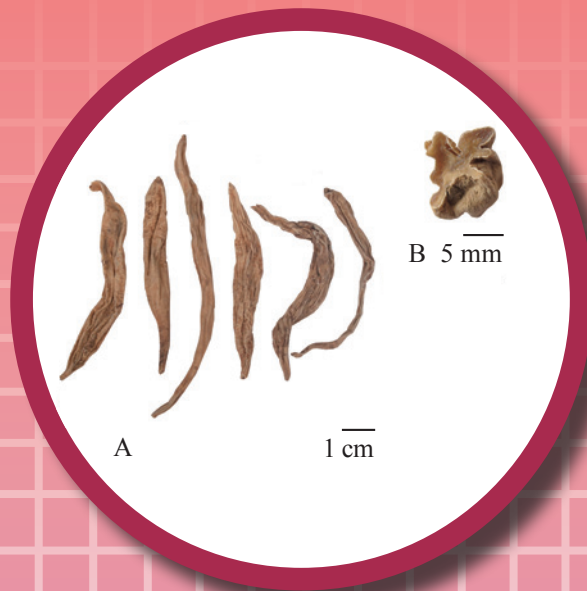
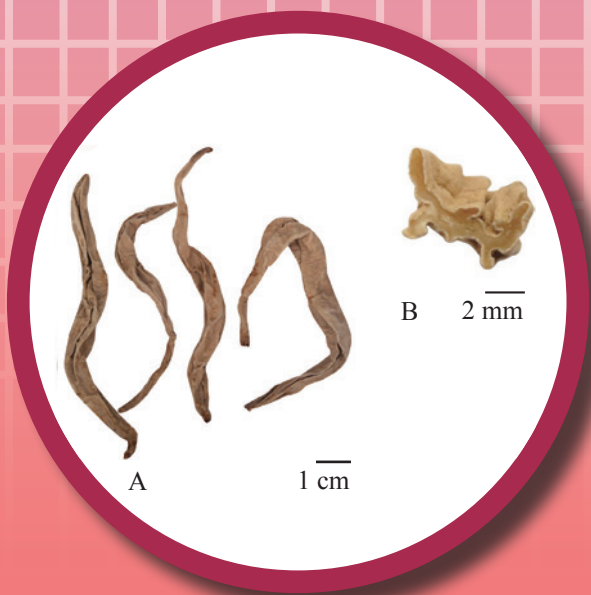


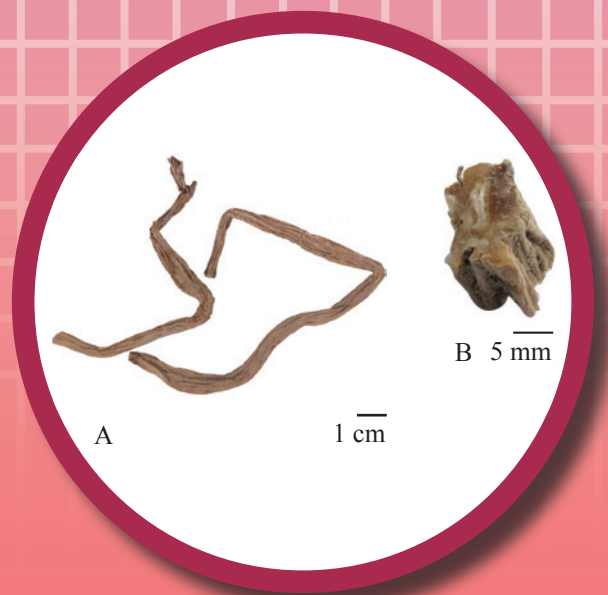
# Stemonae Radix



**Figure 1 (i)** A photograph of dried root tuber of *Stemona sessilifolia* (Miq.) Miq.  
A. Root tubers B. Magnified image of fracture (transverse view)



**Figure 1 (ii)** A photograph of dried root tuber of *Stemona japonica* (Bl.) Miq.  
A. Root tubers  
B. Magnified image of fracture (transverse view)



**Figure 1 (iii)** A photograph of dried root tuber of *Stemona tuberosa* Lour.  
A. Root tubers  
B. Magnified image of fracture (transverse view)

## 1. NAMES

Official Name: *Stemona Radix*

Chinese Name: 百部

Chinese Phonetic Name: Baibu

## 2. SOURCE

*Stemona Radix* is the dried root tuber of *Stemona sessilifolia* (Miq.) Miq., *Stemona japonica* (Bl.) Miq. or *Stemona tuberosa* Lour. (Stemonaceae). The root tuber is collected in spring and autumn, rootlets removed, washed clean, dipped in boiling water briefly or steamed thoroughly, then dried under the sun to obtain *Stemona Radix*.

### Part I Dried root tuber of *Stemona sessilifolia* (Miq.) Miq. and *Stemona japonica* (Bl.) Miq.

## 3. DESCRIPTION

***Stemona sessilifolia* (Miq.) Miq.:** Fusiform, the upper end relatively slender, shrunken and curved, 5-13 cm long, 5-10 mm in diameter. Externally yellowish-white to pale yellowish-brown, with irregular, deep, longitudinal furrows, occasionally with transverse wrinkles. Texture fragile, easily broken. Fracture even, corneous, yellowish-white to yellowish-brown, bark relatively broad, stele compressed. Odour slight; taste sweet and bitter [Fig. 1(i)].

***Stemona japonica* (Bl.) Miq.:** Two ends slightly slender, 5-20 cm long, 5-10 mm in diameter. Externally yellowish-white to yellowish-brown, mostly with irregular folds and transverse wrinkles. Texture fragile, easily broken. Fracture even, corneous, yellowish-white to deep yellowish-brown, bark relatively broad, stele compressed. Odour slight; taste bitter [Fig. 1(ii)].

## 4. IDENTIFICATION

### 4.1 Microscopic Identification (*Appendix III*)

#### Transverse Section

***Stemona sessilifolia* (Miq.) Miq.:** Velamen consists of 3-4 layers of cells, cell wall suberized and lignified, with dense and fine striations. Cortex relatively broad. Endodermis distinct. Stele consists of 13-25 phloem bundles and xylem bundles respectively, arranged alternately. A few non-lignified fibres located in the inner side of phloem. Each xylem bundle consists of 1-9 vessels, vessels subpolygonal, arranged in a radial direction, occasionally penetrating into the pith. Pith scattered with a few small fibres [Fig 2(i)].

