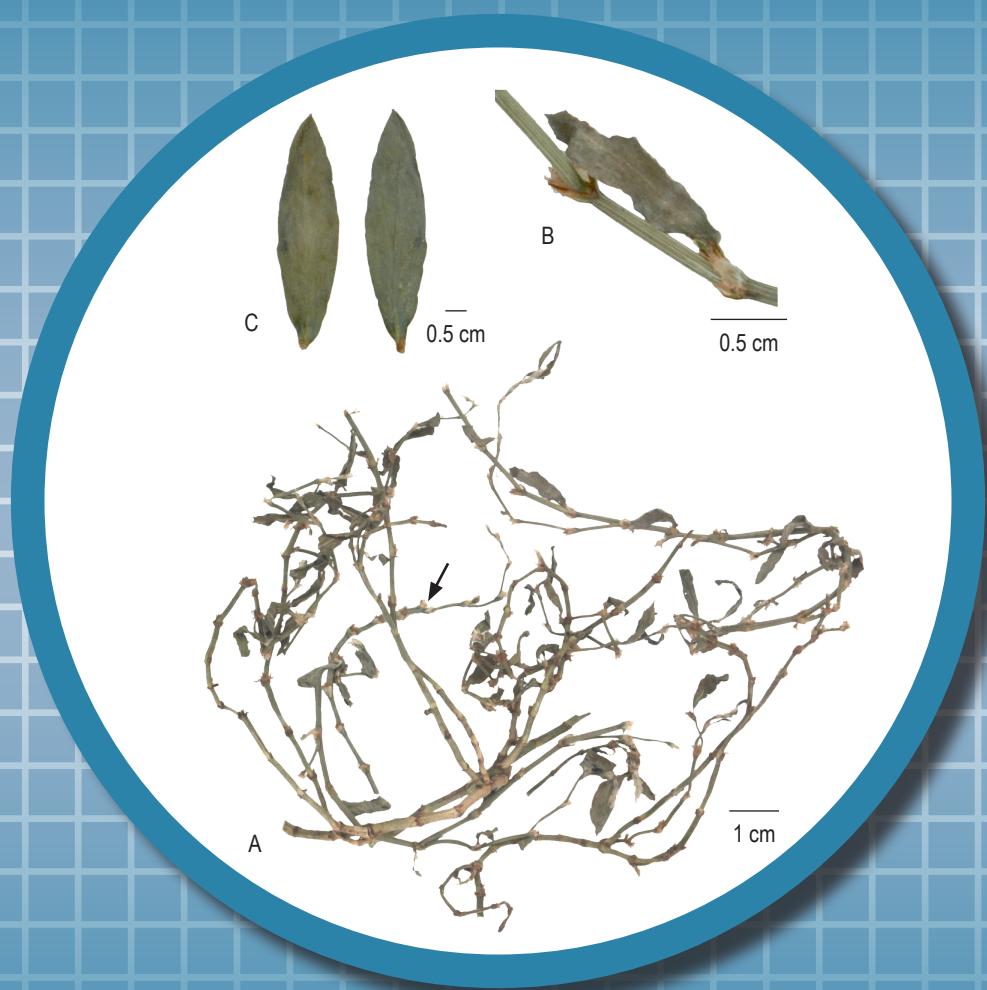


Figure 1 A photograph of *Polygoni Avicularis Herba*

- A. *Polygoni Avicularis Herba* (flower →) B. Stem with nodes and ocrea
C. Upper (left) and lower (right) surface of leaf

Zanthoxyli Radix 紫蘇梗	石菖蒲 兩面針	Triticci Levis Fructus 浮小麥	桃仁 Persicae Semen 絡石藤	金錢草 Lysimachiae Herba
Perillae Caulis 兩面針	Acori Tatarinowii Rhizoma 西紅花 Croci Stigma	巴戟天 Morinda Officinalis Radix	Trachelospermi Caulis et Folium 雞血藤 Spatholobi Caulis	Selaginellae Herba 卷柏 Xanthii Fructus
Polygoni Avicularis Herba 佩蘭	Eupatorii Herba			羅布麻葉 Apocyni Veneti Folium

1. NAMES

Official Name: *Polygoni Avicularis Herba*

Chinese Name: 蔊蓄

Chinese Phonetic Name: Bianxu

2. SOURCE

Polygoni Avicularis Herba is the dried aerial part of *Polygonum aviculare* L. (Polygonaceae). The whole plant is collected in summer when leaves flourish, roots and foreign matter removed, then dried under the sun to obtain *Polygoni Avicularis Herba*.

