Glechomae Herba

Figure 1 A photograph of Glechomae Herba

A. Glechomae Herba B. A single herb of Glechomae Herba
C. Magnified image of upper surface of reniform leaf
D. Magnified image of lower surface of reniform leaf
E. Magnified image of upper surface of subcordate leaf
F. Magnified image of lower surface of subcordate leaf
G. Magnified image of fragment of stem
H. Magnified image of cut surface of stem
I. Stem node with adventitious roots

1. NAMES

Official Name: Glechomae Herba

Chinese Name: 連錢草

Chinese Phonetic Name: Lianqiancao

2. SOURCE

Glechomae Herba is the dried aerial part of *Glechoma longituba* (Nakai) Kupr. (Lamiaceae). The aerial part is collected from spring to autumn, foreign matter removed, then dried under the sun to obtain Glechomae Herba.

3. DESCRIPTION

Herb 4-60 cm long, sparsely pubescent. Stems square-columnar, slender and twisted, 0.9-2.3 mm in diameter; externally yellowish-green to greenish-brown, with adventitious roots at the nodes; texture fragile, easily broken, fracture usually hollow in the centre. Leaves simple, opposite, mostly crumpled, reniform to subcordate, when whole and flattened, 1.5-5.0 cm long, 1.0-3.5 cm wide, greenish-brown to yellowish-green, margin crenate, both upper and lower surfaces sparsely pubescent; petiole slender, 2.5-11 cm long. Odour aromatic on rubbing; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Stem: Non-glandular and glandular hairs found on the surface of epidermis; stomata, remnants of glandular and non-glandular hairs occasionally visible. Non-glandular hairs mostly broken, conical-shaped, cell wall relatively thick. Glandular hairs small, head unicellular, subrounded to oblong, with yellow content; stalk unicellular and short. Epidermis consists of 1 layer of subrectangular to subsquare cells, outer walls serratedly thickened. Collenchyma well developed at four angles of stem. Cortex consists of 3-9 layers of parenchymatous cells, cells subrounded, unequal in size. Endodermis distinct, consisting of 1 layer of tangentially-elongated parenchymatous cells. Pericycle fibre crescent-shaped and in bundles, arranged in an interrupted ring. Phloem located inside the pericycle fibres, consisting of 1-2 layers of flattened parenchymatous cells. Cambium distinct, arranged in an

interrupted ring. Xylem consists of 3-7 vessels, radially arranged, broader along four angles; vessels and xylem fibres alternatively arranged. Pith broad and distinct, usually hollow in the centre [Fig. 2 (i)].

Leaf: Non-glandular hairs found on the surface of both the upper and lower epidermis, bicellular, conical-shaped, cell wall relatively thick. Upper epidermis consists of 1 layer of cells, cells subrectangular to subsquare, covered with cuticle; glandular hairs, glandular scales and stomata occasionally visible. Mesophyll consists of 2-3 layers of subrounded parenchymatous cells, palisade tissue sometimes obscurely visible. Collenchyma consists of several layers of cells located on the upper and lower parts of the midrib. Xylem occupies about 2/3 of the vascular bundle; vessels orderly arranged. Phloem hoof-shaped. Lower epidermis consists of 1 layer of cells, cells subrectangular to subsquare, covered with cuticle; glandular hairs, glandular scales and stomata occasionally visible [Fig. 2 (ii)].

Powder

Colour greyish-green. Non-glandular hairs 3 types: the first type multicellular, the cell wall slightly thickened, some with warty protuberances; the second type multicellular, with 1 to several cells frequently shrunken; the third type unicellular and long-conical, a few contains brownish-yellow contents in cell cavity. Head of glandular hair unicellular, subrounded to elliptic, 12-30 μm in diameter; stalk unicellular. The anticlinal walls of the upper epidermal cells of leaf sinuous, with relatively fine and dense parallel cuticular striations; stomata sometimes observed. The cell walls of the lower epidermal cells of the leaf sinuous, stomata frequently observed, oblong to subrounded in shape, diacytic. Glandular scale subspherical or oblate, with a head of 8 cells, arranged radially; stalk very short, some contain brownish-yellow contents. Vessels mostly spiral and bordered-pitted, 10-39 μm in diameter (Fig. 3).

