Catharanthi Rosei Herba

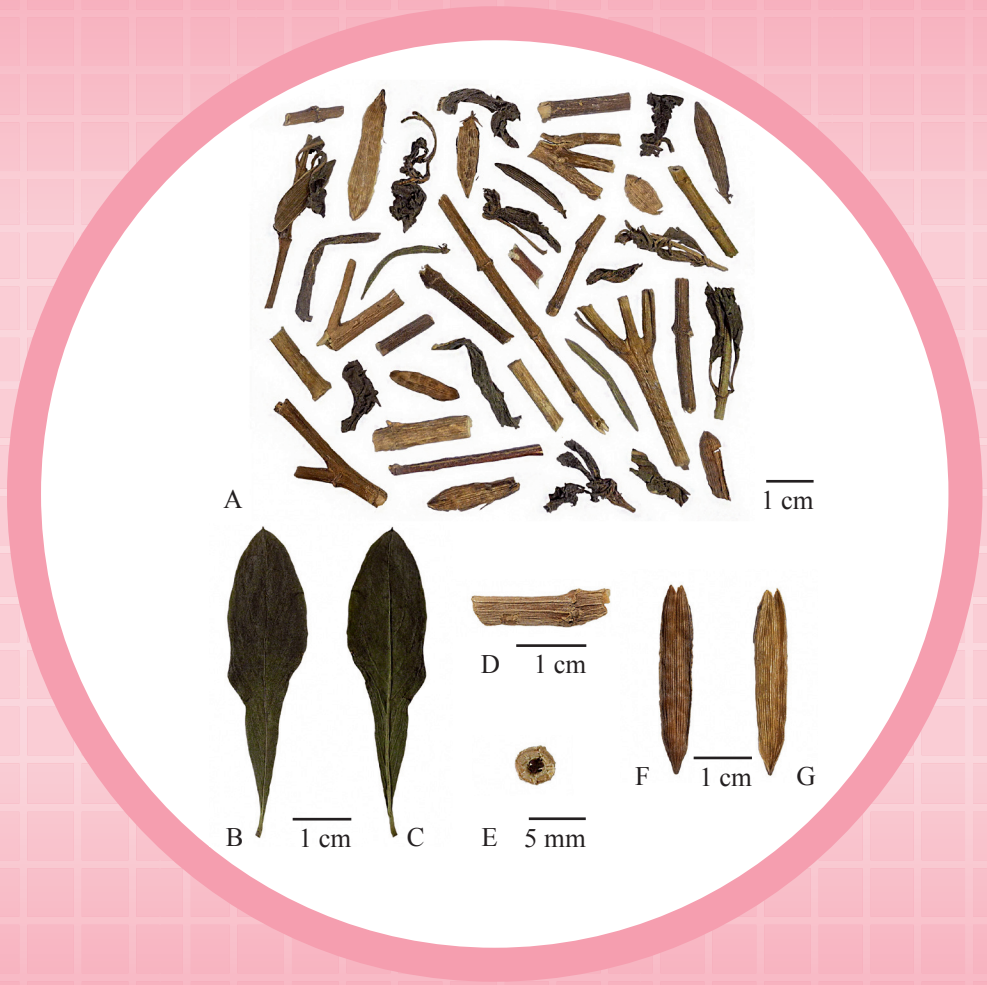


Figure 1 A photograph of Catharanthi Rosei Herba

A. Catharanthi Rosei Herba
B. Magnified image of upper surface of leaf (flattened after soaking into water)
C. Magnified image of lower surface of leaf (flattened after soaking into water)
D. Magnified image of stem E. Magnified image of cut surface of stem
F. Magnified image of external surface of epicarp
G. Magnified image of internal surface of epicarp

1. NAMES

Official name: Catharanthi Rosei Herba

Chinese name: 長春花

Chinese phonetic name: Changchunhua

2. SOURCE

Catharanthi Rosei Herba is the dried aerial part of *Catharanthus roseus* (L.) G. Don (Apocynaceae). The aerial part is collected on sunny days all year round. Lignified stem removed and discarded from the collection; the collected materials cut into sections, then dried under the sun to obtain Catharanthi Rosei Herba.

