Isodonis Herba

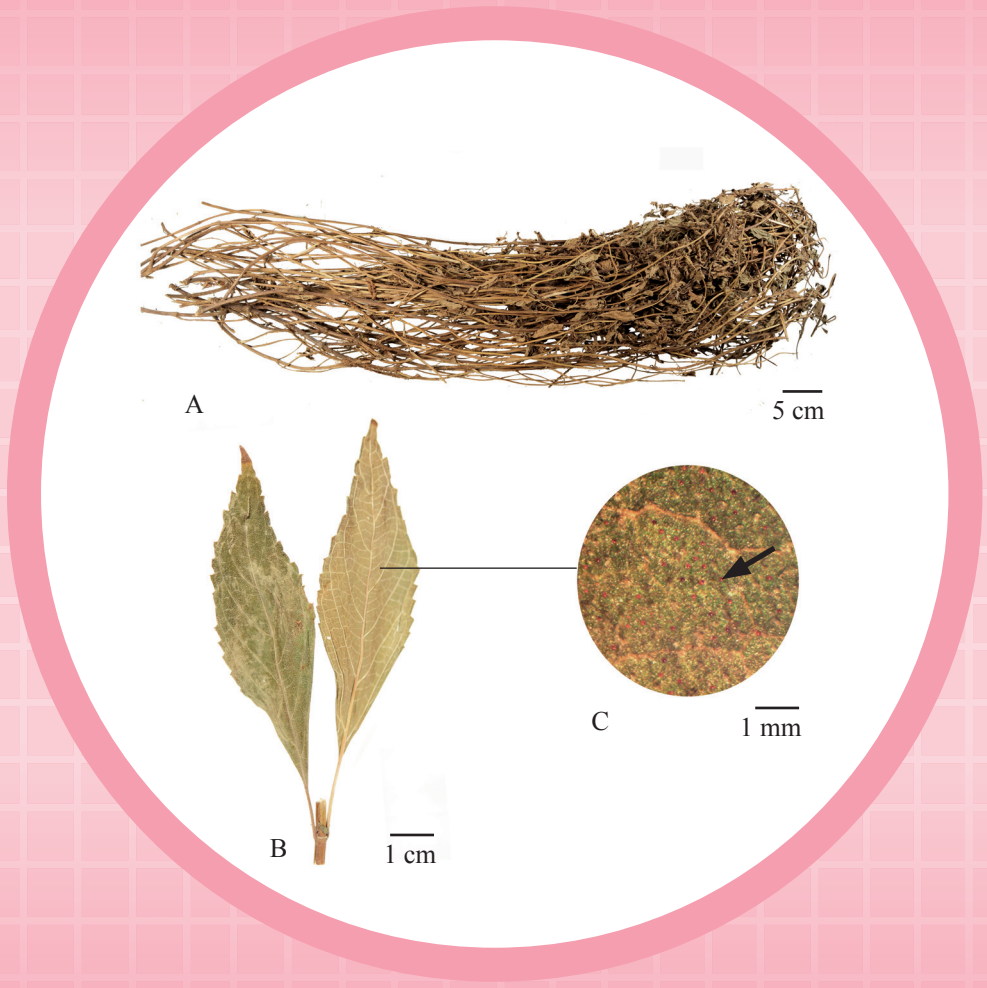

Figure 1 (i) A photograph of dried aerial part of *Isodon lophanthoides* (Buch. -Ham. ex D. Don) H. Hara.

A. Aerial part of herb

B. Magnified image of leaves (Left: upper surface, Right: lower surface)

C. Magnified image of lower surface of leaf (brownish-red dots ⟶)

Figure 1 (ii) A photograph of dried aerial part of *Isodon serra* (Maxim.) Kudo

A. Aerial part of herb

B. Magnified image of leaves (Left: upper surface, Right: lower surface)

C. Magnified image of lower surface of leaf

1. NAMES

Official name: Isodonis Herba

Chinese name: 溪黃草

Chinese phonetic name: Xihuangcao

2. SOURCE

Isodonis Herba is the dried aerial part of *Isodon lophanthoides* (Buch. -Ham. ex D. Don) H. Hara. or *Isodon serra* (Maxim.) Kudo (Lamiaceae). The aerial part is collected during summer and autumn before flowering, foreign matter removed, then dried under the sun to obtain Isodonis Herba.

Part I Dried aerial part of *Isodon lophanthoides* (Buch. -Ham. ex D. Don) H. Hara.

3. DESCRIPTION

Stem square-columnar, arris obtuse, longitudinal grooves distinct, frequently opposite-branched, 30-110 cm long, 2-5 mm in diameter; externally yellowish-green to brown, covered with pubescence and brownish-red glandular dots. Leaves opposite, petiolate, crumpled and easily broken; intact ones ovate to ovate-lanceolate or lanceolate, 4-10 cm long, 1.5-4.5 cm wide; leaf blade acuminate at apex, margin obtusely serrate; both surfaces greyish-green, covered with pubescence and brownish-red glandular dots. After soaked with water, then kneaded with fingers, yellowish-brown juice visible. Odour slight; taste slightly sweet, then bitter [Fig. 1 (i)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Remnants of non-glanlular hair frequently observed on the surface of epidermis. Epidermis consists of 1 layer of subsquare or rectangular cells. Collenchymatous cells 4-9 layers, subpolygonal or irregular polygonal, present at outer parts of cortex near ridges. Cortex narrow. Pericycle fibre bundles beneath the cortex, arranged in an interrupted ring, abundant near ridges. Phloem relatively narrow. Xylem radially arranged, relatively broad near ridges. Pith large, parenchymatous cells large, occasionally hollow in the centre [Fig. 2 (i)].

Leaf: Remnants of non-glandular hair frequently observed on the surface of epidermis. Upper epidermis consists of 1 layer of flat rectangular cells. Palisade tissue consists of 2-3 layers of cells. Spongy tissue consists of several layers of loosely arranged irregular cells. Collenchyma located under the epidermis of midrib. Vascular bundles of midrib collateral; vessels usually several in rows; phloem cells polygonal. Lower epidermis cells relatively small [Fig. 2 (ii)].

Powder

Colour yellowish-brown. Non-glandular hairs composed of 1-3 cells, conical, base relatively broad, 11-92 μm in diameter. Glandular scales numerous; oblate-spherical, with yellowish-brown content, 4-8 cells, 14-49 μm in diameter. Glandular hairs small; head of 1-2 cells, subglobose, 10-40 μm in diameter; stalk unicellular. Upper epidermal cells rectangular or polygonal, anticlinal walls slightly sinuate and thick, larger; compared with the lower epidermal, stomata occasionally observed, paracytic or diacytic. Lower epidermal cells irregular in shape, anticlinal walls usually sinuate, stomata diacytic or anomocytic, subrounded or oblong in shape. Vessels numerous, mainly spiral and reticulate vessels, 8-42 μm in diameter. Brownish-red dots numerous, scattered on the surface of epidermal cells (Fig. 3).