3. DESCRIPTION

Stems cylindrical and somewhat flattened, branched, 1-4 mm in diameter. Externally greyish-green to reddish-brown, with fine and slightly prominent longitudinal striations, nodes slightly swollen, with pale brown membranous ocrea, internodes 0.4-5 cm long. Texture hard and brittle; fracture whitish in the pith. Leaves alternate, almost sessile or short petiole; the lamina usually fallen off, crumpled and broken, but when intact and flattened, lanceolate-elliptic, 0.5-3.8 cm long, 1-7 mm wide, margins entire, both surfaces greyish-green to yellowish-green or brownish-green. Flowers small, fasciculate at leaf axil. Odour slight; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (*Appendix III*)

Transverse section

Stem: Epidermis consists of 1 layer of subrectangular cells covered with cuticle, sometimes with brown to yellowish-brown contents. Hypodermal fibre bundles arranged in an interrupted ring. Cortex consists of several layers of parenchymatous cells, some cells containing clusters of calcium oxalate. Pericyclic fibre bundles arranged in an interrupted ring. Phloem narrow. Cambium in a ring. In xylem, vessels lined up radially. Pith large, consisting of large parenchymatous cells, scattered with clusters of calcium oxalate [Fig. 2 (i)].

Rubiae Radix et Rhizoma 茜草	餘甘子 Phyllanthi Fructus	地膚子 Kochiae Fructus	Farfarae Flos 款冬花	Visci Herba 檵寄生	車前子 Plantaginis Semen	蔓荊子 Viticis Fructus 槐角
Arecae Pericarpium Lophatheri Herba 淡竹葉	Gummi Arecae Cinnamomi Ramulus Hyperici Japonici Herba	桂枝 木蝴蝶 Oroxyl Semen	Cyperi Rhizoma	香附 Dipsaci Radix 續斷	Polygoni Avicularis Herba	Sophorae Fructus 紫菀 Rhizoma

Leaf: Upper and lower epidermis each consists of 1 layer of cells. Palisade tissue present inside the upper and lower epidermis, with some parenchymatous cells containing clusters of calcium oxalate. Vascular bundle of midrib collateral. Sclerenchyma cells present outside the midrib. Collenchyma present on the inner sides of the upper and lower epidermis of the midrib [Fig. 2 (ii)].

Powder

Colour greyish-green to brownish-green. Clusters of calcium oxalate 9-59 μm in diameter; polychromatic under the polarized microscope. Pollen grains yellow to yellowish-white, ellipsoid, subspherical or obtusely triangular, 19-36 μm in diameter, with 3 germinal pores. Epidermal cells of leaf polygonal in surface view, anticlinal wall beaded, stomata anisocytic. Fibres slender, 6-28 μm in diameter; yellowish-white under the polarized microscope. Epidermal cells of stem polygonal or subsquare in surface view, stomata sometimes visible. Vessels mainly spiral and reticulate, 3-51 μm in diameter (Fig. 3).