3. DESCRIPTION

Stems nearly cylindrical, branched, with ridges, 1-12 cm long, 1-9 mm in diameter; externally yellowish-green to reddish-brown; texture fragile, easily broken, fracture fibrous-like, usually hollow. Leaves opposite, mostly crumpled and broken; when whole, obovate-oblong or oblong in shape, 1.5-5.7 cm long, 7-31 mm wide, apex round, with short tips, base cuneate, dark green or greenish-brown, pinnate veins distinct, veins flattened on upper surface and slightly elevated on lower surface, petioles short. Follicles cylindrical, mostly broken, epicarp thickly papyraceous, striped, externally green, yellowish-brown or dark brown, internally pale yellow to yellowish-brown. Odour slight (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Nearly round, with 4 larger protruding ridges. Epidermis consists of 1 layer of cells, cells subrounded, semicircular or irregular in shape, densely arranged, covered with thin cuticle, stomata visible. At the ridge, few layers of collenchyma located underneath the epidermis. Cortex consist of several layers of parenchymatous cells, cells elliptic or polygonal in shape, with slightly thickened wall, the outer 2-3 layers of cells are small, cells become larger gradually inwards. Pericycle fibre bundles arranged in an interrupted ring. Vascular bundle bicollateral; outer phloem relatively narrow; cambium ring consists of few layers of cells; xylem vessels in parallel alignment, arranged radially, xylem rays mostly comprise single row of cells; inner

phloem small. Pith large, hollow in the centre, parenchymatous cells subrounded [Fig. 2 (i)].

Leaf: Upper and lower epidermis consist of 1 layer of polygonal cells, outer wall thickened, stomata visible. Mesophyll consists of 1 layer of palisade tissue and several layers of spongy tissue. Collenchyma consists of several layers of cells, located underneath the upper and lower epidermis of the midrib. Vascular bundle of midrib bicollateral, crescent in shape; xylem lignified, vessels radially arranged [Fig. 2 (ii)].

Powder

Colour light brown to dark brown. Stomata anomocytic, easily observed. Epidermal cells covered with cuticle layer. Vessels mainly spiral type, 4-28 μm in diameter. Fibres mostly in bundles, 7-32 μm in diameter; polychromatic under the polarized microscope. Fragments of secretary canal contain yellowish-brown masses. Clusters of calcium oxalate in rosette aggregate, occasionally visible, subrounded, 11-53 μm in diameter, with short and obtuse angles; polychromatic under the polarized microscope. Non-glandular hairs mainly unicellular (Fig. 3).