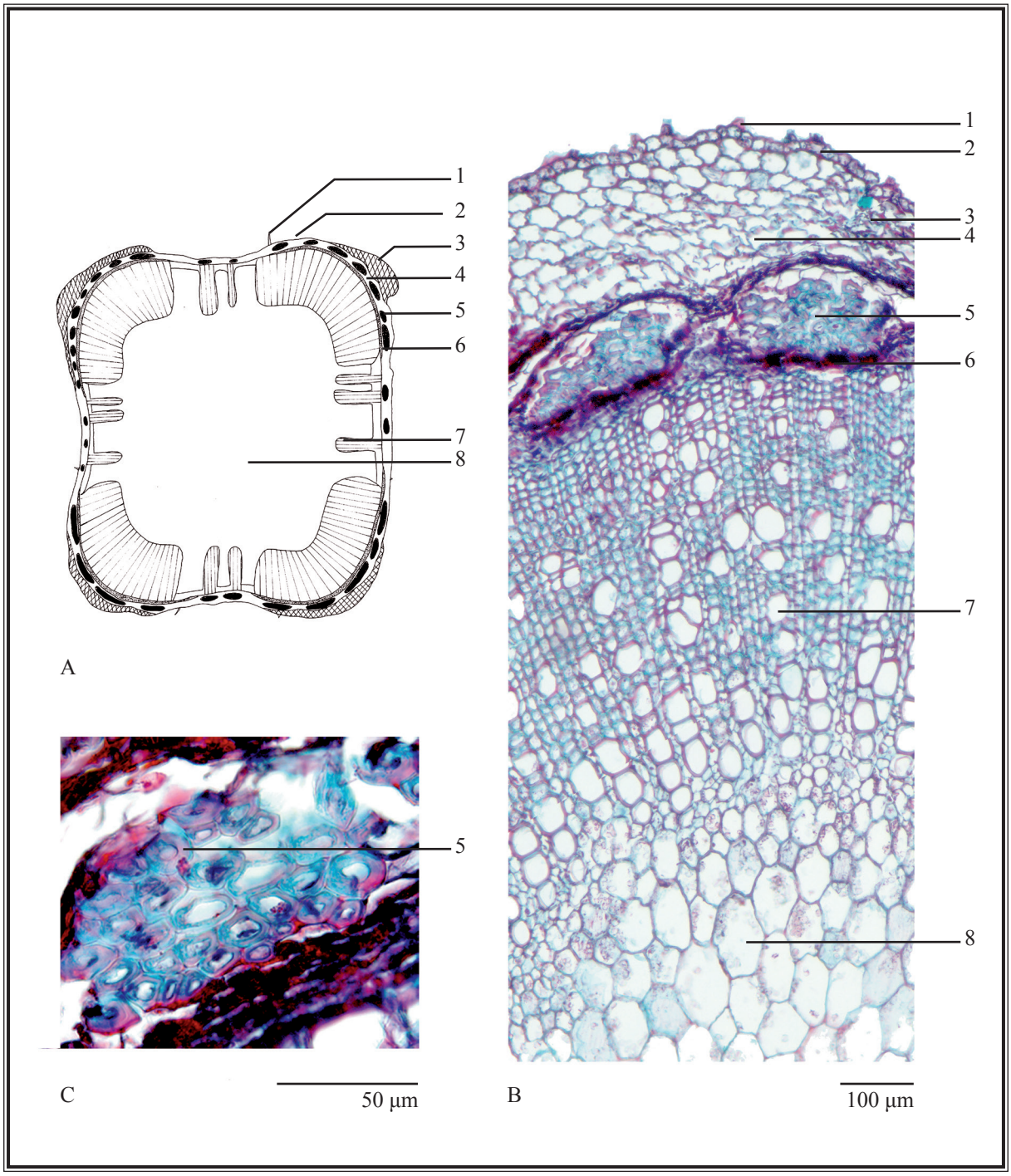

Figure 2 (i) Microscopic features of transverse section of stem of *Isodon lophanthoides* (Buch. -Ham. ex D. Don) H. Hara.