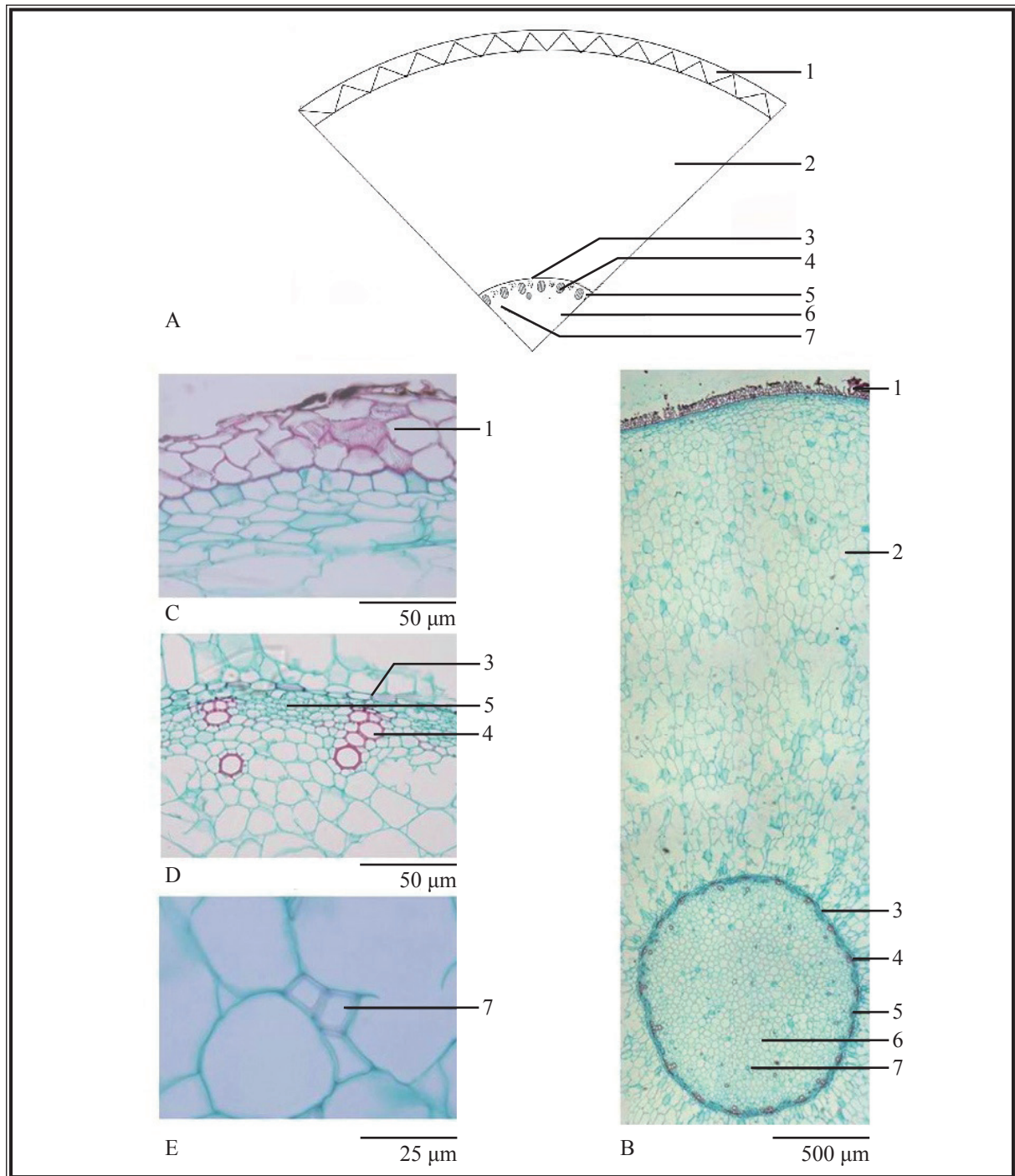
***Stemona japonica* (Bl.) Miq.:** Velamen consists of 3-6 layers of cells. Stele consists of 16-27 phloem bundles and xylem bundles respectively. Phloem fibres lignified. Each xylem bundle consists of 2-8 vessels, usually penetrating into the pith; the vessel bundles on the outer side arranged into 2-3 whorls [Fig 2(ii)].

#### Powder

***Stemona sessilifolia* (Miq.) Miq.:** Colour yellowish-white to brownish-yellow. Velamen cells colourless or pale yellowish-brown, rectangular or long polygonal, walls suberized or lignified, with dense and fine striations on the surface of cell walls. Bordered-pitted vessels frequently visible, 14-83  $\mu\text{m}$  in diameter, spiral, pitted and reticulate vessels occasionally visible. Endodermal cells mostly in pieces, rectangular, with undulantly curved walls. Xylem fibres relatively long, 7-37  $\mu\text{m}$  in diameter, walls relatively thick, lignified, with oblique, V-shaped or bordered-pitted pits, the pits arranged in cross-shaped configuration. Raphides of calcium oxalate rare, mostly in bundles [Fig. 3(i)].

***Stemona japonica* (Bl.) Miq.:** Colour yellowish-white to brownish-yellow. Bordered-pitted vessels frequently visible, 19-108  $\mu\text{m}$  in diameter. Endodermal cells mostly in pieces, rectangular, with undulantly curved walls. Xylem fibres relatively long, 10-54  $\mu\text{m}$  in diameter [Fig. 3(ii)].

**Stemonae Radix**



**Figure 2 (i)** Microscopic features of transverse section of dried root tuber of *Stemona sessilifolia* (Miq.) Miq.

A. Sketch B. Section illustration C. Velamen

D. Phloem bundles and xylem bundles E. Fibres

1. Velamen 2. Cortex 3. Endodermis 4. Xylem 5. Phloem 6. Pith 7. Fibre

山豆根

Saururi Herba 三白草

牡荊葉

車前草

蓮鬚

Saussureae Involucratae Herba

Polygoni Perfoliati Herba

北豆根

Loniceræ Flos

Plantaginis Herba

Bruceae Fructus 鴉膽子

天山雪蓮

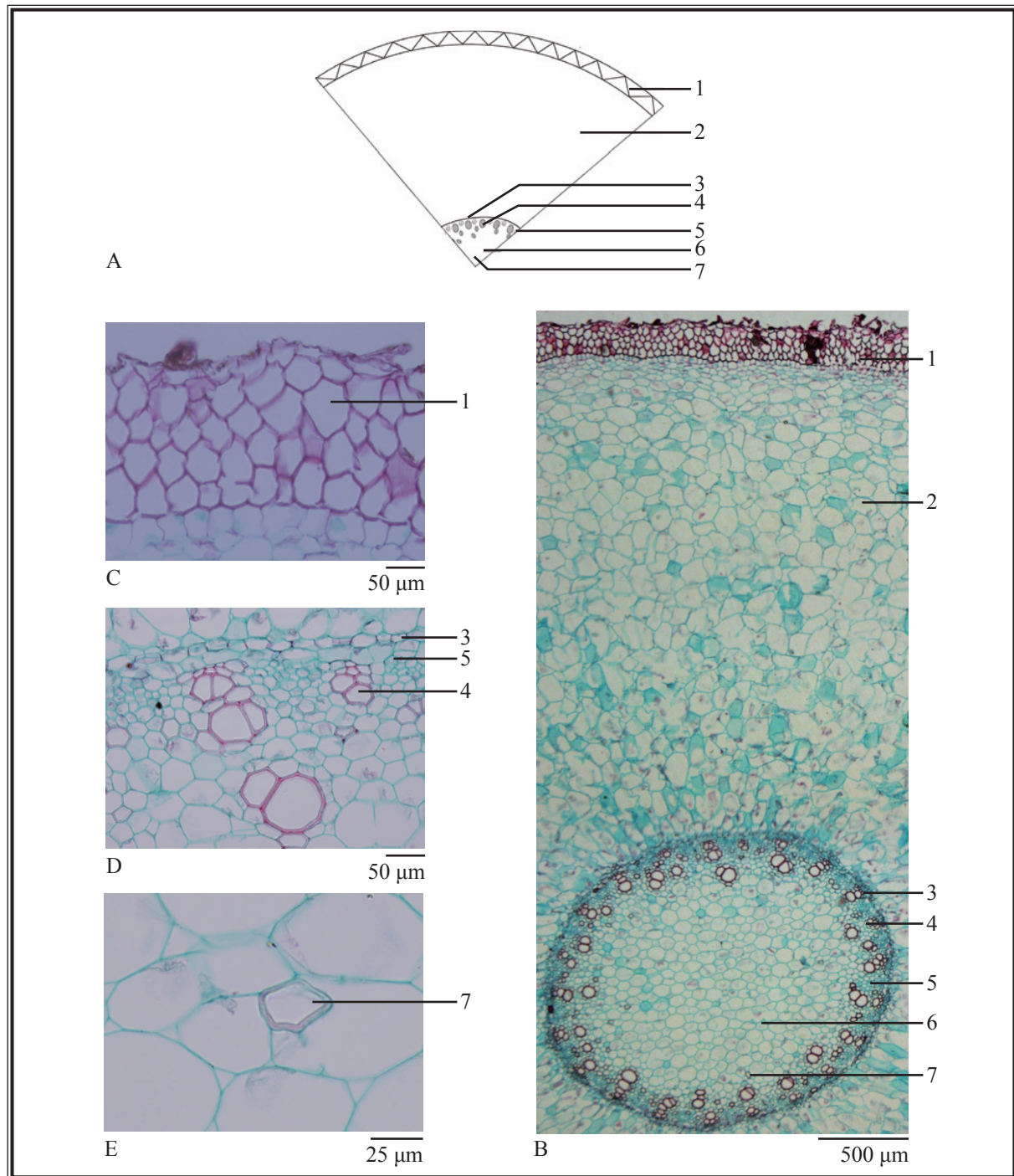
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山銀花