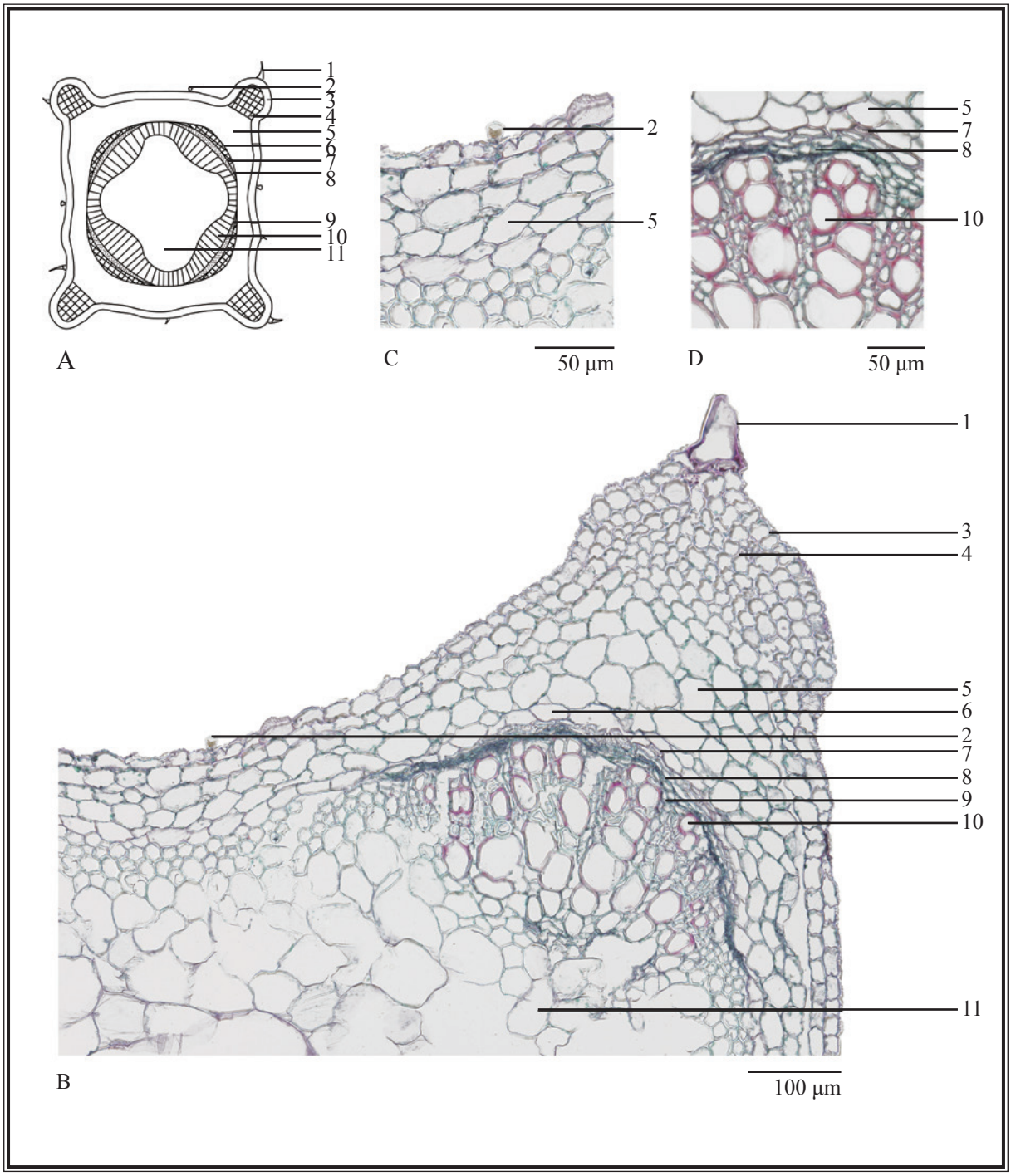


Figure 2 (i) Microscopic features of transverse section of stem of Glechomae Herba

A. Sketch B. Section illustration C-D. Section magnified

1. Non-glandular hair 2. Glandular hair 3. Epidermis 4. Collenchyma
5. Cortex 6. Endodermis 7. Pericycle fibre 8. Phloem 9. Cambium
10. Xylem 11. Pith