Zanthoxyli Radix
兩面針

石菖蒲
Acori Tatarinowii Rhizoma

Triticci Levis Fructus

桃仁 Persicae Semen

金錢草
Lysimachiae Herba

卷柏

紫蘇梗
Perillae Caulis

西紅花 Croci Stigma

Eupatorii Herba

Polygoni Avicularis Herba 佩蘭

巴戟天

Morindaes Officinalis Radix

Trachelospermi Caulis et Folium

絡石藤

Xanthii Fructus

羅布麻葉

蒼耳子

Apocyni Veneti Folium

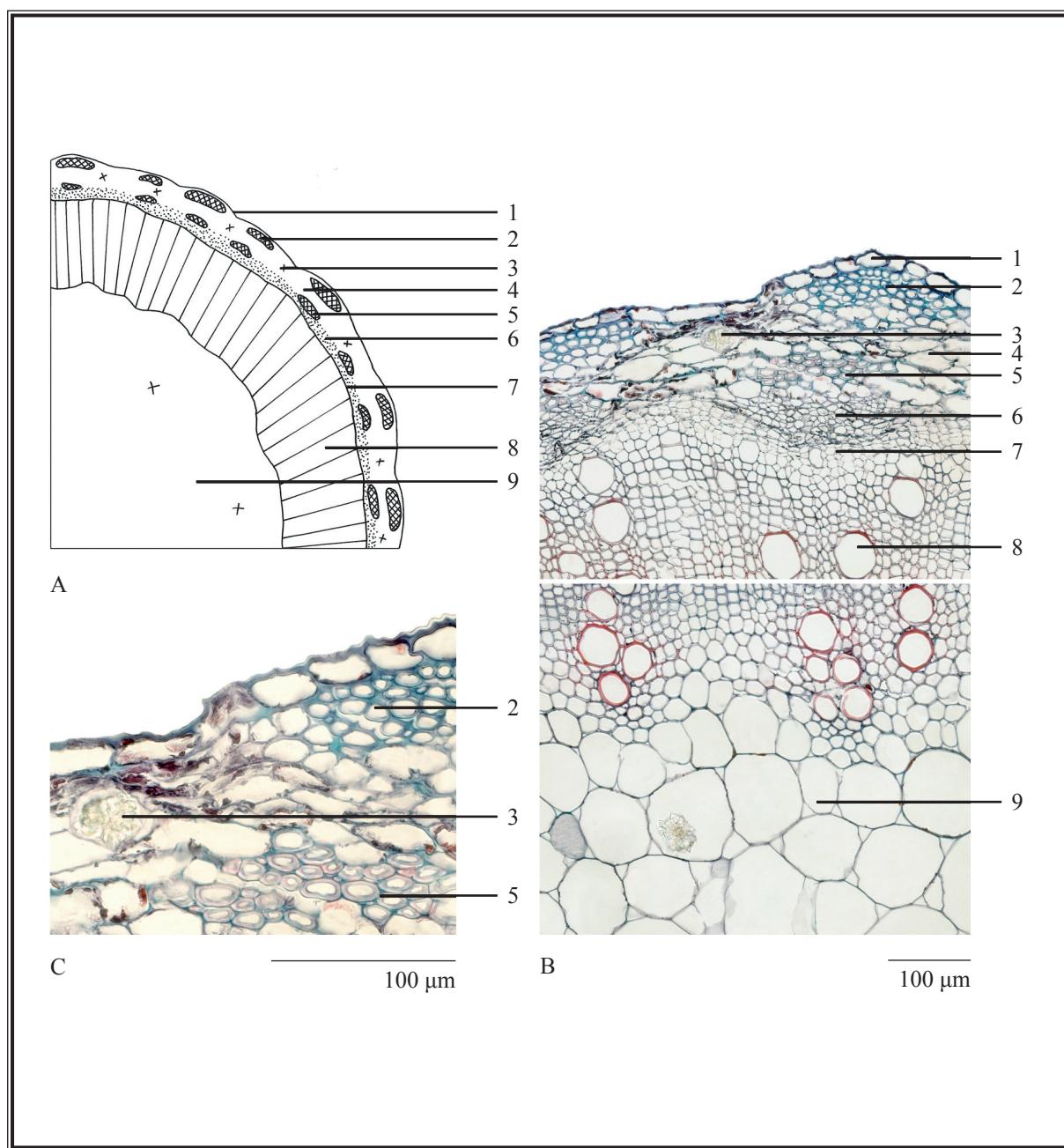


Figure 2 (i) Microscopic features of transverse section of stem of *Polygoni Avicularis Herba*

A. Sketch B. Section illustration C. Cluster of calcium oxalate and fibre bundles

1. Epidermis
2. Hypodermal fibre bundle
3. Cluster of calcium oxalate
4. Cortex
5. Pericyclic fibre bundle
6. Phloem
7. Cambium
8. Xylem
9. Pith