Plumbaginis Zeylanicæ Radix

**Stemona Radix**

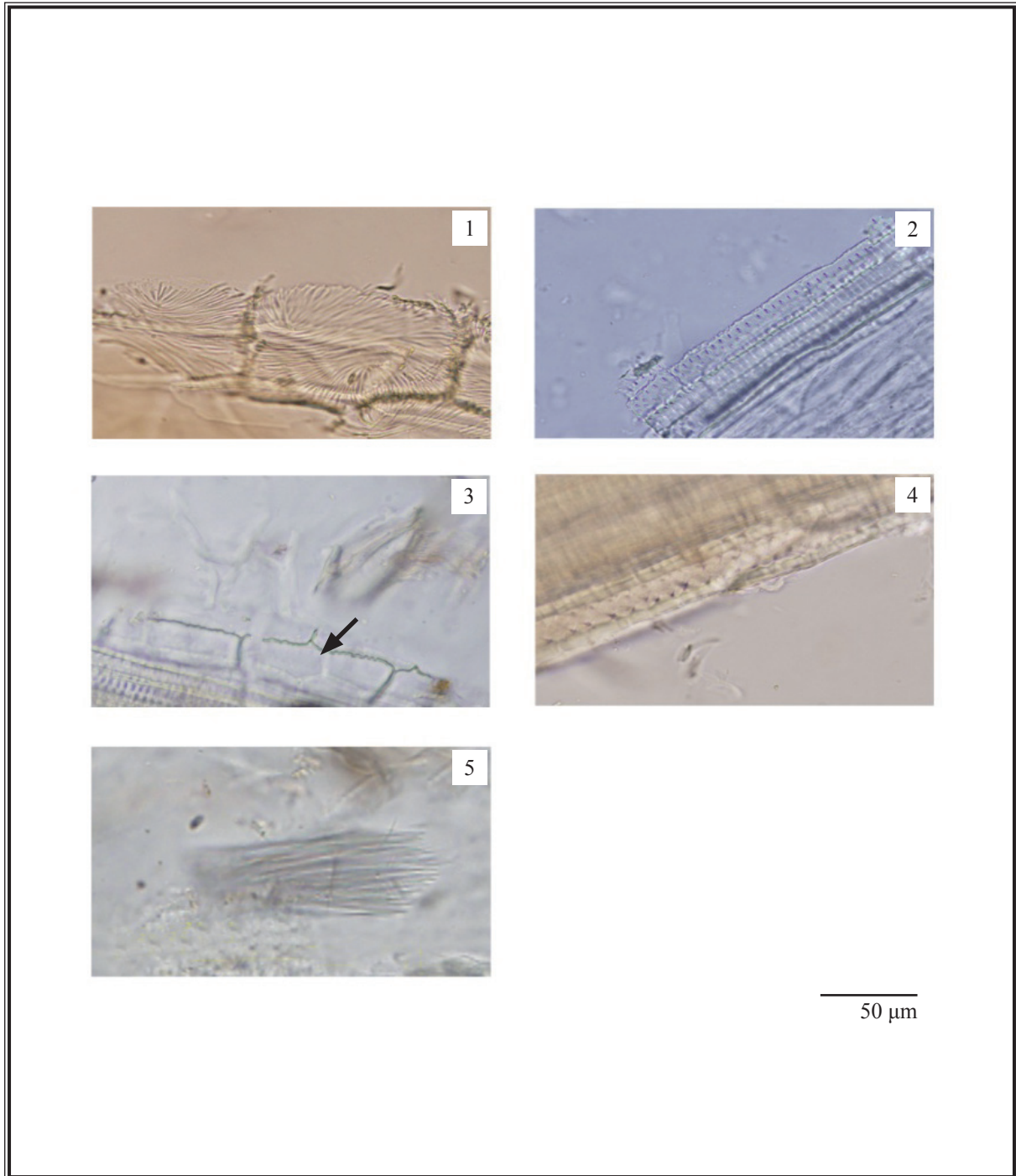
**Figure 2 (ii)** Microscopic features of transverse section of dried root tuber of *Stemona japonica* (Bl.) Miq.

A. Sketch B. Section illustration C. Velamen

D. Phloem bundles and xylem bundles E. Fibre

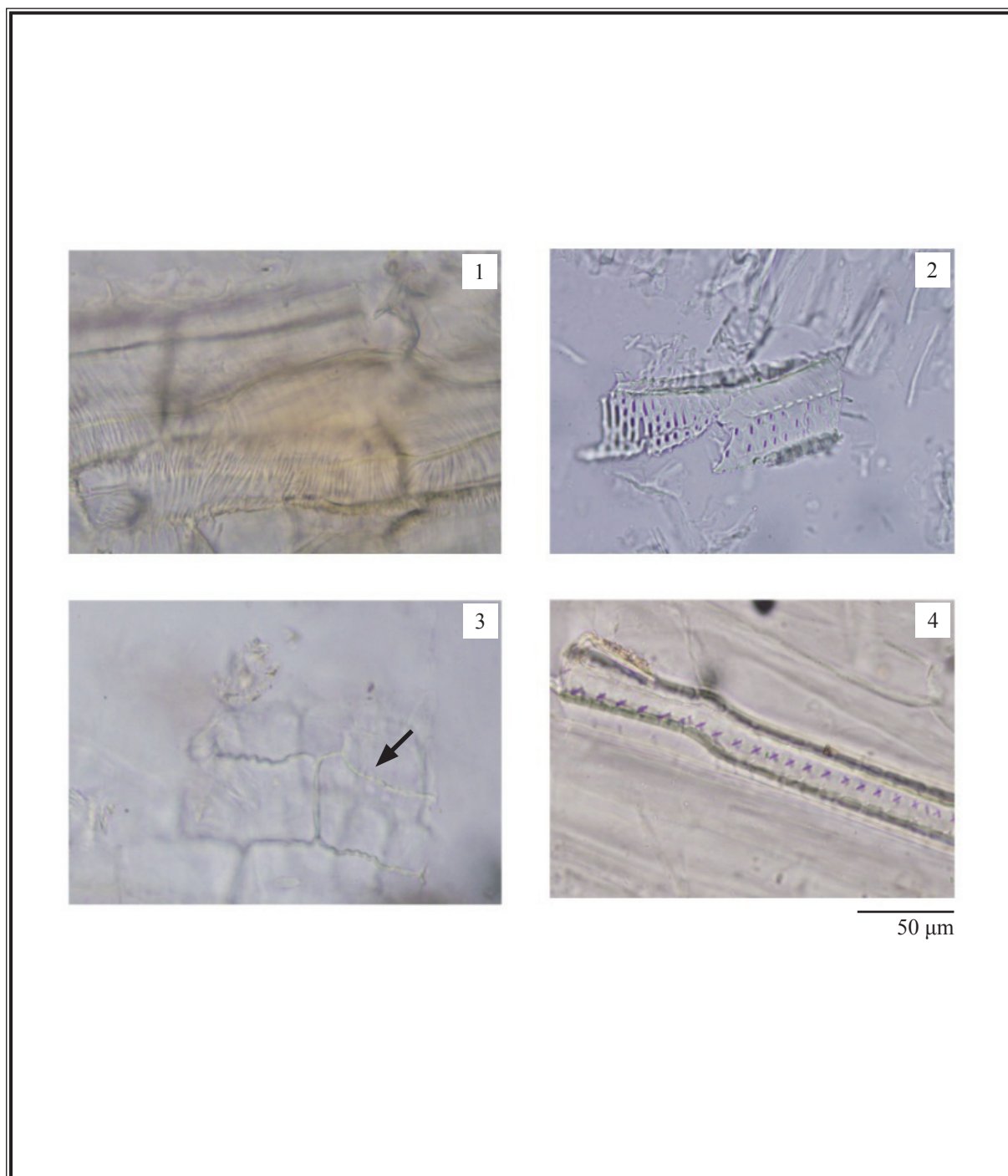
1. Velamen 2. Cortex 3. Endodermis 4. Xylem 5. Phloem 6. Pith 7. Fibre