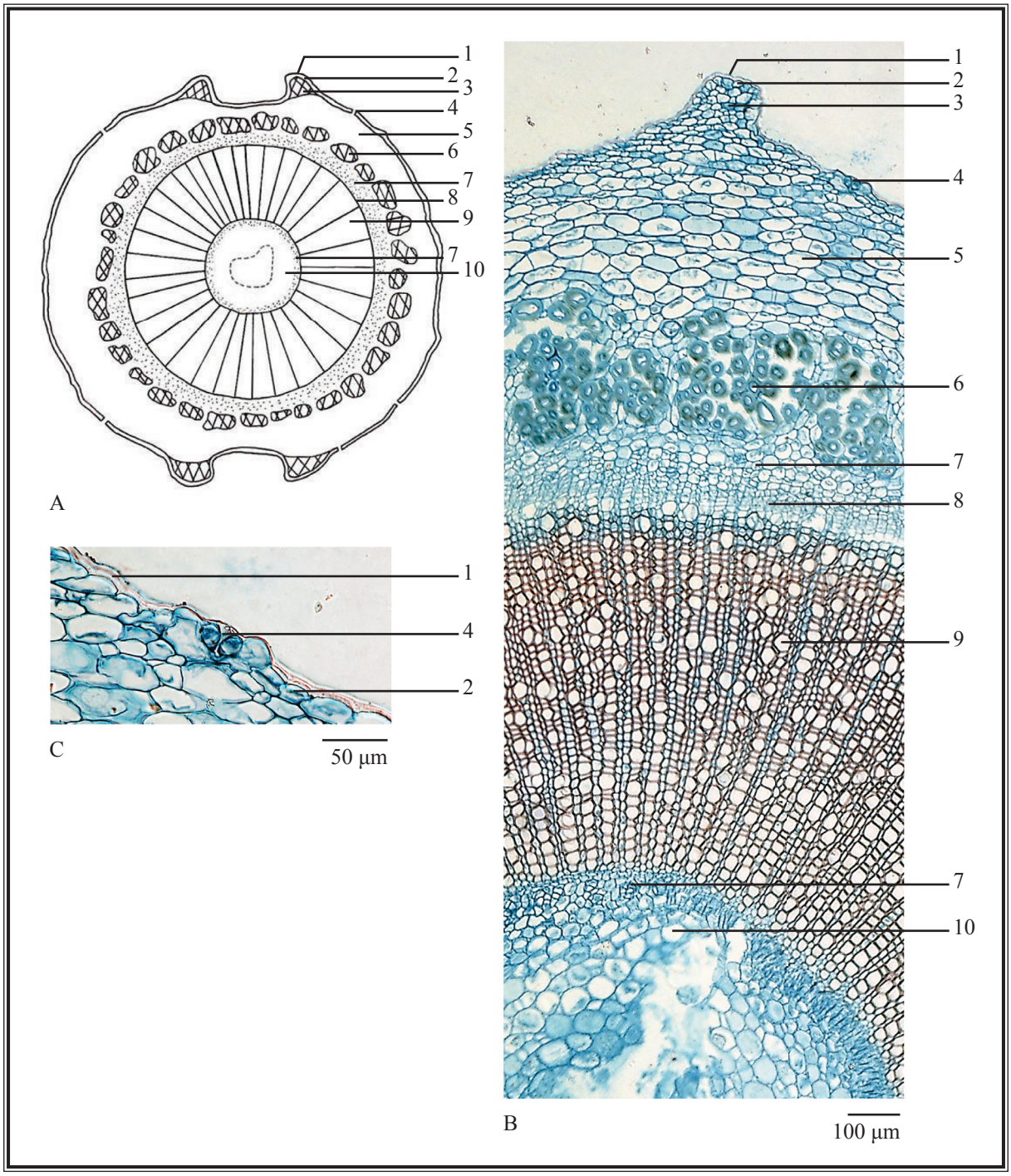


Figure 2 (i) Microscopic features of transverse section of stem of Catharanthi Rosei Herba

A. Sketch B. Section illustration C. Section magnified

1. Cuticle 2. Epidermis 3. Collenchyma 4. Stoma 5. Cortex
6. Pericycle fibre bundle 7. Phloem 8. Cambium 9. Xylem 10. Pith