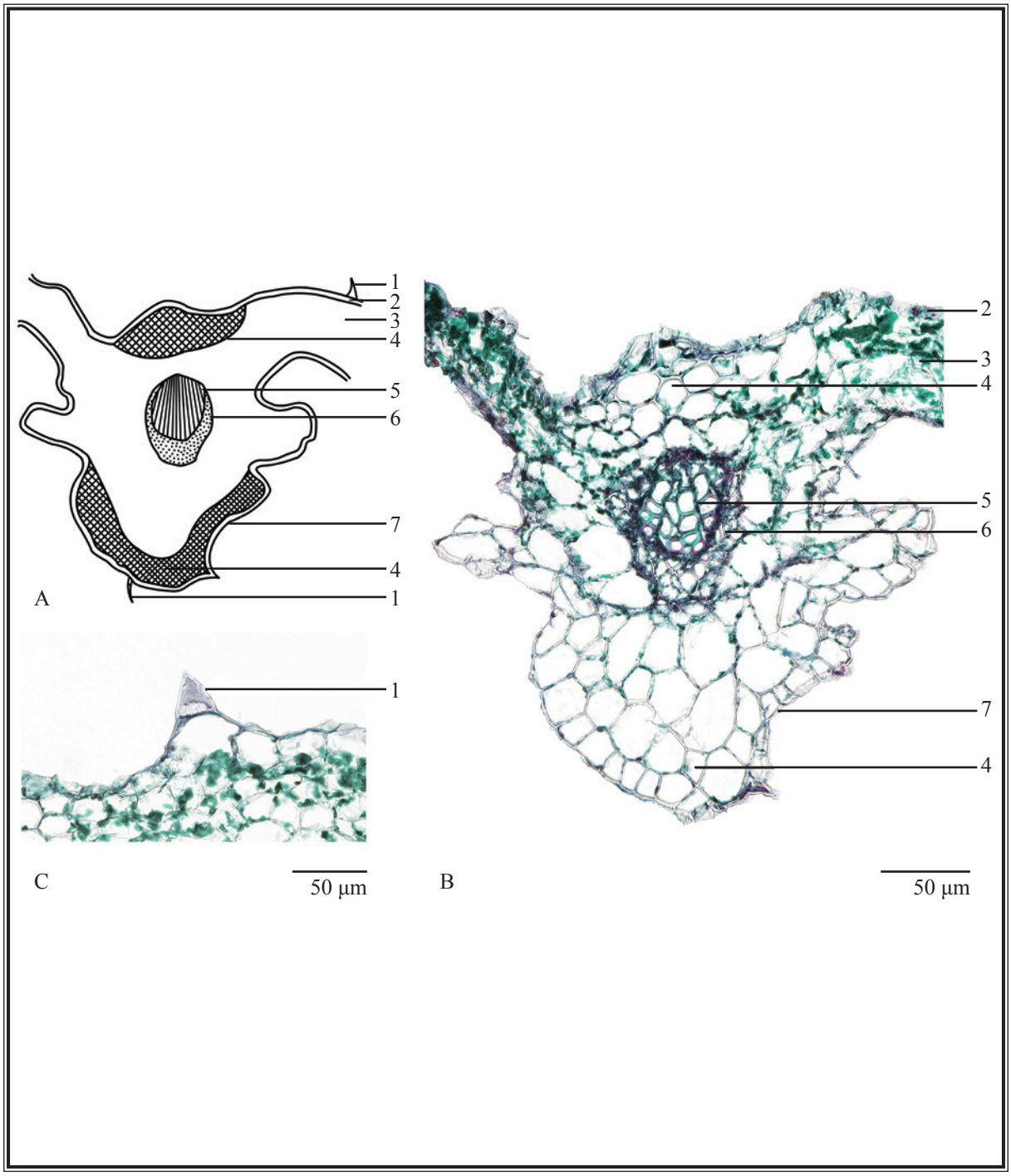


Figure 2 (ii) Microscopic features of transverse section of leaf of Glechomae Herba