**Stemona Radix**



**Figure 3 (i)** Microscopic features of powder of dried root tuber of *Stemona sessilifolia* (Miq.) Miq. (under the light microscope)

1. Velamen cells    2. Vessels    3. Endodermal cells (→)    4. Xylem fibre  
5. Raphides of calcium oxalate



**Figure 3 (ii)** Microscopic features of powder of dried root tuber of *Stemona japonica* (Bl.) Miq. (under the light microscope)

1. Velamen cells    2. Vessel    3. Endodermal cells (→)    4. Xylem fibre

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

### Standard solution

#### *Protostemonine standard solution*

Weigh 1.0 mg of protostemonine CRS (Fig. 4) and dissolve in 2 mL of ethanol (95%).

### Developing solvent system

Prepare a mixture of ammonium hydroxide solution (25%, v/v), acetone, ethyl acetate and cyclohexane (1:4:4:6, v/v). Use the upper layer.

### 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 1*

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.

#### *Spray reagent 2*

Weigh 5 g of sodium nitrite and dissolve in 100 mL of ethanol (70%).

### Test solution

Weigh 1.0 g of the powdered sample and place it in a 100-mL conical flask, then add 1 mL of ammonium hydroxide solution (25%, v/v). Allow to stand for 30 min. Add 50 mL of diethyl ether. Sonicate (360 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol (95%).

### Procedure

Carry out the method by using a HPTLC silica gel G plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately protostemonine standard solution and the test solution (5  $\mu$ L 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 8 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Spray the



山豆根

Saururi Herba 三白草

牡荊葉

車前草

蓮鬚

Saussureae Involucratae Herba

Polygoni Perfoliati Herba

Lonicerae Flos

Plantaginis Herba

Bruceae Fructus 鴉膽子

天山雪蓮

白花丹

杠板歸

北豆根

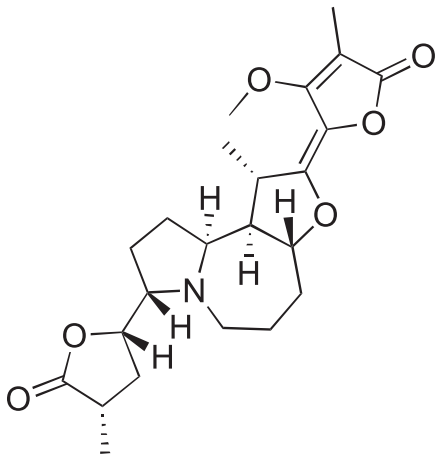
山銀花

Plumbaginis Zeylanicae Radix

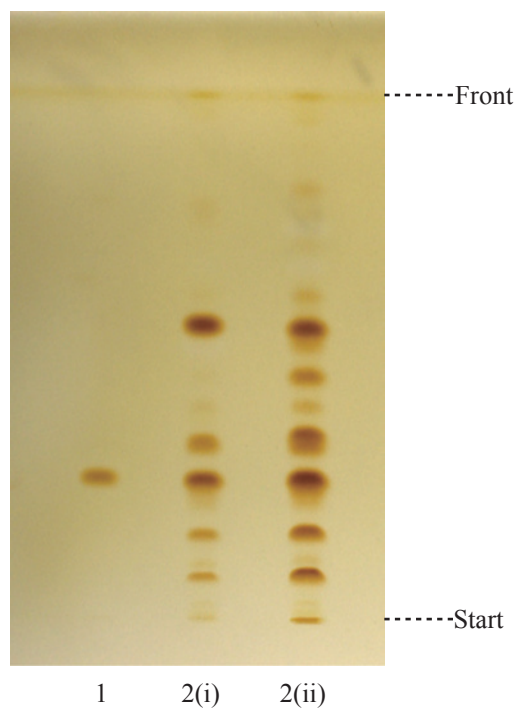
Menispermii Rhizoma

**Stemonaе Radix**

plate evenly with the spray reagent 1 and the spray reagent 2, then dry in air. Examine the plate under visible light. Calculate the  $R_f$  value by using the equation as indicated in Appendix IV (A).



**Figure 4** Chemical structure of protostemonine



**Figure 5** A reference HPTLC chromatogram of *Stemonaе Radix* extract observed under visible light after staining

1. Protostemonine standard solution
2. Test solution of
  - (i) dried root tuber of *Stemona sessilifolia* (Miq.) Miq.
  - (ii) dried root tuber of *Stemona japonica* (Bl.) Miq.

For positive identification, the sample must give spot or band with chromatographic characteristics, including the colour and the  $R_f$  value, corresponding to that of protostemonine (Fig. 5).

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

#### Standard solution

*Protostemonine standard solution for fingerprinting, Std-FP (60 mg/L)*

Weigh 0.6 mg of protostemonine CRS and dissolve in 10 mL of methanol. Keep at about 4°C.

#### Test solution

Weigh 1.0 g of the powdered sample and place it in a cellulose extraction thimble. Place the cellulose extraction thimble in soxhlet extractor. Add 4 mL of ammonium hydroxide solution (25%, v/v). Allow to stand for 30 min. Add 100 mL of methanol to a 250-mL round-bottomed flask. Perform the soxhlet extraction for 4 h at 80°C. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 25-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45- $\mu$ m PTFE filter.

#### Chromatographic system

The liquid chromatograph is equipped with a DAD (306 nm) and a column (4.6  $\times$  250 mm) packed with phenyl hexyl bonded silica gel (5  $\mu$ m particle size and 110 Å pore size). The column temperature is maintained at 35°C during the separation. The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 0.1% triethylamine and acetonitrile (68:32, v/v). The elution time is about 60 min.

#### System suitability requirements

Perform at least five replicate injections, each using 10  $\mu$ L of protostemonine Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of protostemonine should not be more than 5.0%; the RSD of the retention time of protostemonine peak should not be more than 2.0%; the column efficiency determined from protostemonine peak should not be less than 10000 theoretical plates.

The  $R$  value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.5 [Fig. 6 (i) or (ii)].