A. Sketch B. Section illustration C. Section magnified

1. Non-glandular hair 2. Epidermis 3. Collenchyma 4. Cortex
5. Pericycle fibre bundle 6. Phloem 7. Xylem 8. Pith

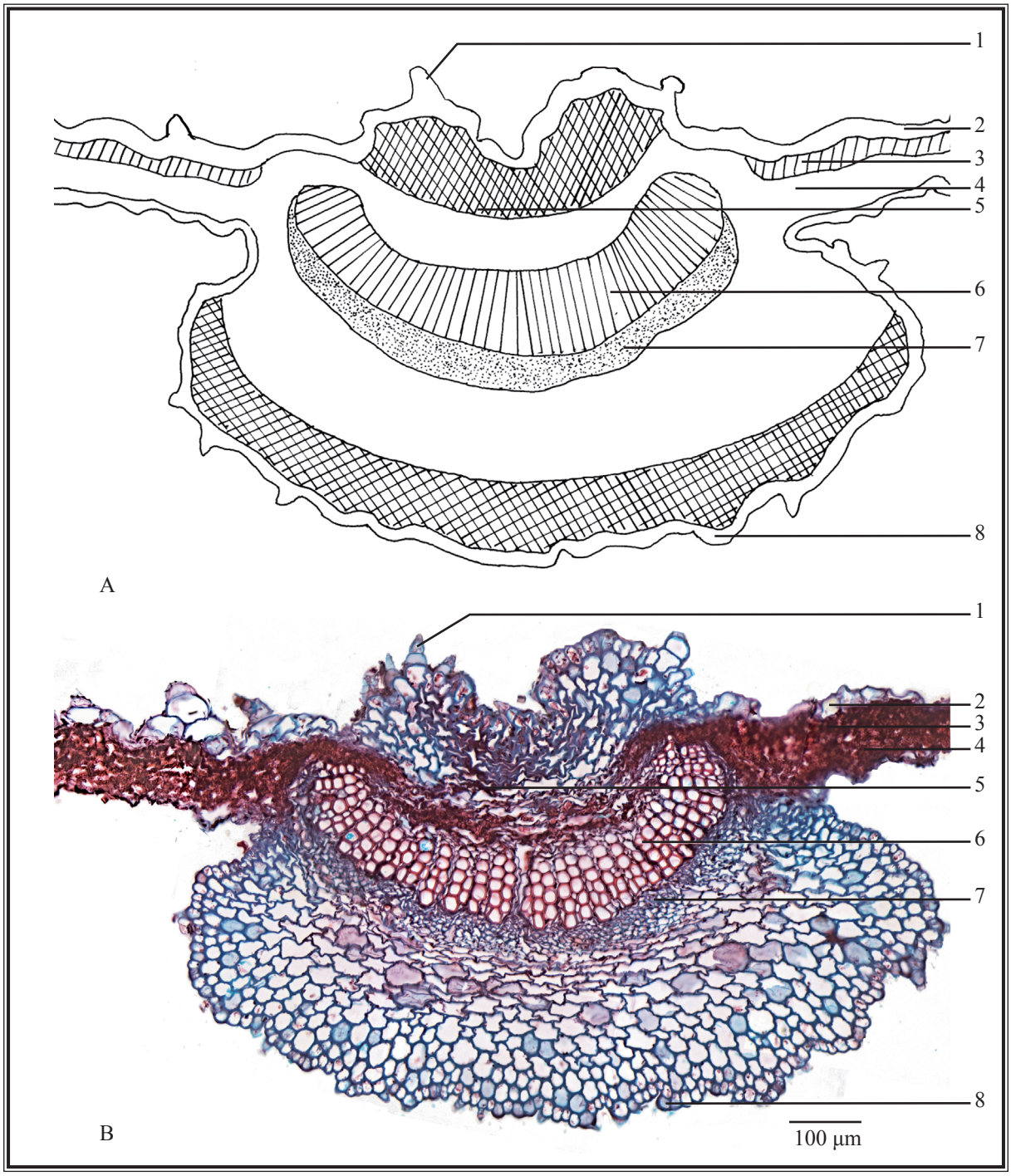

Figure 2 (ii) Microscopic features of transverse section of leaf of *Isodon lophanthoides* (Buch. -Ham. ex D. Don) H. Hara.

A. Sketch B. Section illustration

1. Non-glandular hair 2. Upper epidermis 3. Palisade tissue 4. Spongy tissue
5. Collenchyma 6. Xylem 7. Phloem 8. Lower epidermis

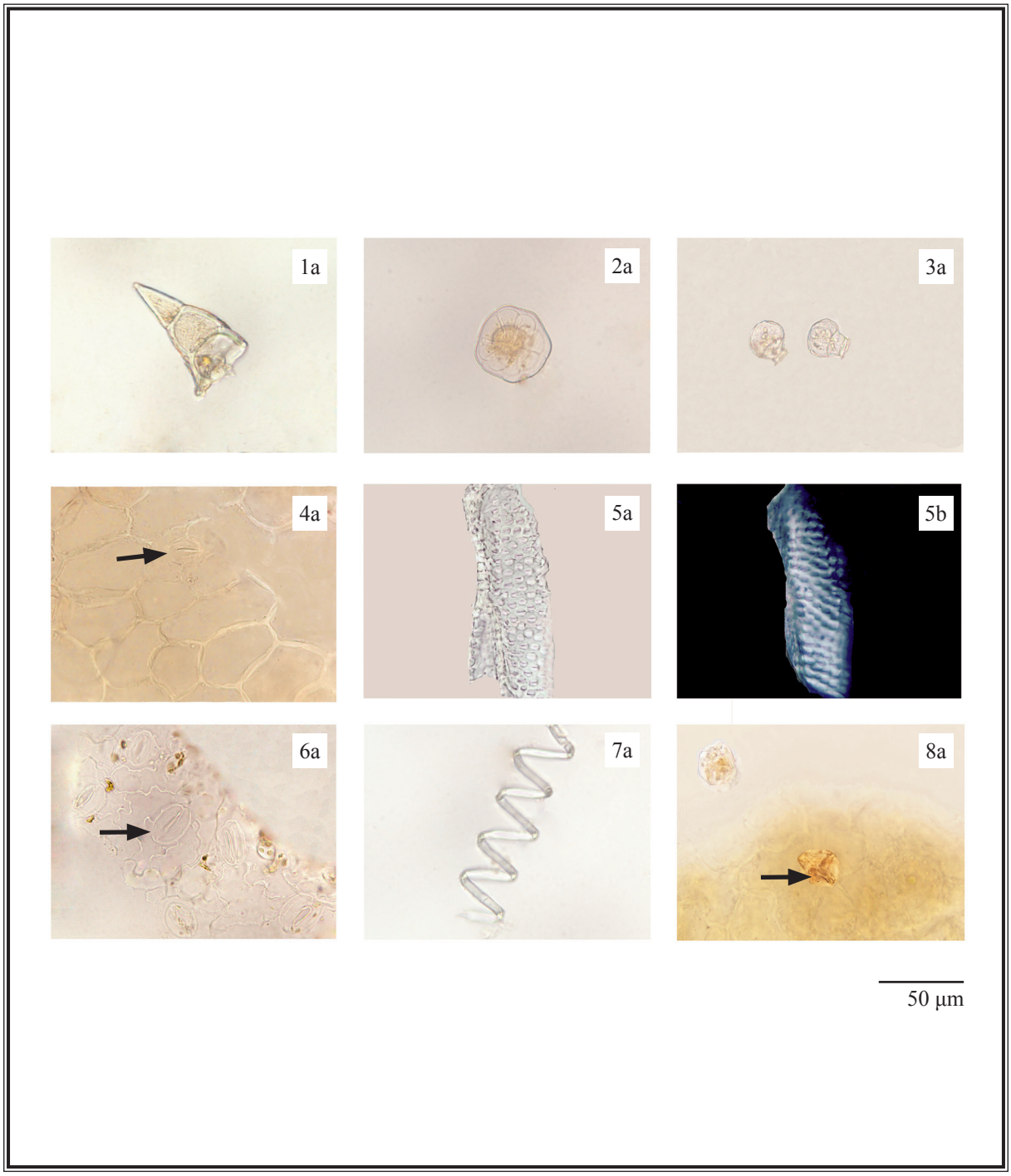

Figure 3 Microscopic features of powder of *Isodon lophanthoides* (Buch. -Ham. ex D. Don) H. Hara.

1. Non-glandular hair 2. Glandular scale 3. Glandular hairs
4. Upper epidermal cells with stoma (→) 5. Reticulate vessel
6. Lower epidermal cells with stomata (→)
7. Spiral vessel 8. Lower epidermal cells with brownish-red dots (→)

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

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

Standard solution

Rosmarinic acid standard solution

Weigh 2.0 mg of rosmarinic acid CRS (Fig. 4) and dissolve in 2 mL of ethanol (50%).