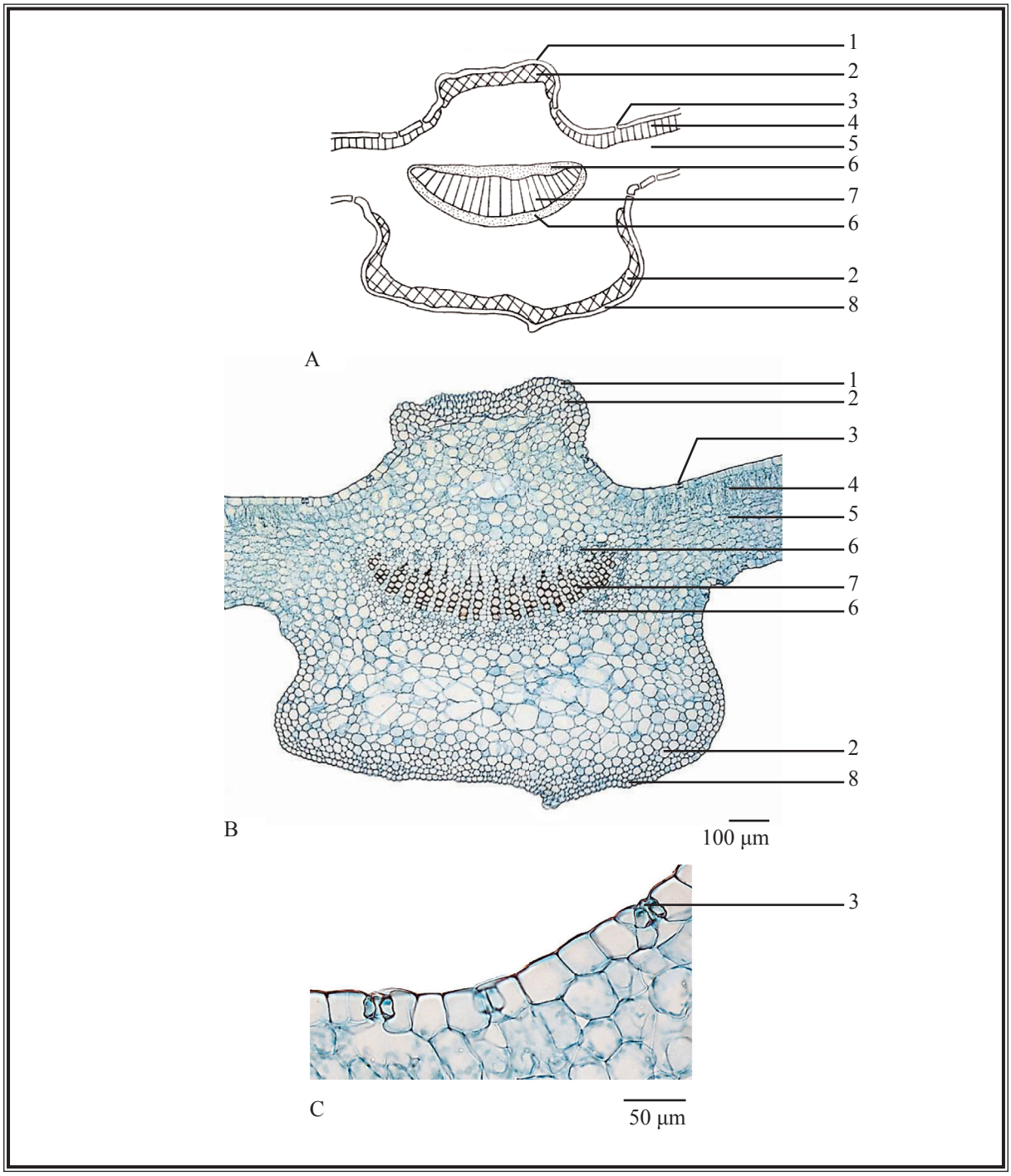


Figure 2 (ii) Microscopic features of transverse section of leaf of Catharanthi Rosei Herba