#### Procedure

Separately inject protostemonine Std-FP and the test solution (10  $\mu$ L each) into the HPLC system and record the chromatograms. Measure the retention time of protostemonine peak in

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天山雪蓮

白花丹

Polygoni Perfoliati Herba

杠板歸

北豆根

Menispermii Rhizoma

Lonicerae Flos

山銀花

Plantaginis Herba

Bruceae Fructus 鴉膽子

Plumbaginis Zeylanicae Radix

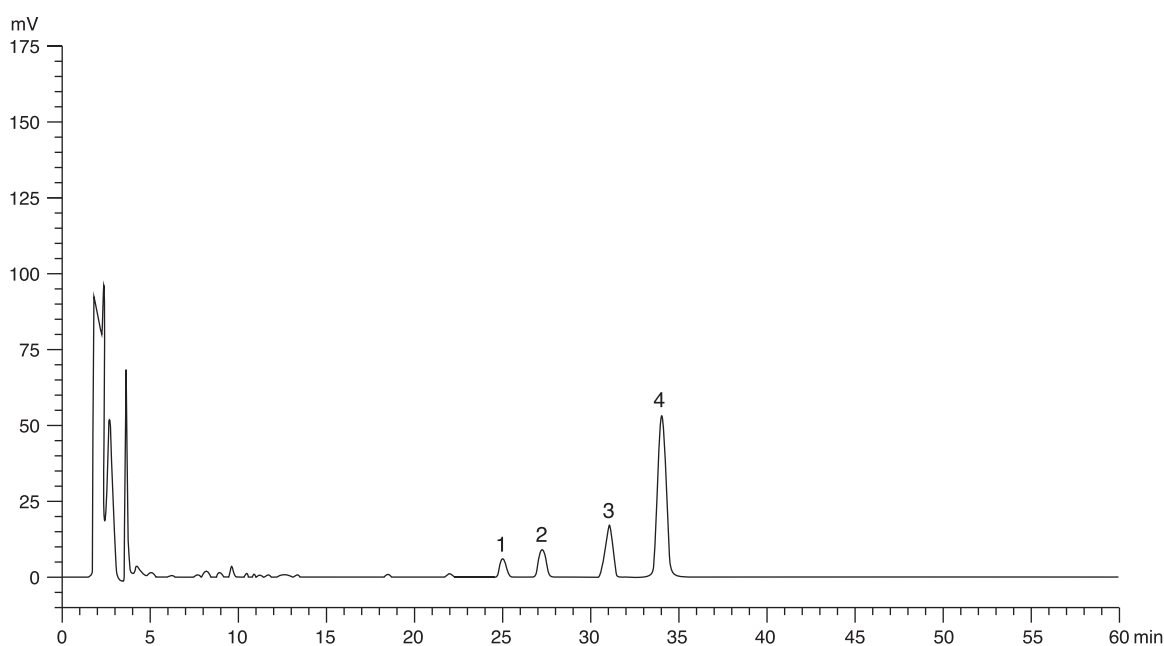
**Stemona Radix**

the chromatogram of protostemonine Std-FP and the retention times of the four characteristic peaks [Fig. 6 (i) or (ii)] in the chromatogram of the test solution. Identify protostemonine peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of protostemonine Std-FP. The retention times of protostemonine 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.

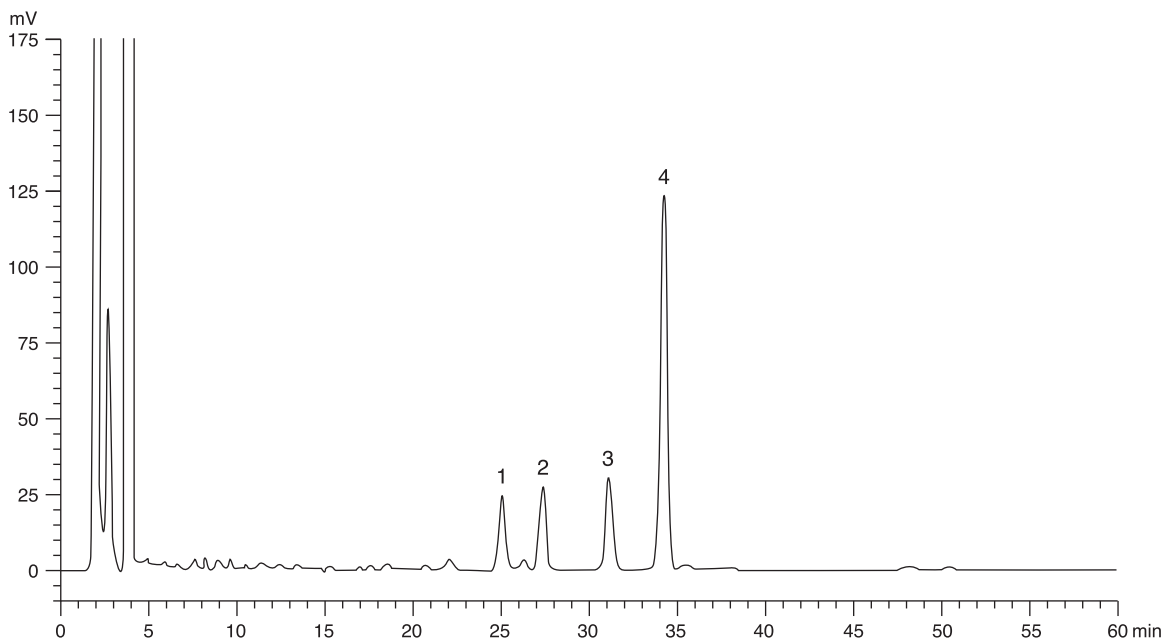
The RRTs and acceptable ranges of the four characteristic peaks of dried root tuber of *Stemona sessilifolia* (Miq.) Miq. extract and *Stemona japonica* (Bl.) Miq. extract are listed in Table 1.

**Table 1** The RRTs and acceptable ranges of the four characteristic peaks of dried root tuber of *Stemona sessilifolia* (Miq.) Miq. extract and *Stemona japonica* (Bl.) Miq. extract.

Peak No.	RRT	Acceptable Range
1	0.73	± 0.03
2	0.80	± 0.03
3	0.91	± 0.03
4 (marker, protostemonine)	1.00	-



**Figure 6 (i)** A reference fingerprint chromatogram of dried root tuber of *Stemona sessilifolia* (Miq.) Miq. extract



**Figure 6 (ii)** A reference fingerprint chromatogram of dried root tuber of *Stemona japonica* (Bl.) Miq. 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 respective reference fingerprint chromatograms [Fig. 6 (i) or (ii)].

## 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 8.0%.

**5.6 Ash** (*Appendix IX*)

Total ash: not more than 6.5%.

Acid-insoluble ash: not more than 2.5%.

**5.7 Water Content** (*Appendix X*)

Oven dried method: not more than 13.0%.

## 6. EXTRACTIVES (*Appendix XI*)

Water-soluble extractives (hot extraction method): not less than 55.0%.

Ethanol-soluble extractives (hot extraction method): not less than 49.0%.

## 7. ASSAY

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

### Standard solution

*Protostemonine standard stock solution, Std-Stock (1000 mg/L)*

Weigh accurately 5.0 mg of protostemonine CRS and dissolve in 5 mL of methanol. Keep at about 4°C.

*Protostemonine standard solution for assay, Std-AS*

Measure accurately the volume of the protostemonine Std-Stock, dilute with methanol to produce a series of solutions of 10, 20, 50, 100, 200 mg/L for protostemonine. Keep at about 4°C.

### Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a cellulose extraction thimble. Place the cellulose extraction thimble in soxhlet extractor. Add 4 mL of ammonium hydroxide solution (25%, v/v). Allow to stand for 30 min. Add 100 mL of methanol to a 250-mL round-bottomed flask. Perform the soxhlet extraction for 4 h at 80°C. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 25-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45- $\mu$ m PTFE filter.

### Chromatographic system

The liquid chromatograph is equipped with a DAD (306 nm) and a column (4.6  $\times$  250 mm) packed with phenyl hexyl bonded silica gel (5  $\mu$ m particle size and 110 Å pore size). The column temperature is maintained at 35°C during the separation. The flow rate is about 1.0 mL/min. The mobile phase is a mixture of 0.1% triethylamine and acetonitrile (68:32, v/v). The elution time is about 60 min.