Developing solvent system

Prepare a mixture of cyclohexane, ethyl acetate, ethanol and formic acid (8:3:1.5:0.5, v/v).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 25 mL of ethanol (50%). Sonicate (150 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol (50%). Filter the extract. Make an appropriate dilution if necessary.

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 rosmarinic acid standard solution (2 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. Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

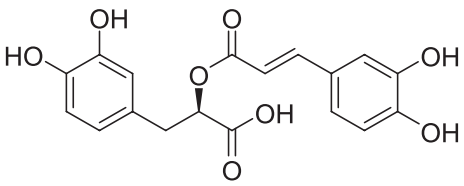

Figure 4 Chemical structure of rosmarinic acid

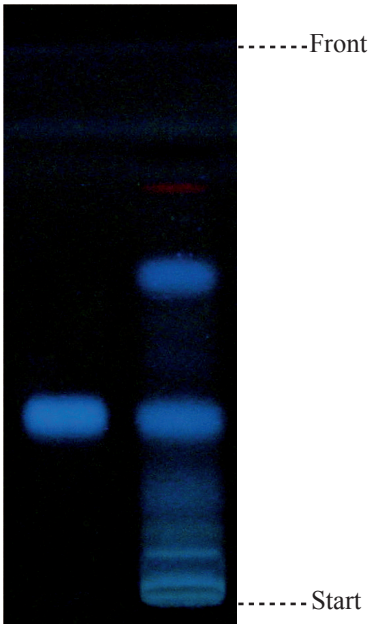

Figure 5 A reference HPTLC chromatogram of dried aerial part of *Isodon lophanthoides* (Buch. -Ham. ex D. Don) H. Hara. extract observed under UV light (366 nm)

1. Rosmarinic acid 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 rosmarinic acid (Fig. 5).

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

Standard solution

Rosmarinic acid standard solution for fingerprinting, Std-FP (10 mg/L)

Weigh 0.1 mg of rosmarinic acid CRS and dissolve in 10 mL of ethanol (50%).

Test solution

Weigh 0.7 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of ethanol (50%). Sonicate (150 W) the mixture for 1 h. Centrifuge at about 4000 × *g* for 5 min. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (329 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.1% Formic acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 40	85 → 60	15 → 40	linear gradient
40 – 60	60	40	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 μL of rosmarinic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of rosmarinic acid should not be more than 5.0%; the RSD of the retention time of rosmarinic acid peak should not be more than 2.0%; the column efficiency determined from rosmarinic acid peak should not be less than 60000 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. 6).

Procedure

Separately inject rosmarinic acid Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of rosmarinic acid peak in the chromatogram of rosmarinic acid Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify rosmarinic acid peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of rosmarinic acid Std-FP. The retention times of rosmarinic acid 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 aerial part of *Isodon lophanthoides* (Buch. -Ham. ex D. Don) H. Hara. extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of dried aerial part of *Isodon lophanthoides* (Buch. -Ham. ex D. Don) H. Hara. extract

Peak No.	RRT	Acceptable Range
1	0.38	± 0.03
2	0.77	± 0.03
3 (marker, rosmarinic acid)	1.00	-

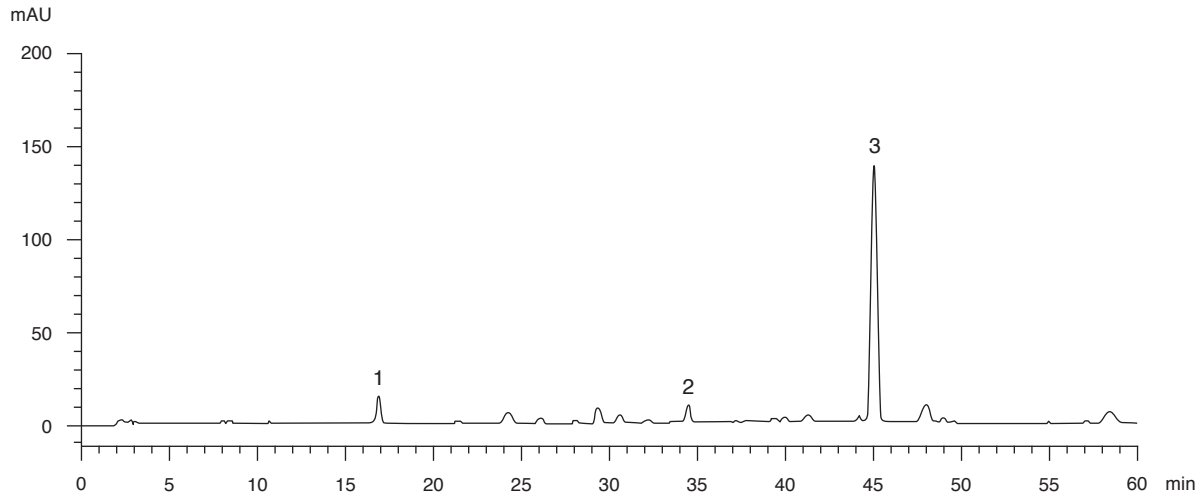

Figure 6 A reference fingerprint chromatogram of dried aerial part of *Isodon lophanthoides* (Buch. -Ham. ex D. Don) H. Hara. extract

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 7.0%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 13.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 10.0%.
Ethanol-soluble extractives (cold extraction method): not less than 8.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 1.0 mg of rosmarinic acid CRS and dissolve in 10 mL of ethanol (50%).

Rosmarinic acid standard solution for assay, Std-AS

Measure accurately the volume of the rosmarinic acid Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 1, 2, 20, 40, 60 mg/L for rosmarinic acid.

Test solution

Weigh accurately 0.3 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of ethanol (50%). Reflux the mixture for 1 h. Cool down to room temperature. Transfer the extract to a 50-mL centrifuge tube. Centrifuge at about 4000 × *g* for 5 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction one more time. Combine the supernatants and make up to the mark with ethanol (50%). Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (329 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Formic acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 20	70 → 60	30 → 40	linear gradient
20 – 30	60	40	isocratic

System suitability requirements

Perform at least five replicate injections, each using 10 µL of rosmarinic acid Std-AS (20 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of rosmarinic acid should not be more than 5.0%; the RSD of the retention time of rosmarinic acid peak should not be more than 2.0%; the column efficiency determined from rosmarinic acid peak should not be less than 15000 theoretical plates.