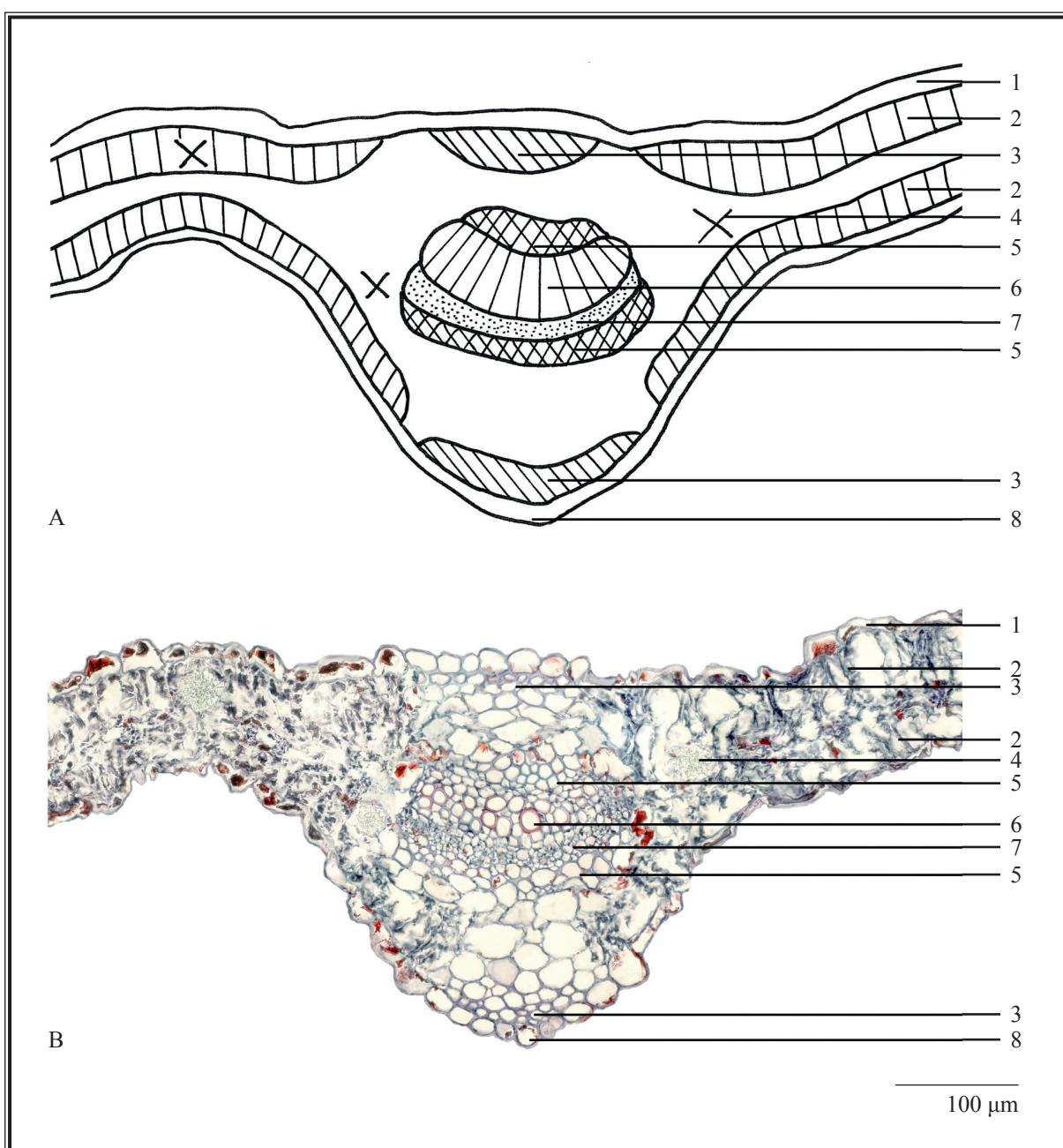


Figure 2 (ii) Microscopic features of transverse section of leaf of *Polygoni Avicularis Herba*

A. Sketch B. Section illustration

1. Upper epidermis 2. Palisade tissue 3. Collenchyma
4. Cluster of calcium oxalate 5. Sclerenchyma 6. Xylem
7. Phloem 8. Lower epidermis