### System suitability requirements

Perform at least five replicate injections, each using 10  $\mu$ L of protostemonine Std-AS (50 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of protostemonine should not be more than 5.0%; the RSD of the retention time of protostemonine peak should not be more than 2.0%; the column efficiency determined from protostemonine peak should not be less than 10000 theoretical plates.

**Stemona Radix**

The *R* value between protostemonine 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 protostemonine Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of protostemonine against the corresponding concentrations of protostemonine Std-AS. Obtain the slope, y-intercept and the *r*<sup>2</sup> value from the 5-point calibration curve.

**Procedure**

Inject 10 μL of the test solution into the HPLC system and record the chromatogram. Identify protostemonine peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of protostemonine Std-AS. The retention times of protostemonine 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 protostemonine in the test solution, and calculate the percentage content of protostemonine in the sample by using the equations as indicated in Appendix IV (B).

**Limits**

The dried root tuber of *Stemona sessilifolia* (Miq.) Miq. and *Stemona japonica* (Bl.) Miq. contains not less than 0.098% of protostemonine (C<sub>23</sub>H<sub>31</sub>NO<sub>6</sub>), calculated with reference to the dried substance.

## Part II Dried root tuber of *Stemona tuberosa* Lour.

### 3. DESCRIPTION

Long fusiform or long slit-shaped, 8-32 cm long, 6-17 mm in diameter. Externally yellowish-white to yellowish-brown, with shallow longitudinal wrinkles or irregular longitudinal furrows. Texture hard and compact. Fracture yellowish-white to dark brown, stele relatively large, pith whitish. Odour slight; taste bitter [Fig. 1(iii)].

### 4. IDENTIFICATION

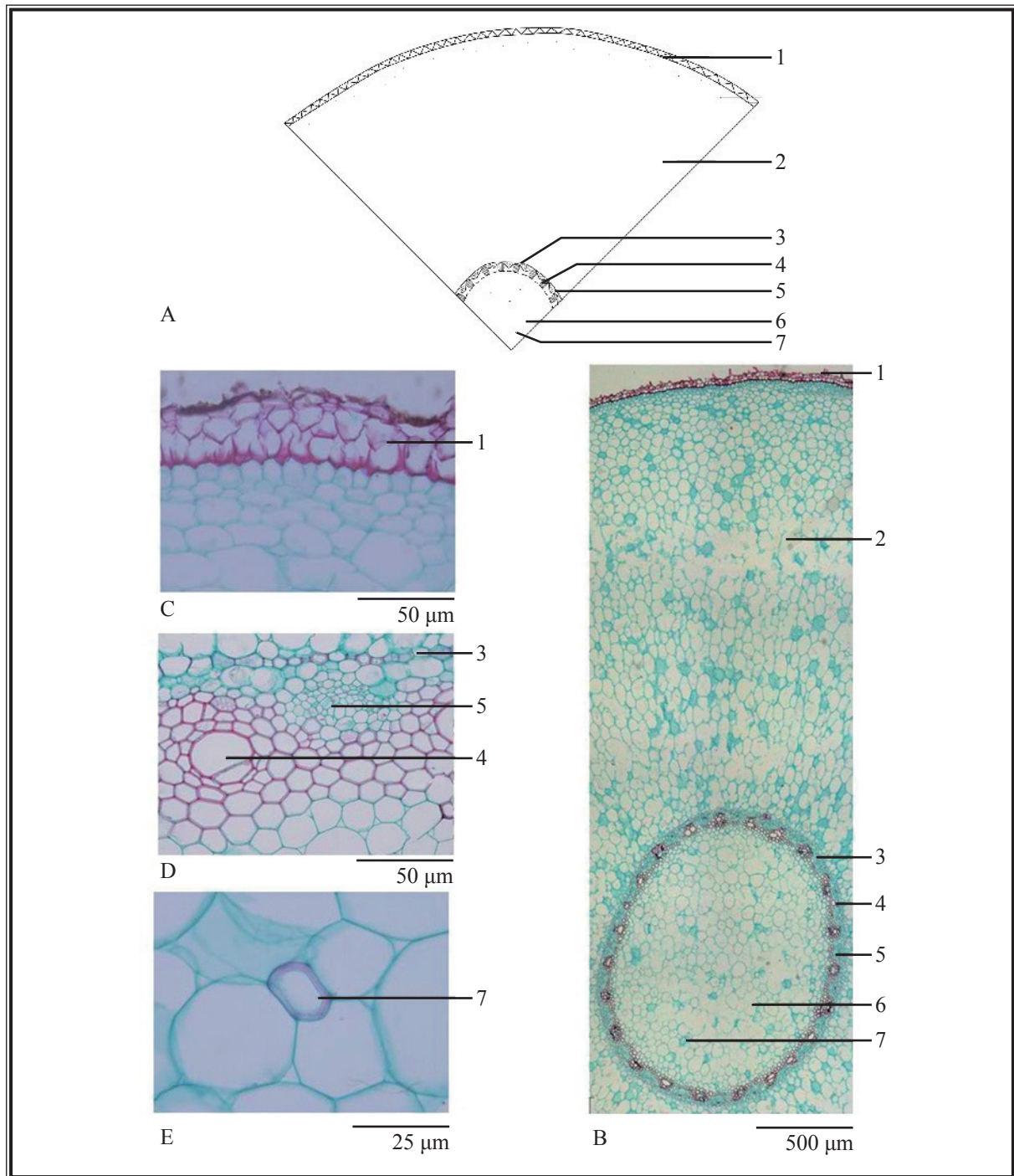
#### 4.1 Microscopic Identification (*Appendix III*)

##### Transverse Section

Velamen consists of 3 layers of cells, cell wall without fine striations, the inner walls of the innermost layers extremely thickened. Fibres scattered in the outer side of cortex, subsquare, with slightly lignified walls. Stele consists of 23-26 phloem bundles and xylem bundles respectively. Xylem bundles consist of 1-5 vessels, vessels rounded-polygonal, arranged in a radial direction, the inner side of xylem bundle arranged with xylem fibres and slightly lignified parenchymatous cells in a ring. Pith scattered with a few small fibres (Fig. 7).

##### Powder

Colour yellowish-white to brownish-yellow. Velamen cells pale yellowish-brown, subpolygonal or subsquare, walls relatively thick and lignified, without dense and fine striations on the surface of cell walls. Bordered-pitted vessels 24-110  $\mu\text{m}$  in diameter. Endodermal cells mostly in pieces, subrectangular, with undulantly curved walls. Xylem fibres relatively long, 13-81  $\mu\text{m}$  in diameter, walls relatively thick and lignified, with oblique, V-shaped or bordered-pitted pits. Gelatinized starch granules or starch granules can be found (samples processed by dipping in boiling water briefly) (Fig. 8).