The *R* value between rosmarinic acid 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 rosmarinic acid Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of rosmarinic acid against the corresponding concentrations of rosmarinic acid 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 rosmarinic acid peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of rosmarinic acid Std-AS. The retention times of rosmarinic acid 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 rosmarinic acid in the test solution, and calculate the percentage content of rosmarinic acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The dried aerial part of *Isodon lophanthoides* (Buch. -Ham. ex D. Don) H. Hara. contains not less than 0.11% of rosmarinic acid ($C_{18}H_{16}O_8$), calculated with reference to the dried substance.

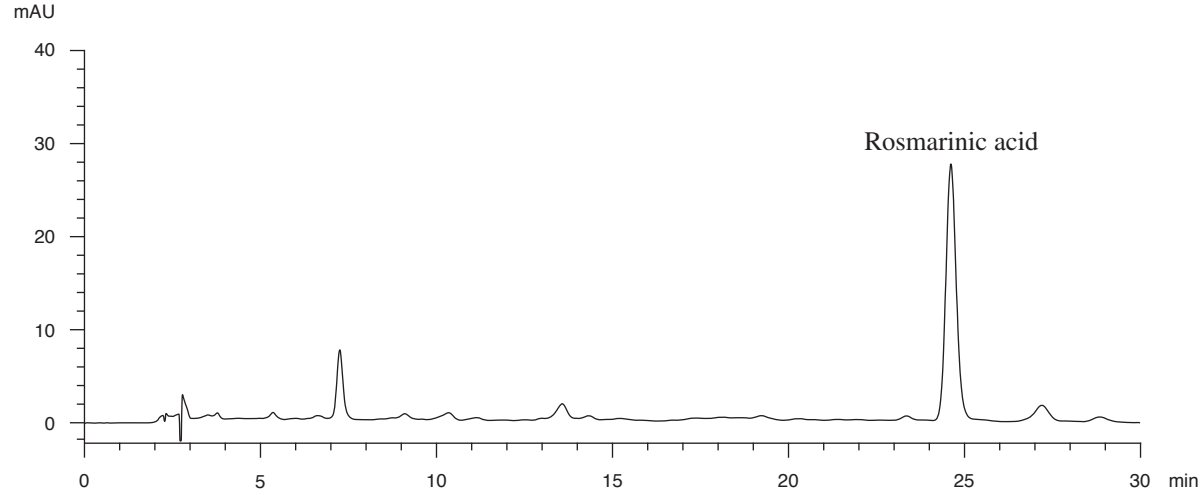

Figure 7 A reference assay chromatogram of dried aerial part of *Isodon lophanthoides* (Buch. -Ham. ex D. Don) H. Hara. extract

Part II Dried aerial part of *Isodon serra* (Maxim.) Kudo.

3. DESCRIPTION

Stem square-columnar, arris obtuse, longitudinal grooves distinct, frequently opposite-branched, 80-190 cm long, 2-10 mm in diameter with purplish-brown stripes. Leaves opposite, petiolate, crumpled and easily broken; intact ones ovate-lanceolate to lanceolate, 5.5-12 cm long, 2-4.5 cm wide; lamina thin, margin coarsely incurved-serrate, pubescence sparse, brownish-red glandular dots absent; petiole broad-alate toward apex. After soaked with water, then kneaded with fingers, no yellowish-brown juice visible. Odour slight; taste slightly sweet, then bitter [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Remnants of non-glandular hair occasionally observed on the surface of epidermis. Epidermis consists of 1 layer of subsquare or rectangular cells. Collenchymatous cells 4-9 layers, subpolygonal or irregular polygonal, present at outer parts of cortex near ridges. Cortex narrow. Pericycle fibre bundles beneath the cortex, arranged in an interrupted ring, abundant near ridges. Phloem relatively narrow. Xylem radially arranged, relatively broad near ridges. Pith large, parenchymatous cells large, usually hollow in the centre. The depression between the two edges is evident [Fig. 8 (i)].

Leaf: Remnants of non-glandular hair frequently observed on the surface of epidermis. Upper epidermis consists of 1 layer of flat rectangular cells. Palisade tissue consists of 2-3 layers of cells. Spongy tissue consists of several layers of loosely arranged irregular cells. Collenchyma under the upper epidermis of midrib inexistent or indistinct. Phloem fibres surrounded, arranged in an interrupted ring. Vascular bundles of midrib collateral; vessels usually several in rows; phloem cells polygonal. Lower epidermis cells relatively small [Fig. 8 (ii)].

Powder

Colour yellowish-brown. Non-glandular hairs composed of 1-7 cells, relatively slender, base 9-29 μm in diameter. Glandular scales numerous; oblate-spherical, yellowish-brown content rare or absent, 4 cells, 15-39 μm in diameter. Glandular hairs small; head mainly unicellular, subglobose, 10-34 μm in diameter; stalk of 1-2 cells. Upper and lower epidermal cells irregular in shape, anticlinal walls slightly sinuate and thick, stomata occasionally observed, diacytic or anomocytic. Vessels numerous, mainly spiral and reticulate vessels, 5-68 μm in diameter. Fine raphides of calcium oxalate sometimes observed, 2-16 μm long; polychromatic under the polarized microscope. No brownish-red dots observed (Fig. 9).