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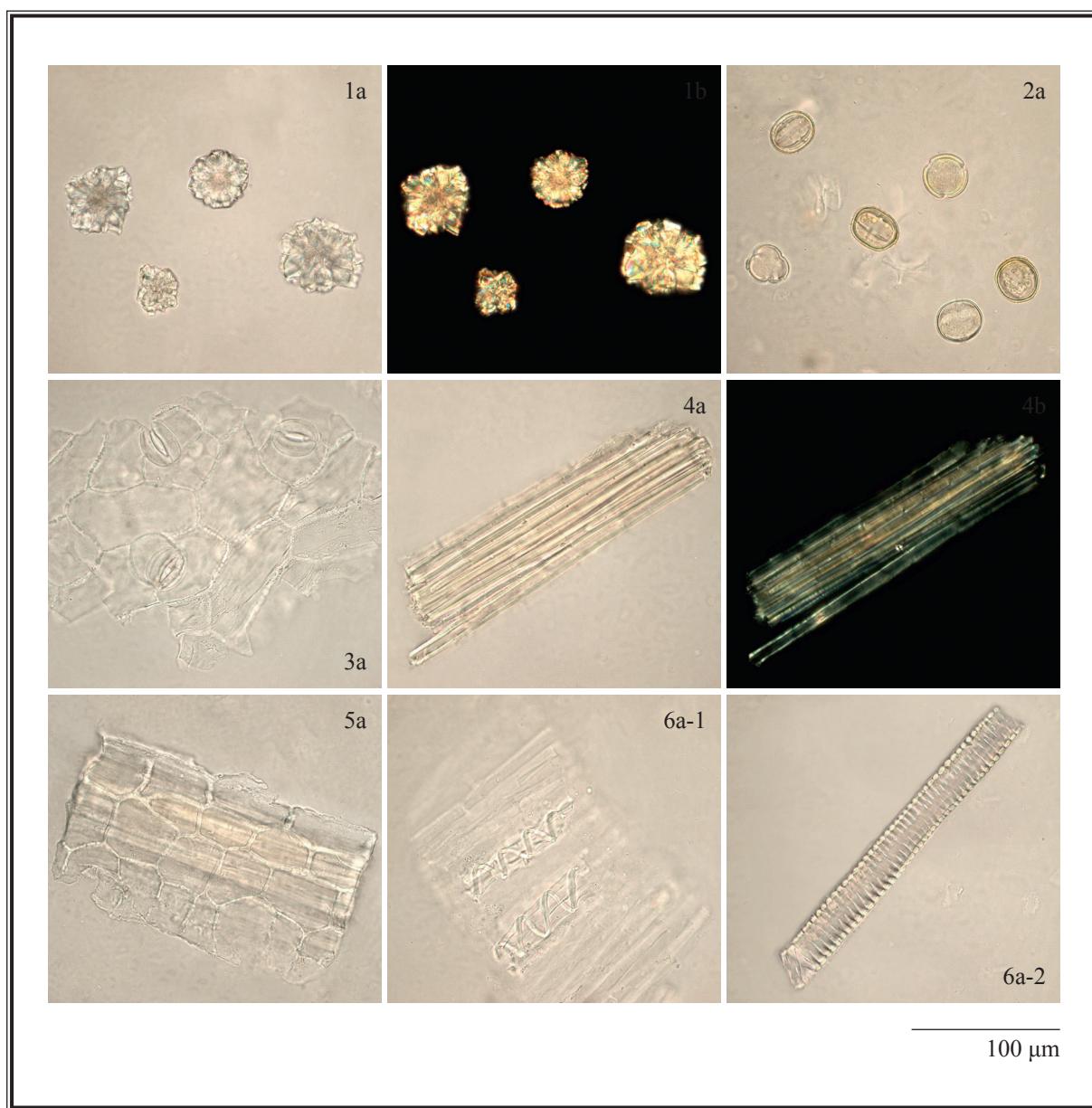


Figure 3 Microscopic features of powder of *Polygoni Avicularis Herba*

1. Clusters of calcium oxalate 2. Pollen grains 3. Epidermal cells of leaf 4. Fibres
5. Epidermal cells of stem 6. Vessels (6-1 spiral vessels, 6-2 reticulate vessel)

a. Features under the light microscope b. Features under the polarized microscope

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Arecae Pericarpium Lophatheri Herba	桂枝 Cinnamomi Ramulus	木蝴蝶 Oroxyl Semen	Cyperi Rhizoma	Dipsaci Radix	紫菀 續斷 Polygoni Avicularis Herba	槐角 Rhizoma
淡竹葉 田基黃 Hyperici Japonici Herba						

4.2 Thin-Layer Chromatographic Identification [Appendix IV(A)]

Standard solution

Myricitrin standard solution

Weigh 1.0 mg of myricitrin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, formic acid, methanol and water (25:1:1:1, v/v).