A. Sketch B. Section illustration C. Epidermis and stomata

1. Upper epidermis 2. Collenchyma 3. Stoma 4. Palisade tissue
5. Spongy tissue 6. Phloem 7. Xylem 8. Lower epidermis

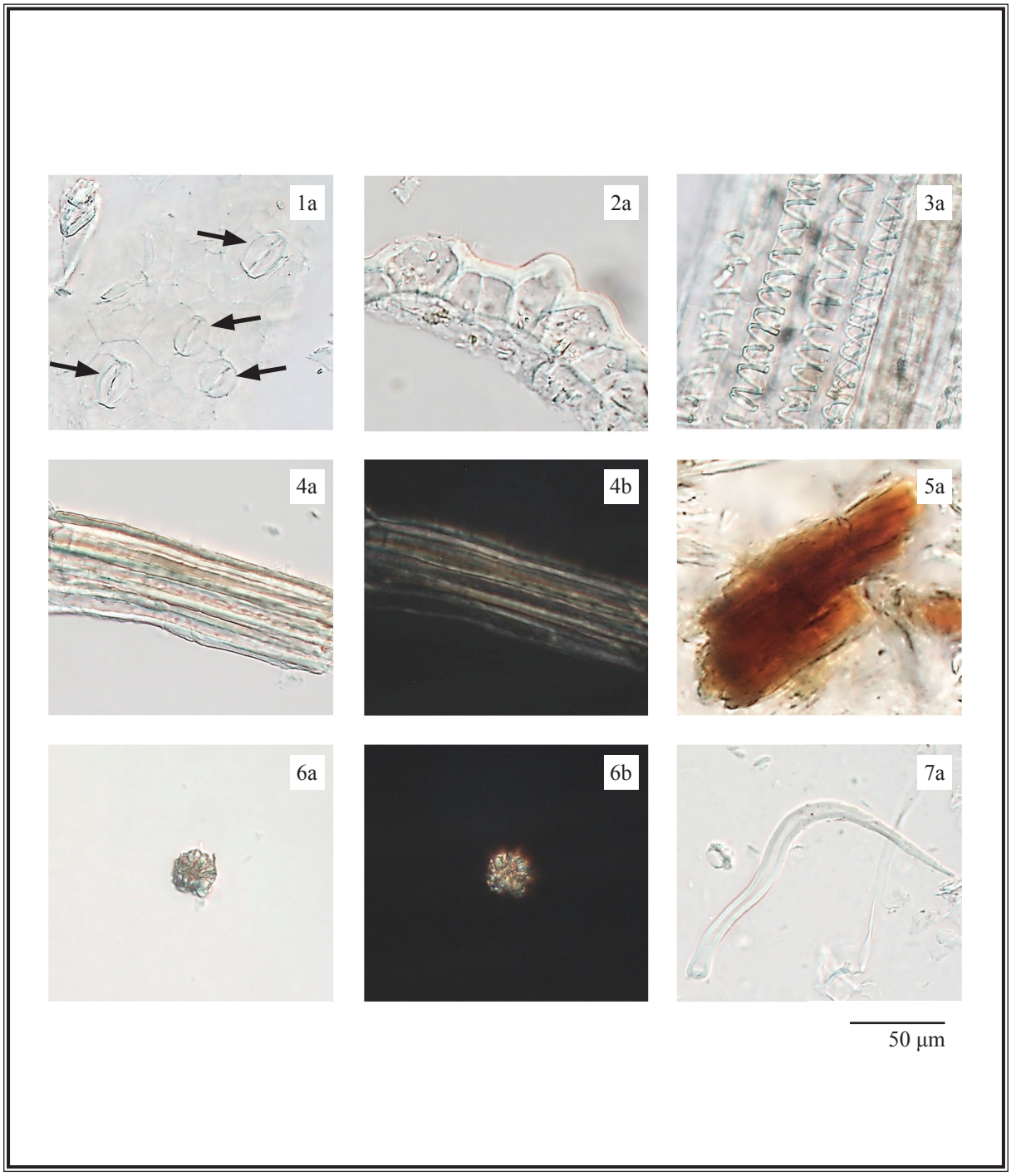


Figure 3 Microscopic features of powder of Catharanthi Rosei Herba

1. Anomocytic stomata (→) 2. Epidermis fragment with cuticle
3. Spiral vessels 4. Fibres 5. Fragment of secretory canal
6. Cluster of calcium oxalate 7. Non-glandular hair

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

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

Standard solutions

Catharanthine hemitartrate standard solution

Weigh 2.0 mg of catharanthine hemitartrate CRS (Fig. 4) and dissolve in 1 mL of methanol.

Vindoline standard solution

Weigh 2.0 mg of vindoline CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of petroleum ether (60 – 80°C), acetone, diethyl ether and ethanol (7:4:1:0.2, v/v).

Spray reagent

Solution A

Weigh 0.85 g of bismuth oxynitrate and dissolve in a mixture of 10 mL of glacial acetic acid and 40 mL of water.

Solution B

Weigh 4 g of potassium iodide and dissolve in 10 mL of water.

Spray reagent

Transfer 5 mL of Solution A, 5 mL of Solution B and 20 mL of glacial acetic acid into a 100-mL volumetric flask and make up to the mark with water. Freshly prepare the reagent.