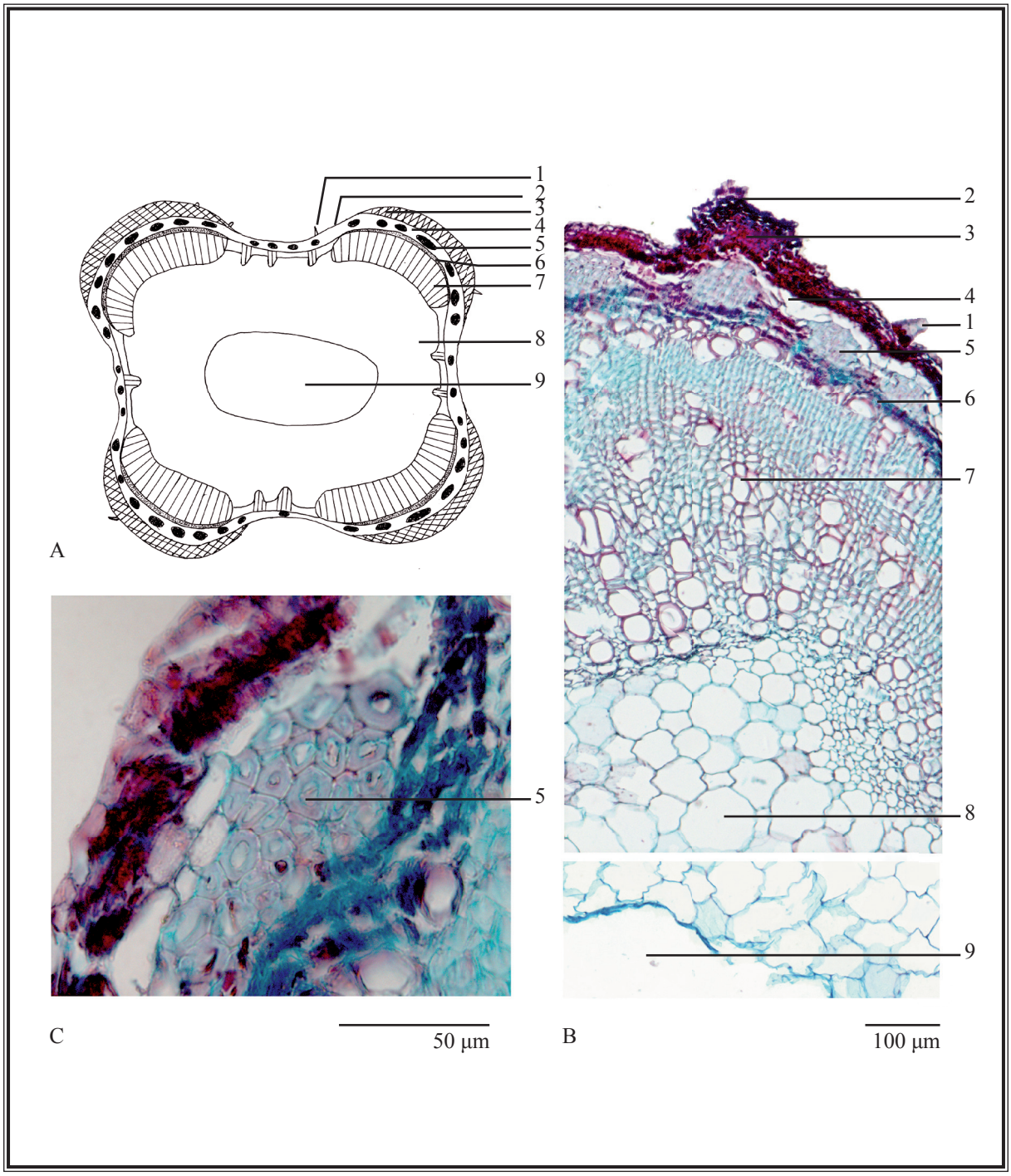

Figure 8 (i) Microscopic features of transverse section of stem of *Isodon serra* (Maxim.) Kudo

A. Sketch B. Section illustration C. Section magnified

1. Non-glandular hair 2. Epidermis 3. Collenchyma 4. Cortex
5. Pericycle fibre bundle 6. Phloem 7. Xylem 8. Pith 9. Hollow cavity

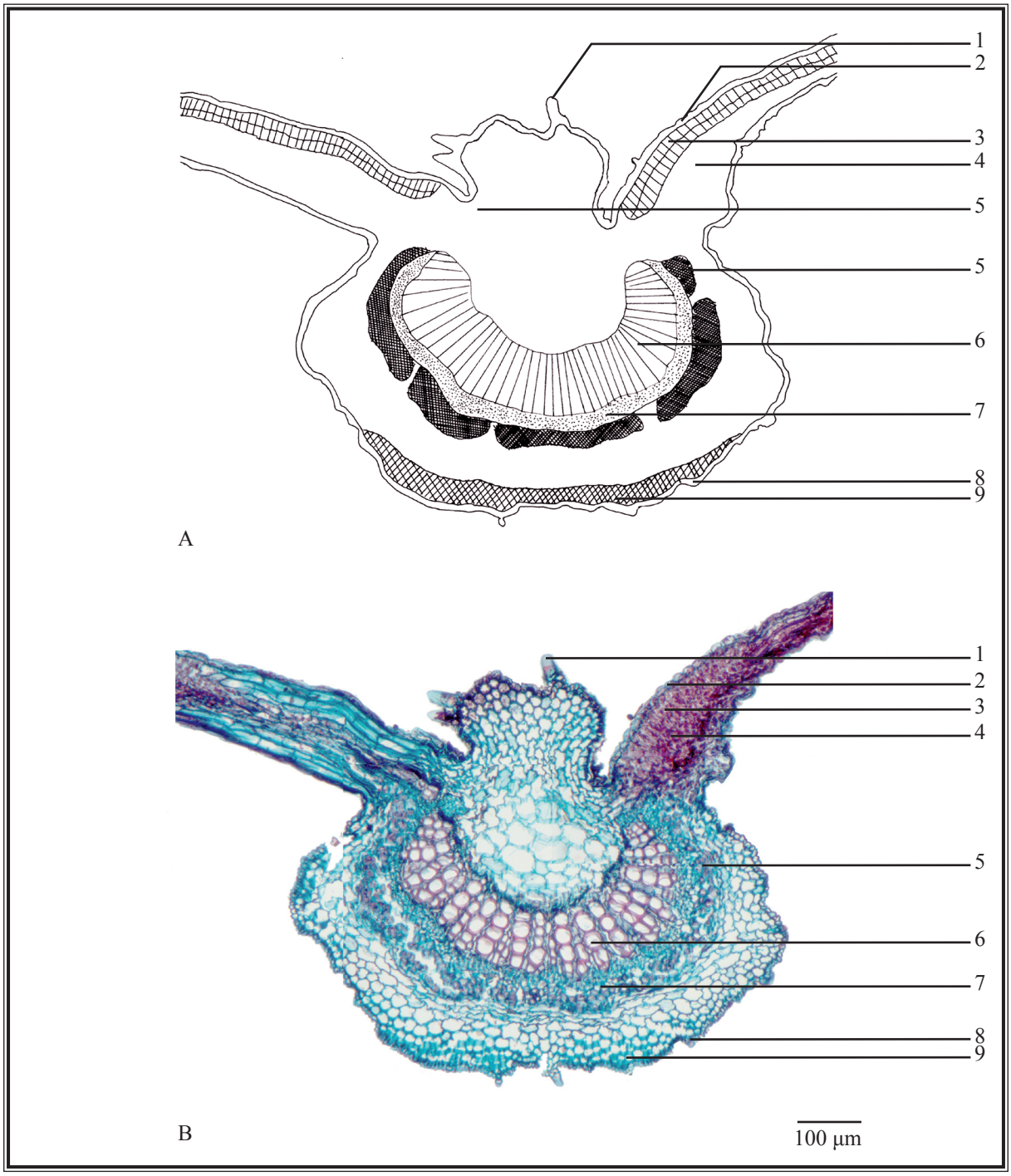

Figure 8 (ii) Microscopic features of transverse section of leaf of *Isodon serra* (Maxim.) Kudo