Spray reagent

Weigh 2.5 g of aluminium trichloride and dissolve in 100 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 15-mL centrifuge tube, then add 10 mL of methanol (70%). Sonicate (140 W) the mixture for 30 min. Centrifuge at about $2800 \times g$ for 10 min. Transfer the supernatant to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of methanol (70%). Filter through a 0.45-μm nylon filter.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately myricitrin standard solution and the test solution (2 μL each) to the plate. Before the development, add the developing solvent to one of the troughs of the chamber and place the HPTLC plate in the other trough. Cover the chamber with a lid and let equilibrate for about 15 min. Carefully tilt the chamber to allow sufficient solvent to pass from the trough containing the solvent to the other containing the HPTLC plate for development. Develop over a path of about 6 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Spray the plate evenly with the spray reagent and dry it in air until the spots or bands become visible (about 5 min). Examine the plate under UV light (254 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

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Xanthii Fructus

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蒼耳子

Apocyni Veneti Folium

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f value, corresponding to those of myricitrin.

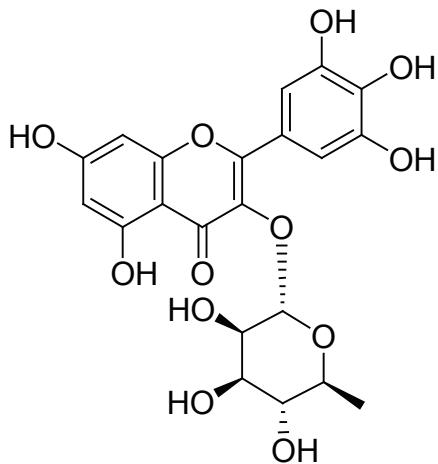


Figure 4 Chemical structure of myricitrin

4.3 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solution

Myricitrin standard solution for fingerprinting, Std-FP (40 mg/L)

Weigh 2.0 mg of myricitrin CRS and dissolve in 50 mL of methanol (60%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 45 mL of methanol (60%). Soak the mixture for 1 h. Reflux the mixture for 30 min. Cool down to room temperature. Centrifuge at about $3800 \times g$ for 10 min. Transfer the supernatant to a 50-mL volumetric flask and make up to the mark with methanol (60%). Filter through a 0.45- μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size, 190 Å pore size and 12% carbon loading). The column temperature is maintained at 35°C during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

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大腹皮 淡竹葉	田基黃 Hyperici Japonici Herba	Oroxyl Semen		續斷 <i>Polygoni Avicularis</i> Herba	紫菀 Rhizoma

Table 1 Chromatographic system conditions

Time (min)	0.2% Acetic acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 45	80 → 40	20 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of myricitrin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of myricitrin should not be more than 5.0%; the RSD of the retention time of myricitrin peak should not be more than 2.0%; the column efficiency determined from myricitrin peak should not be less than 20000 theoretical plates.

The *R* value between peak 1 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

Procedure

Separately inject myricitrin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of myricitrin peak in the chromatogram of myricitrin Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify myricitrin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of myricitrin Std-FP. The retention times of myricitrin peaks from the two chromatograms should not differ by more than 2.0%. Calculate the RRTs of the characteristic peaks by using the equation as indicated in Appendix XII.

The RRTs and acceptable ranges of the four characteristic peaks of *Polygoni Avicularis* Herba extract are listed in Table 2.

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Xanthii Fructus

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Table 2 The RRTs and acceptable ranges of the four characteristic peaks of *Polygoni Avicularis* Herba extract

Peak No.	RRT	Acceptable Range
1 (marker, myricitrin)	1.00	-
2	1.12	± 0.03
3	1.25	± 0.03
4 (quercitrin)	1.30	± 0.03