A. Sketch B. Section illustration C. Section magnified

1. Non-glandular hair 2. Upper epidermis 3. Mesophyll 4. Collenchyma
5. Xylem 6. Phloem 7. Lower epidermis

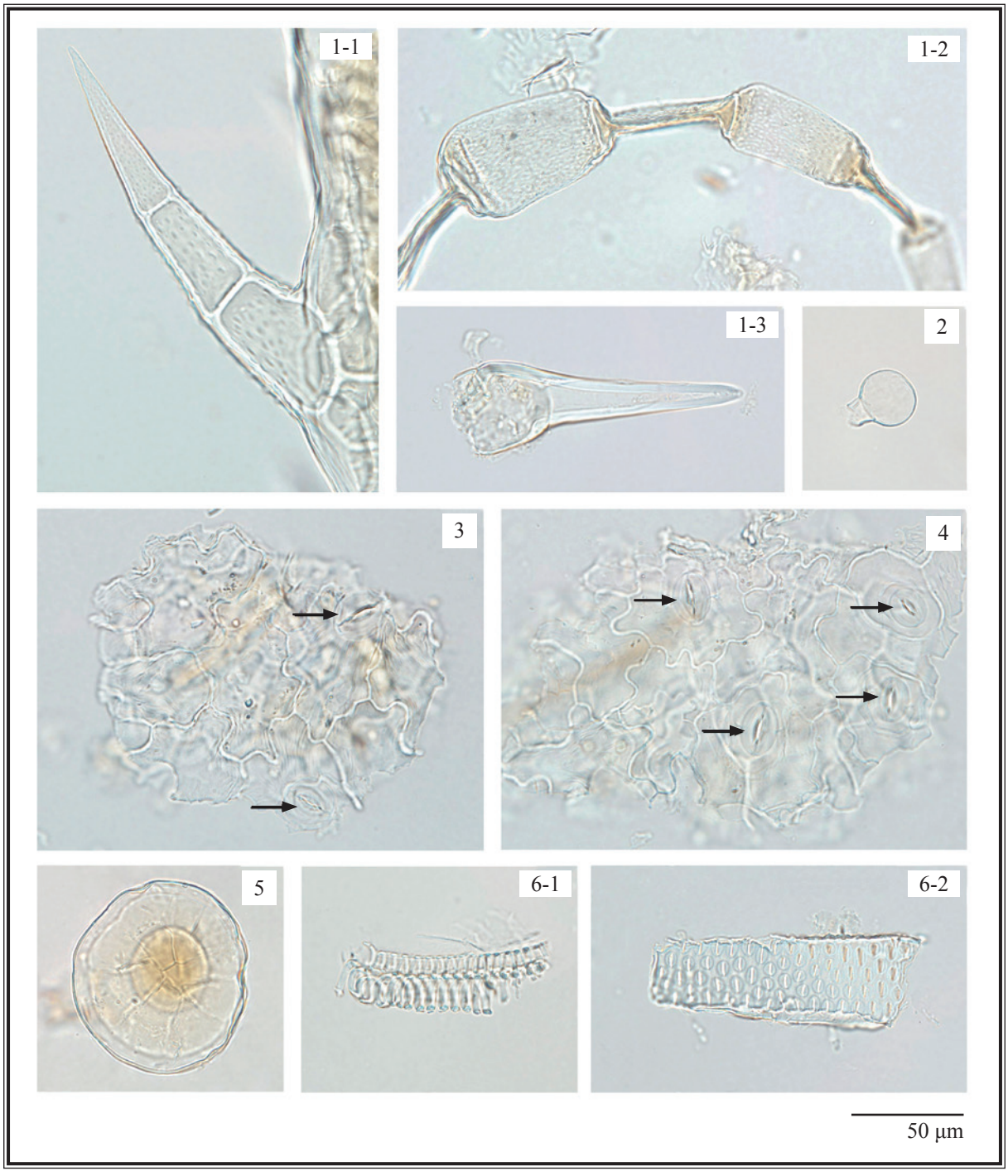


Figure 3 Microscopic features of powder of Glechomae Herba (under light microscope)

1. Non-glandular hairs (1-1 multicellular, 1-2 with shrunken cells, 1-3 unicellular)
2. Glandular hair 3. Upper epidermal cells of leaf with stomata (→)
4. Lower epidermal cells of leaf with stomata (→) 5. Glandular scale
6. Vessels (6-1 spiral vessels, 6-2 bordered-pitted vessel)

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

Standard solutions

Caffeic acid standard solution

Weigh 1.0 mg of caffeic acid CRS (Fig. 4) and dissolve in 10 mL of methanol.

Rosmarinic acid standard solution

Weigh 1.0 mg of rosmarinic acid CRS (Fig. 4) and dissolve in 5 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, *n*-hexane and formic acid (10:5:1, v/v).

Spray reagent

Weigh 1 g of aluminium trichloride and dissolve in 100 mL of ethanol.