A. Sketch B. Section illustration

1. Non-glandular hair 2. Upper epidermis 3. Palisade tissue 4. Spongy tissue
5. Phloem fibre 6. Xylem 7. Phloem 8. Lower epidermis 9. Collenchyma

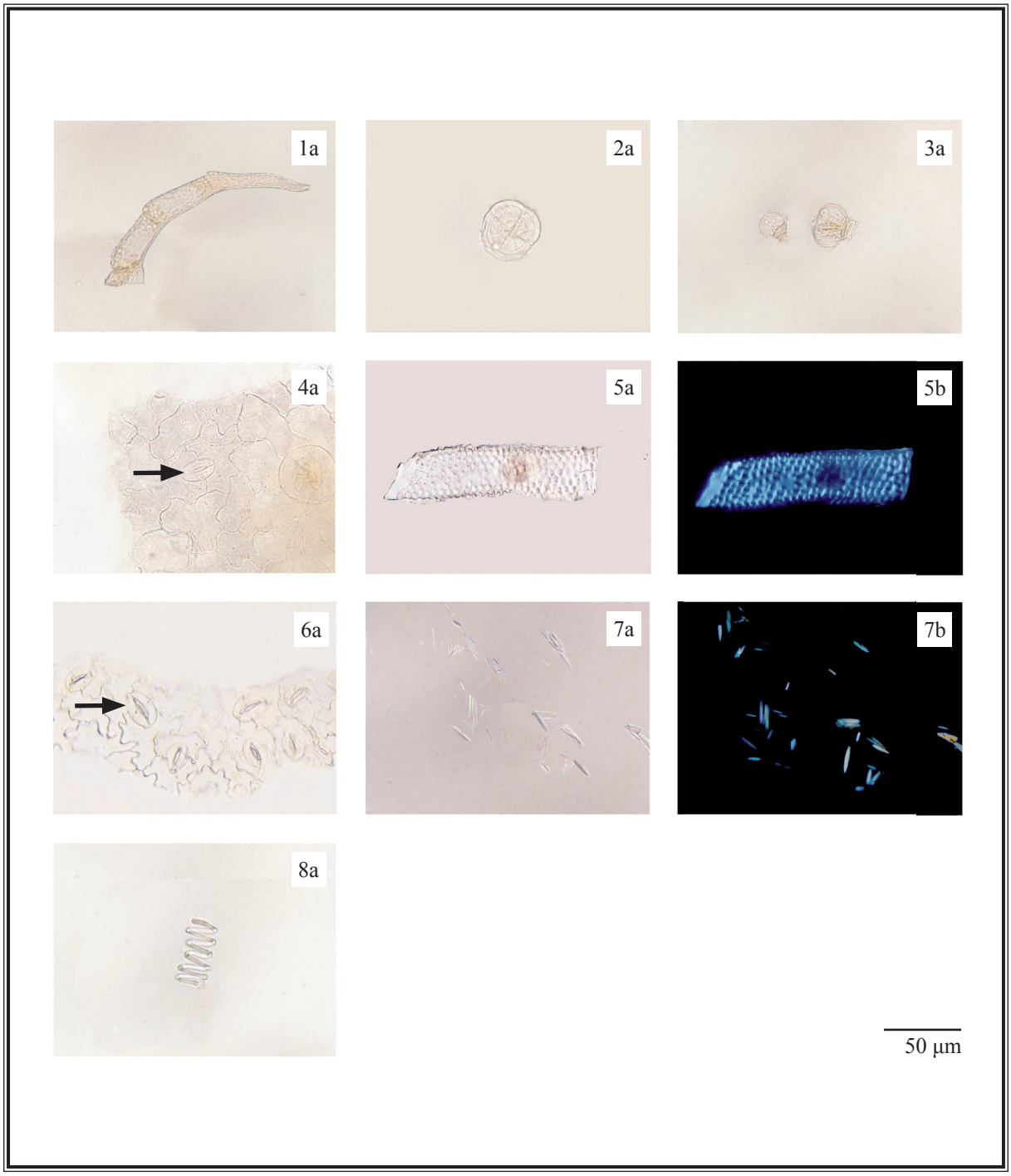

Figure 9 Microscopic features of powder of *Isodon serra* (Maxim.) Kudo

1. Non-glandular hair 2. Glandular scale 3. Glandular hairs
4. Upper epidermal cells with stomata (⟶) 5. Reticulate vessel
6. Lower epidermal cells with stomata (⟶) 7. Raphides of calcium oxalate 8. Spiral vessel

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

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

Standard solution

Nodosin standard solution

Weigh 1.0 mg of nodosin CRS (Fig. 10) and dissolve in 2 mL of methanol (70%).

Developing solvent system

Prepare a mixture of dichloromethane, ethyl acetate and formic acid (9:1:0.5, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol (70%). Sonicate (150 W) the mixture for 30 min. Filter the mixture.

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 nodosin standard solution (2 μL) and the test solution (20 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 and heat at about 105ºC (about 3 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

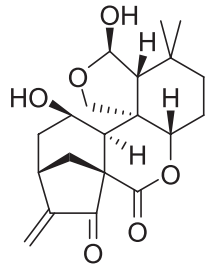

Figure 10 Chemical structure of nodosin

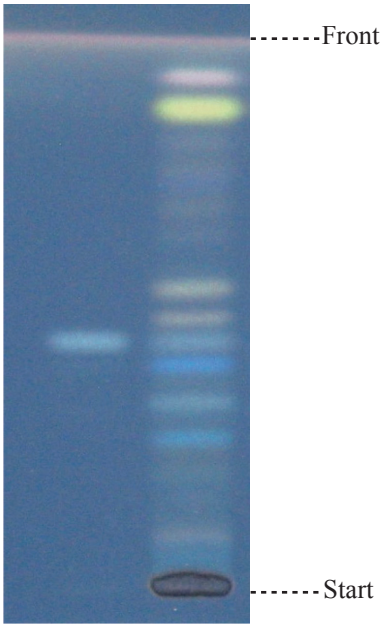

Figure 11 A reference HPTLC chromatogram of dried aerial part of *Isodon serra* (Maxim.) Kudo extract observed under UV light (366 nm) after staining

1. Nodosin 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 nodosin (Fig. 11).

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

Standard solution

Nodosin standard solution for fingerprinting, Std-FP (10 mg/L)

Weigh 0.1 mg of nodosin CRS and dissolve in 10 mL of methanol (70%).

Test solution

Weigh 0.4 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (70%). Sonicate (300 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction one more time. Combine the supernatants and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (240 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). 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% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	85 → 70	15 → 30	linear gradient
30 – 40	70 → 60	30 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of nodosin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of nodosin should not be more than 5.0%; the RSD of the retention time of nodosin peak should not be more than 2.0%; the column efficiency determined from nodosin peak should not be less than 60000 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. 12).