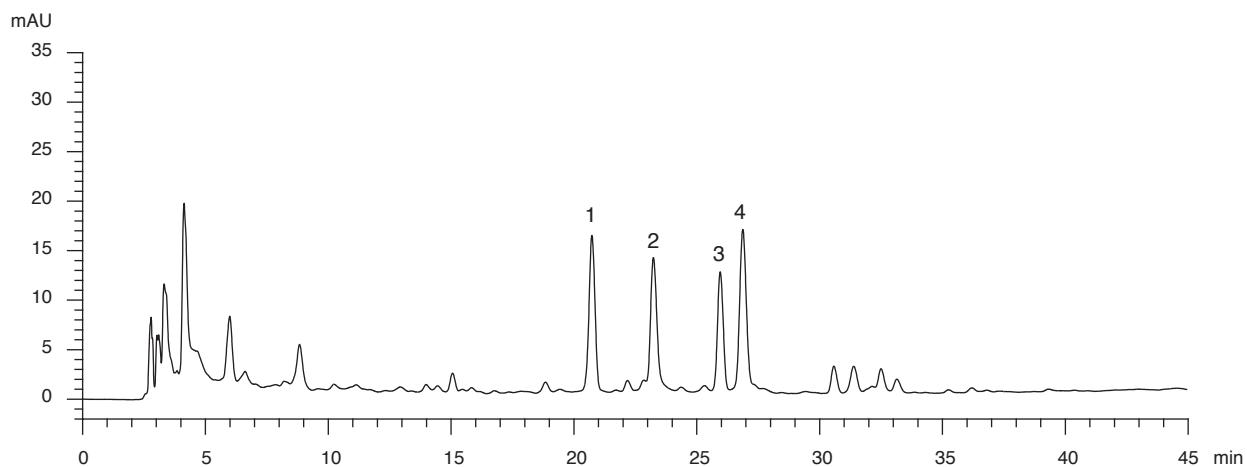


Figure 5 A reference fingerprint chromatogram of *Polygoni Avicularis* Herba extract

For positive identification, the sample must give the above four characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 5).

5. TESTS

5.1 Heavy Metals (*Appendix V*): meet the requirements.

5.2 Pesticide Residues (*Appendix VI*): meet the requirements.

5.3 Mycotoxins (*Appendix VII*): meet the requirements.

5.4 Foreign Matter (*Appendix VIII*): not more than 1.0%.

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大腹皮 淡竹葉	田基黃 Hyperici Japonici Herba	Oroxyl Semen		續斷 Dipsaci Radix	紫菀 <i>Polygoni Avicularis</i> Herba Rhizoma

5.5 Ash (Appendix IX)

Total ash: not more than 10.5%.

Acid-insoluble ash: not more than 1.5%.

5.6 Water Content (Appendix X)

Oven dried method: not more than 12.0%.

6. EXTRACTIVES (Appendix XI)

Water-soluble extractives (hot extraction method): not less than 15.0%.

Ethanol-soluble extractives (cold extraction method): not less than 11.0%.

7. ASSAY

Carry out the method as directed in Appendix IV(B).

Standard solution

Myricitrin standard stock solution, Std-Stock (100 mg/L)

Weigh accurately 1.0 mg of myricitrin CRS and dissolve in 10 mL of methanol (60%).

Myricitrin standard solution for assay, Std-AS

Measure accurately the volume of the myricitrin Std-Stock, dilute with methanol (60%) to produce a series of solutions of 1, 5, 10, 20, 40 mg/L for myricitrin.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 45 mL of methanol (60%). Soak the mixture for 1 h. Reflux the mixture for 30 min. Cool down to room temperature. Centrifuge at about $3800 \times g$ for 10 min. Transfer the supernatant to a 50-mL volumetric flask and make up to the mark with methanol (60%). Filter through a 0.45- μm PTFE filter.

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Chromatographic system

The liquid chromatograph is equipped with a DAD (254 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size, 190 Å pore size and 12% carbon loading). The column temperature is maintained at 35°C during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.2% Acetic acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 45	80 → 40	20 → 60	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of myricitrin Std-AS (10 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of myricitrin should not be more than 5.0%; the RSD of the retention time of myricitrin peak should not be more than 2.0%; the column efficiency determined from myricitrin peak should not be less than 20000 theoretical plates.

The *R* value between myricitrin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curve

Inject a series of myricitrin Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of myricitrin against the corresponding concentrations of myricitrin Std-AS. Obtain the slope, y-intercept and the *r*² value from the 5-point calibration curve.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify myricitrin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of myricitrin Std-AS. The retention times of myricitrin peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak area and calculate the concentration (in milligram per litre) of myricitrin in the test solution, and calculate the percentage content of myricitrin in the sample by using the equations as indicated in Appendix IV(B).

Limits

The sample contains not less than 0.051% of myricitrin ($C_{21}H_{20}O_{12}$), calculated with reference to the dried substance.