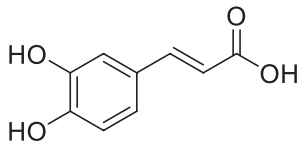
Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 30 mL of methanol. Sonicate (220 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 methanol.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate and a freshly prepared developing solvent system as described above. Apply separately caffeic acid standard solution (1 µL), rosmarinic acid standard solution (3 µL) and the test solution (1 µL) to the plate. 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 5 min). Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

(i)



(ii)

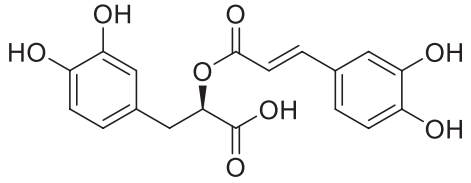


Figure 4 Chemical structures of (i) caffeic acid and (ii) rosmarinic acid

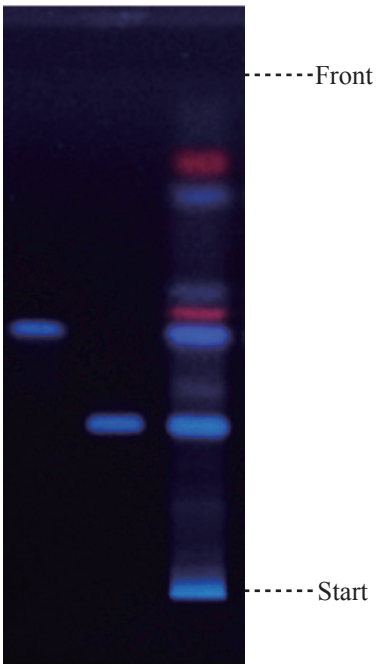


Figure 5 A reference HPTLC chromatogram of Glechomae Herba extract observed under UV light (366 nm) after staining

1. Caffeic acid standard solution 2. Rosmarinic acid 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 caffeic acid and rosmarinic acid (Fig. 5).

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

Standard solutions

Caffeic acid standard solution for fingerprinting, Std-FP (5 mg/L)

Weigh 0.5 mg of caffeic acid CRS and dissolve in 100 mL of methanol (70%).

Rosmarinic acid standard solution for fingerprinting, Std-FP (25 mg/L)

Weigh 0.5 mg of rosmarinic acid CRS and dissolve in 20 mL of methanol (70%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 80 mL of methanol (70%). Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL volumetric flask. Wash the residue for four times each with 5 mL of methanol (70%). Combine the solutions 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 (330 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle 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 1) –

Table 1 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 70	80 → 50	20 → 50	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of caffeic acid Std-FP and rosmarinic acid Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of caffeic acid and rosmarinic acid should not be more than 5.0%; the RSD of the retention times of caffeic acid and rosmarinic acid peaks should not be more than 2.0%; the column efficiencies determined from caffeic acid and rosmarinic acid peaks should not be less than 17000 and 80000 theoretical plates respectively.

The *R* value between peak 3 and the closest peak; and the *R* value between peak 6 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject caffeic acid Std-FP, rosmarinic acid Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of caffeic acid and rosmarinic acid peaks in the chromatograms of caffeic acid Std-FP, rosmarinic acid Std-FP and the retention times of the six characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify caffeic acid and rosmarinic acid peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of caffeic acid Std-FP and rosmarinic acid Std-FP. The retention times of caffeic acid and rosmarinic acid 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 six characteristic peaks of Glechomae Herba extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Glechomae Herba extract

Peak No.	RRT	Acceptable Range
1	0.31	± 0.03
2 (chlorogenic acid)	0.33	± 0.03
3 (caffeic acid)	0.41	± 0.03
4	0.73	± 0.04
5	0.86	± 0.03
6 (marker, rosmarinic acid)	1.00	-

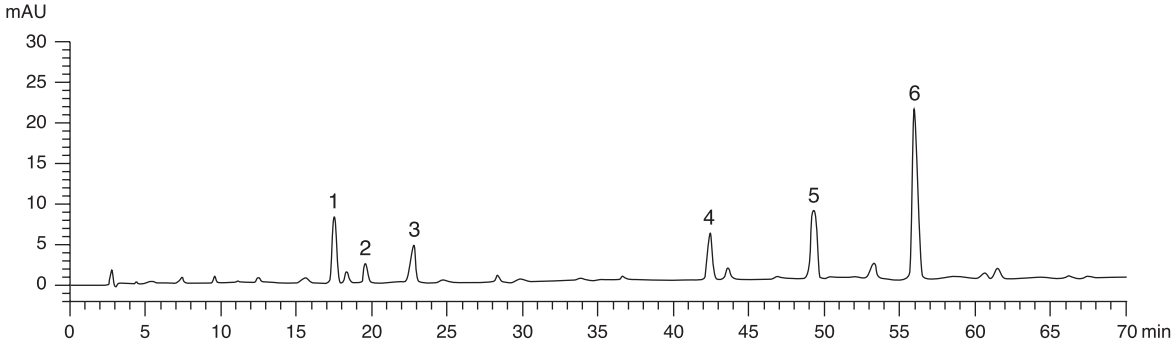


Figure 6 A reference fingerprint chromatogram of Glechomae Herba extract

For positive identification, the sample must give the above six 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 – Aflatoxins *(Appendix VII)*: meet the requirements.