**Figure 7** Microscopic features of transverse section of dried root tuber of *Stemona tuberosa* Lour.

A. Sketch B. Section illustration C. Velamen D. Phloem bundles and xylem bundles E. Fibre

1. Velamen 2. Cortex 3. Endodermis 4. Xylem 5. Phloem 6. Pith 7. Fibre



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Saururi Herba 三白草

牡荊葉

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Saussureae Involucratae Herba

Polygona Perfoliati Herba

Lonicerae Flos

Plantaginis Herba

Bruceae Fructus 鴉膽子

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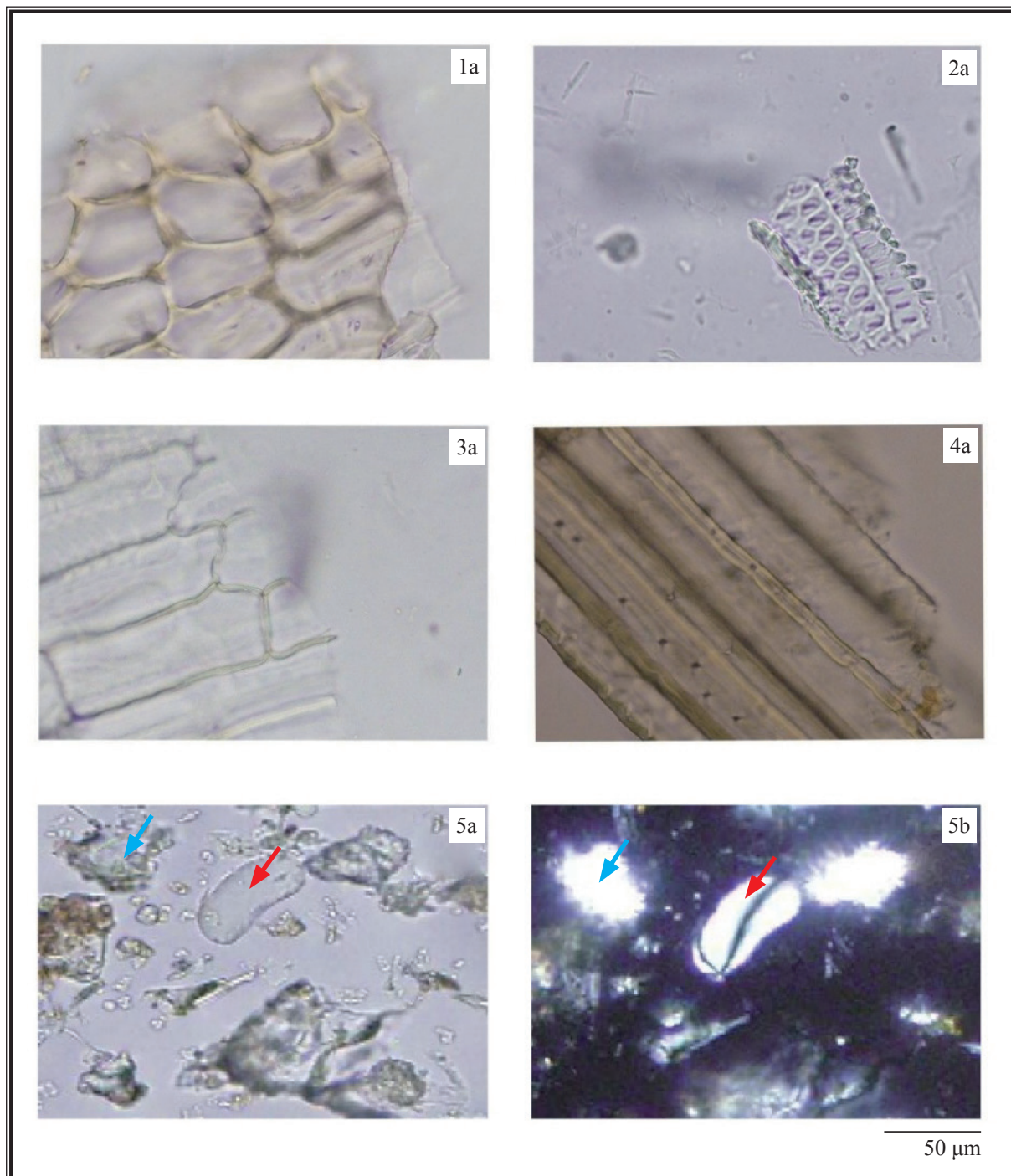
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Menispermii Rhizoma

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Plumbaginis Zeylanicae Radix

**Stemona Radix**

**Figure 8** Microscopic features of powder of dried root tuber of *Stemona tuberosa* Lour.

1. Velamen cells    2. Vessels    3. Endodermal cells    4. Xylem fibres  
5. Starch granules ( → ) and gelatinized starch granules ( → )

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

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

### Standard solution

*Tuberostemonine trifluoroacetate standard solution*

Weigh 1.0 mg of tuberostemonine trifluoroacetate CRS (Fig. 9) and dissolve in 2 mL of ethanol (95%).

### Developing solvent system

Prepare a mixture of ammonium hydroxide solution (25%, v/v), acetone, ethyl acetate and cyclohexane (1:4:4:6, v/v). Use the upper layer.

### 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 1*

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.

*Spray reagent 2*

Weigh 5 g of sodium nitrite and dissolve in 100 mL of ethanol (70%).

### Test solution

Weigh 1.0 g of the powdered sample and place it in a 100-mL conical flask, then add 1 mL of ammonium hydroxide solution (25%, v/v). Allow to stand for 30 min. Add 50 mL of diethyl ether. Sonicate (360 W) the mixture for 30 min. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol (95%).

### Procedure

Carry out the method by using a HPTLC silica gel G plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately tuberostemonine trifluoroacetate standard solution and the test solution (5  $\mu$ L 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 8 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Spray the

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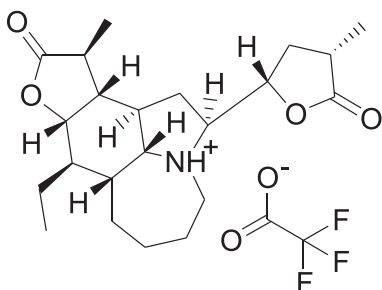
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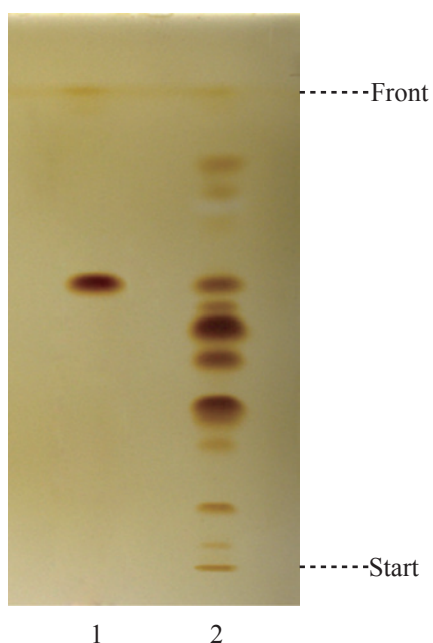
Plumbaginis Zeylanicae Radix

**Stemonae Radix**

plate evenly with the spray reagent 1 and the spray reagent 2, then dry in air. Examine the plate under visible light. Calculate the  $R_f$  value by using the equation as indicated in Appendix IV (A).



**Figure 9** Chemical structure of tuberostemonine trifluoroacetate



**Figure 10** A reference HPTLC chromatogram of dried root tuber of *Stemona tuberosa* Lour. extract observed under visible light after staining

1. Tuberostemonine trifluoroacetate standard solution    2. Test solution

For positive identification, the sample must give spot or band with chromatographic characteristics, including the colour and the  $R_f$  value, corresponding to that of tuberostemonine (Fig. 10).

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

#### Standard solution

*Tuberostemonine trifluoroacetate standard solution for fingerprinting, Std-FP (1200 mg/L)*

Weigh 1.2 mg of tuberostemonine trifluoroacetate CRS and dissolve in 1 mL of methanol. Keep at about 4°C.

#### Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 4 mL of ammonium hydroxide solution (25%, v/v). Allow to stand for 30 min. Add 25 mL of a mixture of isopropanol and methanol (1:1, v/v). Sonicate (300 W) the mixture for 30 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Repeat the extraction for one more time. Combine the filtrates and evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 10-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45- $\mu$ m PTFE filter.