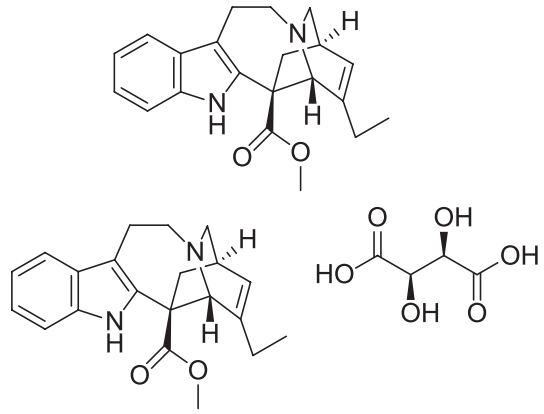
Test solution

Weigh 5.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 30 mL of methanol. Sonicate (320 W) the mixture for 1 h. Centrifuge at about 2000 × *g* for 5 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction two more times. Combine the supernatants. Evaporate the combined supernatants to dryness at reduced pressure in a rotary evaporator in a water bath at 60°C. Dissolve the residue in 10 mL of methanol. Transfer the extract to a 250-mL separating funnel. Add 10 mL of water and 10 mL of 0.3% hydrochloric acid. Extract the aqueous layer three times each with 30 mL of *n*-hexane. Discard the *n*-hexane layer. Adjust the pH to 9.0 with ammonium hydroxide solution (28%, v/v). Extract the aqueous layer three times each with 30 mL of dichloromethane. Collect the dichloromethane layer. Evaporate the combined extracts to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 5 mL of methanol.

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 catharanthine hemitartrate standard solution (5 µL), vindoline standard solution (5 µL) and the test solution (10 µL) 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 8 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 until the spots or bands become visible. Examine the plate under visible light. Calculate the R_f values by using the equation as indicated in Appendix IV (A).

(i)



(ii)

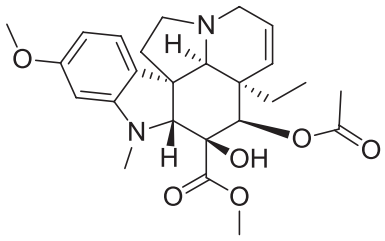


Figure 4 Chemical structures of (i) catharanthine hemitartrate and (ii) vindoline

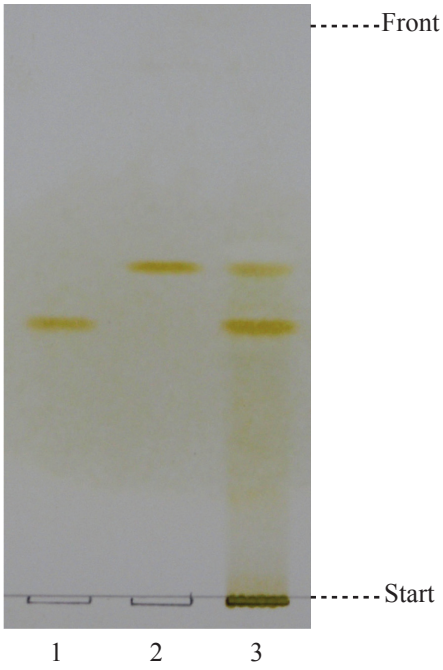


Figure 5 A reference HPTLC chromatogram of Catharanthi Rosei Herba extract observed under visible light after staining

1. Vindoline standard solution 2. Catharanthine hemitartrate standard solution
3. Test solution

For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the R_f values, corresponding to those of catharanthine and vindoline (Fig. 5).

4.3 High-Performance Liquid Chromatographic Fingerprinting *(Appendix XII)*

Reagent

0.005 M Potassium dihydrogen phosphate solution (pH 6.0)

Weigh 0.34 g of potassium dihydrogen phosphate and dissolve in 500 mL of water. Adjust the pH value to 6.0 with ammonium hydroxide solution (28%, v/v).

Standard solutions

Catharanthine hemitartrate standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 0.1 mg of catharanthine hemitartrate CRS and dissolve in 1 mL of methanol.

Vindoline standard solution for fingerprinting, Std-FP (100 mg/L)

Weigh 0.1 mg of vindoline CRS and dissolve in 1 mL of methanol.

Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 5 mL of ammonium hydroxide solution (28%, v/v). Allow the mixture to stand for 30 min. Add 40 mL of methanol. Sonicate (320 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 10 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction one more time with 40 mL of methanol. Combine the supernatants. Evaporate the combined supernatants to dryness at reduced pressure in a rotary evaporator in a water bath at 60ºC. Dissolve the residue in methanol. Transfer the extract to a 10-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (215 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size, 90 Å pore size and 14.8% carbon loading). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 0.005 M potassium dihydrogen phosphate solution (pH 6.0) and methanol (36:64, v/v). The elution time is about 40 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of catharanthine hemitartrate Std-FP and vindoline Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of catharanthine and vindoline should not be more than 5.0%; the RSD of the retention times of catharanthine and vindoline peaks should not be more than 2.0%; the column efficiencies determined from catharanthine and vindoline peaks should not be less than 5000 theoretical plates.

The *R* value between peak 2 and the closest peak; and the *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 6).

Procedure

Separately inject catharanthine hemitartrate Std-FP, vindoline Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of catharanthine and vindoline peaks in the chromatograms of catharanthine hemitartrate Std-FP, vindoline Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify catharanthine and vindoline peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of catharanthine hemitartrate Std-FP and vindoline Std-FP. The retention times of catharanthine and vindoline peaks in the chromatograms of the test solution and the corresponding Std-FP 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 three characteristic peaks of Catharanthi Rosei Herba extract are listed in Table 1.

Table 1 The RRTs and acceptable ranges of the three characteristic peaks of Catharanthi Rosei Herba extract

Peak No.	RRT	Acceptable Range
1	0.48	± 0.04
2 (vindoline)	0.68	± 0.05
3 (marker, catharanthine)	1.00	-

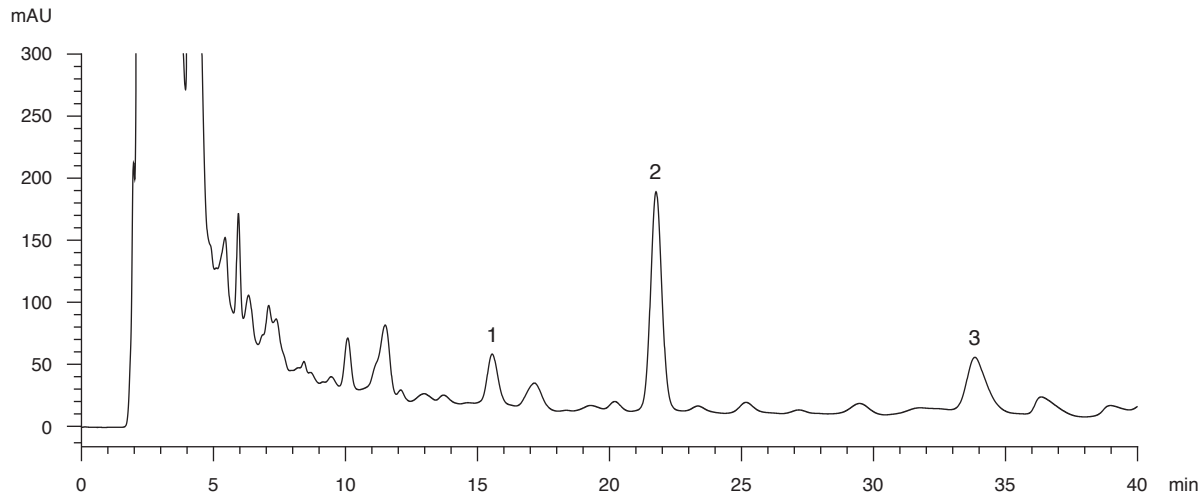


Figure 6 A reference fingerprint chromatogram of Catharanthi Rosei Herba extract

For positive identification, the sample must give the above three characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 6).

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 Sulphur Dioxide Residues *(Appendix XVI)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 9.5%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 11.0%.
Ethanol-soluble extractives (cold extraction method): not less than 10.0%.

7. ASSAY

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

Reagent

0.005 M Potassium dihydrogen phosphate solution (pH 6.0)

Weigh 0.34 g of potassium dihydrogen phosphate and dissolve in 500 mL of water. Adjust the pH value to 6.0 with ammonium hydroxide solution (28%, v/v).