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

5.5 Foreign Matter *(Appendix VIII)*: not more than 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 11.5%.
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 (cold extraction method): not less than 22.0%.
Ethanol-soluble extractives (cold extraction method): not less than 12.0%.

7. ASSAY

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

Standard solution

Mixed caffeic acid and rosmarinic acid standard stock solution, Std-Stock (200 mg/L each)

Weigh accurately 1.0 mg of caffeic acid CRS and 1.0 mg of rosmarinic acid CRS, and dissolve in 5 mL of methanol (70%).

Mixed caffeic acid and rosmarinic acid standard solution for assay, Std-AS

Measure accurately the volume of the mixed caffeic acid and rosmarinic acid Std-Stock, dilute with methanol (70%) to produce a series of solutions of 0.1, 0.2, 0.5, 1, 5 mg/L for caffeic acid and 0.25, 0.5, 1, 2.5, 25 mg/L for rosmarinic acid.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 80 mL of methanol (70%). Reflux the mixture for 1 h. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL volumetric flask. Wash the residue for four times each with 5 mL of methanol (70%). Combine the solutions 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 (330 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle 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 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Phosphoric acid (%, v/v)	Methanol (%, v/v)	Elution
0 – 70	80 → 50	20 → 50	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed caffeic acid and rosmarinic acid Std-AS (0.5 mg/L for caffeic acid and 1 mg/L for rosmarinic acid). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of caffeic acid and rosmarinic acid should not be more than 5.0%; the RSD of the retention times of caffeic acid and rosmarinic acid peaks should not be more than 2.0%; the column efficiencies determined from caffeic acid and rosmarinic acid peaks should not be less than 17000 and 80000 theoretical plates respectively.

The *R* value between caffeic acid peak and the closest peak; and 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 curves

Inject a series of the mixed caffeic acid and rosmarinic acid Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of caffeic acid and rosmarinic acid against the corresponding concentrations of the mixed caffeic acid and rosmarinic acid Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify caffeic acid and rosmarinic acid peaks (Fig. 7) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed caffeic acid and rosmarinic acid Std-AS. The retention times of caffeic acid and rosmarinic acid peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of caffeic acid and rosmarinic acid in the test solution, and calculate the percentage contents of caffeic acid and rosmarinic acid in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.049% of the total content of caffeic acid ($C_9H_8O_4$) and rosmarinic acid ($C_{18}H_{16}O_8$), calculated with reference to the dried substance.

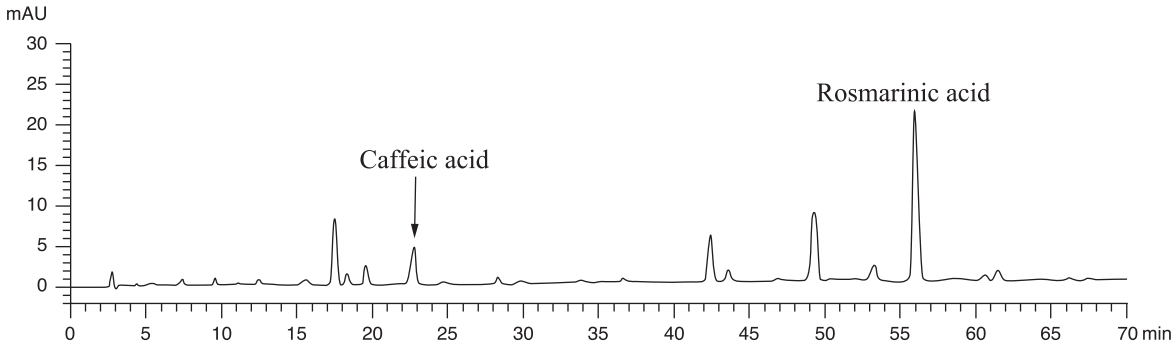


Figure 7 A reference assay chromatogram of Glechomae Herba extract