#### Chromatographic system

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 105°C; nebulizer gas (N<sub>2</sub>) flow rate: 3.0 L/min] and a column (4.6  $\times$  250 mm) packed with phenyl hexyl bonded silica gel (5  $\mu$ m particle size and 110 Å pore size). The column temperature is maintained at 30°C during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 2) –

**Table 2** Chromatographic system conditions

Time (min)	0.1% Triethylamine (% v/v)	Acetonitrile (% v/v)	Elution
0 – 10	90 → 60	10 → 40	linear gradient
10 – 20	60 → 50	40 → 50	linear gradient
20 – 40	50 → 25	50 → 75	linear gradient

#### System suitability requirements

Perform at least five replicate injections, each using 5  $\mu$ L of tuberostemonine trifluoroacetate Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of tuberostemonine should not be more than 5.0%; the RSD of the retention time of tuberostemonine peak should not be more than 2.0%; the column efficiency determined from tuberostemonine peak should not be less than 100000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 11).

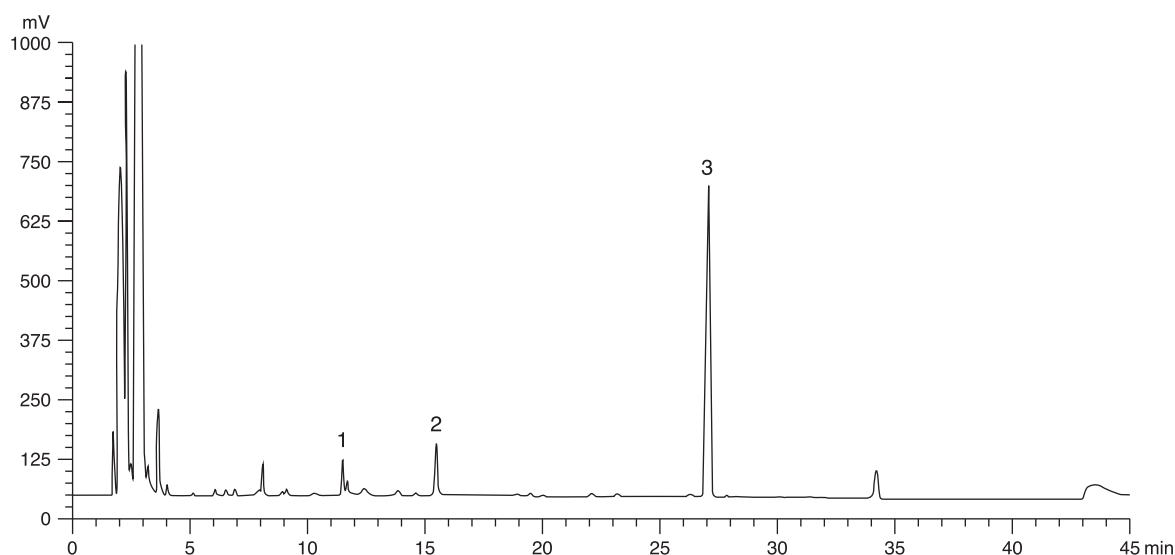
### Procedure

Separately inject tuberostemonine trifluoroacetate Std-FP and the test solution (5  $\mu$ L each) into the HPLC system and record the chromatograms. Measure the retention time of tuberostemonine peak in the chromatogram of tuberostemonine trifluoroacetate Std-FP and the retention times of the three characteristic peaks (Fig. 11) in the chromatogram of the test solution. Identify tuberostemonine peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of tuberostemonine trifluoroacetate Std-FP. The retention times of tuberostemonine 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 three characteristic peaks of dried root tuber of *Stemona tuberosa* Lour. extract are listed in Table 3.

**Table 3** The RRTs and acceptable ranges of the three characteristic peaks of dried root tuber of *Stemona tuberosa* Lour. extract

Peak No.	RRT	Acceptable Range
1	0.42	$\pm 0.03$
2	0.55	$\pm 0.03$
3 (marker, tuberostemonine)	1.00	-



**Figure 11** A reference fingerprint chromatogram of dried root tuber of *Stemona tuberosa* Lour. 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. 11).

## 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 8.0%.

**5.6 Ash** (*Appendix IX*)

Total ash: not more than 6.5%.

Acid-insoluble ash: not more than 2.5%.

**5.7 Water Content** (*Appendix X*)

Oven dried method: not more than 13.0%.

## 6. EXTRACTIVES (*Appendix XI*)

Water-soluble extractives (hot extraction method): not less than 55.0%.

Ethanol-soluble extractives (hot extraction method): not less than 49.0%.

## 7. ASSAY

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

### Standard solution

*Tuberostemonine trifluoroacetate standard stock solution, Std-Stock (4000 mg/L)*

Weigh accurately 4.0 mg of tuberostemonine trifluoroacetate CRS and dissolve in 1 mL of methanol.

Keep at about 4°C.

*Tuberostemonine trifluoroacetate standard solution for assay, Std-AS*

Measure accurately the volume of the tuberostemonine trifluoroacetate Std-Stock, dilute with methanol to produce a series of solutions of 200, 400, 800, 1200, 1600 mg/L for tuberostemonine trifluoroacetate. Keep at about 4°C.

**Test solution**

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 4 mL of ammonium hydroxide solution (25%, v/v). Allow to stand for 30 min. Add 25 mL of a mixture of isopropanol and methanol (1:1, v/v). Sonicate (300 W) the mixture for 30 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Repeat the extraction for one more time. Combine the filtrates and evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the solution to a 10-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45- $\mu$ m PTFE filter.

**Chromatographic system**

The liquid chromatograph is equipped with an ELSD [drift tube temperature: 105°C; nebulizer gas (N<sub>2</sub>) flow rate: 3.0 L/min] and a column (4.6  $\times$  250 mm) packed with phenyl hexyl bonded silica gel (5  $\mu$ m particle size and 110 Å pore size). The column temperature is maintained at 30°C during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 4) –

**Table 4** Chromatographic system conditions

Time (min)	0.1% Triethylamine (% v/v)	Acetonitrile (% v/v)	Elution
0 – 10	90 $\rightarrow$ 60	10 $\rightarrow$ 40	linear gradient
10 – 20	60 $\rightarrow$ 50	40 $\rightarrow$ 50	linear gradient
20 – 40	50 $\rightarrow$ 25	50 $\rightarrow$ 75	linear gradient

**System suitability requirements**

Perform at least five replicate injections, each using 5  $\mu$ L of tuberostemonine trifluoroacetate Std-AS (800 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of tuberostemonine should not be more than 5.0%; the RSD of the retention time of tuberostemonine peak should not be more than 2.0%; the column efficiency determined from tuberostemonine peak should not be less than 100000 theoretical plates.

The *R* value between tuberostemonine 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 tuberostemonine trifluoroacetate Std-AS (5 µL each) into the HPLC system and record the chromatograms. Plot the natural logarithm of peak areas of tuberostemonine against the natural logarithm of the corresponding concentrations of tuberostemonine trifluoroacetate Std-AS. Obtain the slope, y-intercept and the  $r^2$  value from the 5-point calibration curve.

### Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify tuberostemonine peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of tuberostemonine trifluoroacetate Std-AS. The retention times of tuberostemonine 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 tuberostemonine trifluoroacetate in the test solution, and calculate the percentage content of tuberostemonine (the percentage content of tuberostemonine trifluoroacetate  $\times 0.77$ , where 0.77 is the molar mass ratio of tuberostemonine and tuberostemonine trifluoroacetate) in the sample by using the equations as indicated in Appendix IV (B).

### Limits

The dried root tuber of *Stemona tuberosa* Lour. contains not less than 0.47% of tuberostemonine ( $C_{22}H_{33}NO_4$ ), calculated with reference to the dried substance.