Standard solution

Vindoline standard stock solution, Std-Stock (540 mg/L)

Weigh accurately 5.4 mg of vindoline CRS and dissolve in 10 mL of methanol.

Vindoline standard solution for assay, Std-AS

Measure accurately the volume of the vindoline Std-Stock, dilute with methanol to produce a series of solutions of 4.3, 10.8, 54, 108, 270 mg/L for vindoline.

Test solution

Weigh accurately 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 5 mL of ammonium hydroxide solution (28%, v/v). Allow the mixture to stand for 30 min. Add 40 mL of methanol. Sonicate (320 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 10 min. Transfer the supernatant to a 250-mL round-bottomed flask. Repeat the extraction one more time with 40 mL of methanol. Combine the supernatants. Evaporate the combined supernatants to dryness at reduced pressure in a rotary evaporator in a water bath at 60ºC. Dissolve the residue in methanol. Transfer the extract to a 10-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (215 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size, 90 Å pore size and 14.8% carbon loading). The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 0.005 M potassium dihydrogen phosphate solution (pH 6.0) and methanol (36:64, v/v). The elution time is about 40 min.

System suitability requirements

Perform at least five replicate injections, each using 10 μL of vindoline Std-AS (54 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of vindoline should not be more than 5.0%; the RSD of the retention time of vindoline peak should not be more than 2.0%; the column efficiency determined from vindoline peak should not be less than 5000 theoretical plates.

The *R* value between vindoline peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

Calibration curve

Inject a series of vindoline Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of vindoline against the corresponding concentrations of vindoline 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 vindoline peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of vindoline Std-AS. The retention times of vindoline peaks from the two chromatograms should not differ by more than 5.0%. Measure the peak area and calculate the concentration (in milligram per litre) of vindoline in the test solution, and calculate the percentage content of vindoline in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.011% of vindoline ($C_{25}H_{32}N_2O_6$), calculated with reference to the dried substance.

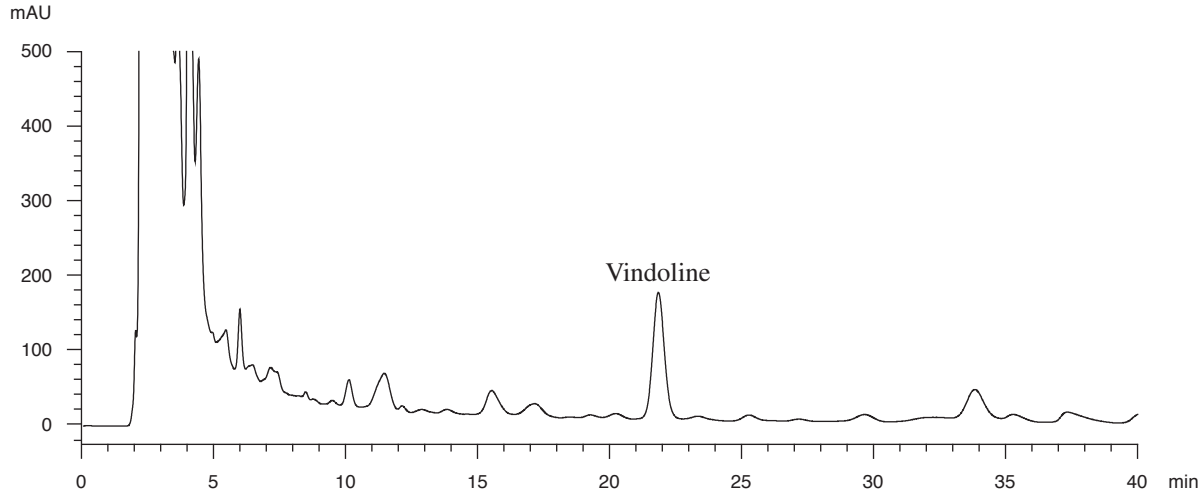


Figure 7 A reference assay chromatogram of Catharanthi Rosei Herba extract