Procedure

Separately inject nodosin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of nodosin peak in the chromatogram of nodosin Std-FP and the retention times of the three characteristic peaks (Fig. 12) in the chromatogram of the test solution. Identify nodosin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of nodosin Std-FP. The retention times of nodosin 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 aerial part of *Isodon serra* (Maxim.) Kudo extract are listed in Table 5.

Table 5 The RRTs and acceptable ranges of the three characteristic peaks of dried aerial part of *Isodon serra* (Maxim.) Kudo extract

Peak No.	RRT	Acceptable Range
1	0.60	± 0.03
2	0.71	± 0.03
3 (marker, nodosin)	1.00	-

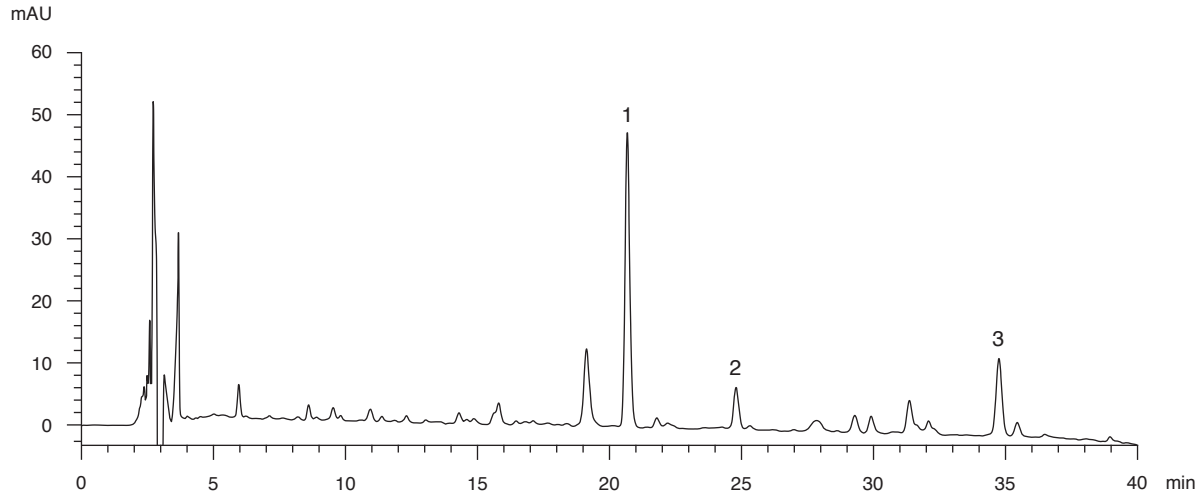

Figure 12 A reference fingerprint chromatogram of dried aerial part of *Isodon serra* (Maxim.) Kudo extract

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. 12).

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 4.5%.
Acid-insoluble ash: not more than 0.5%.

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 5.0%.
Ethanol-soluble extractives (cold extraction method): not less than 4.0%.

7. ASSAY

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

Standard solution

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

Weigh accurately 1.0 mg of nodosin CRS and dissolve in 10 mL of methanol (70%).

Nodosin standard solution for assay, Std-AS

Measure accurately the volume of the nodosin Std-Stock, dilute with methanol (70%) to produce a series of solutions of 1, 2, 5, 10, 50 mg/L for nodosin.

Test solution

Weigh accurately 0.4 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (70%). Sonicate (300 W) the mixture for 30 min. Centrifuge at about 3000 × *g* for 5 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction one more time. Combine the supernatants and make up to the mark with methanol (70%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (240 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 6) –

Table 6 Chromatographic system conditions

Time (min)	0.1% Formic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 30	85 → 70	15 → 30	linear gradient
30 – 40	70 → 60	30 → 40	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of nodosin Std-AS (5 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of nodosin should not be more than 5.0%; the RSD of the retention time of nodosin peak should not be more than 2.0%; the column efficiency determined from nodosin peak should not be less than 60000 theoretical plates.

The *R* value between nodosin peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 13).

Calibration curve

Inject a series of nodosin Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of nodosin against the corresponding concentrations of nodosin 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 nodosin peak (Fig. 13) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of nodosin Std-AS. The retention times of nodosin 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 nodosin in the test solution, and calculate the percentage content of nodosin in the sample by using the equations as indicated in Appendix IV(B).

Limits

The dried aerial part of *Isodon serra* (Maxim.) Kudo contains not less than 0.027% of nodosin ($C_{20}H_{26}O_6$), calculated with reference to the dried substance.

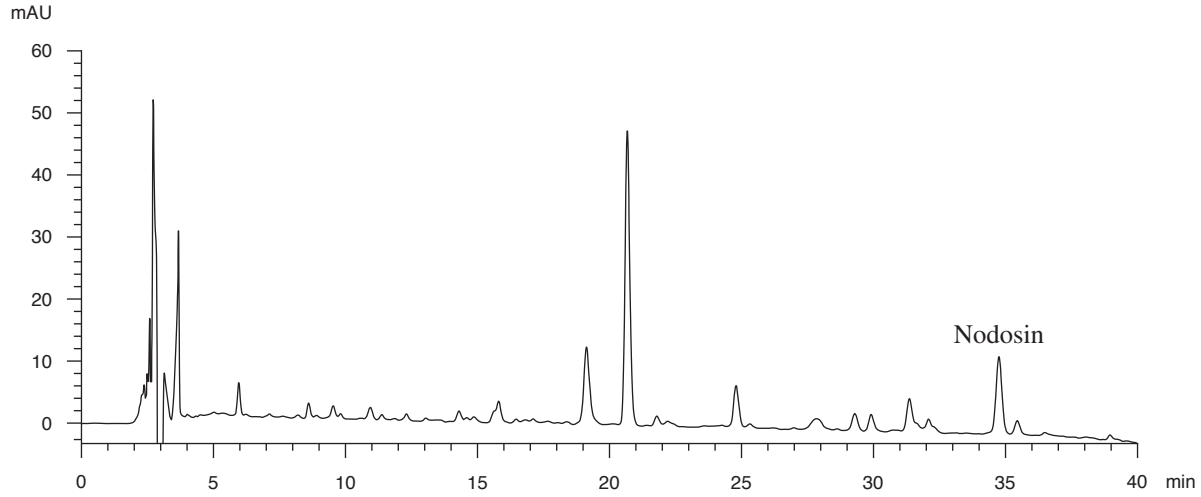

Figure 13 A reference assay chromatogram of dried aerial part of *Isodon serra* (Maxim.) Kudo extract