Houttuyniae Herba

Figure 1 (i) A photograph of Houttuyniae Herba

Figure 1 (ii) A photograph of spikes and leaves

1. NAMES

Official Name: Houttuyniae Herba

Chinese Name: 魚腥草

Chinese Phonetic Name: Yuxingcao

2. SOURCE

Houttuyniae Herba is the dried aerial part of *Houttuynia cordata* Thunb. (Saururaceae). The aerial part of herb is collected in summer when leaves and spikes flourish, foreign matter removed, then the aerial part is dried under the sun to obtain Houttuyniae Herba.

3. DESCRIPTION

Stems flattish-cylindrical, somewhat twisted, 15-32 cm long, 1.5-4.5 mm in diameter; externally brownish-yellow to brown, with several longitudinal ridges; nodes prominent, with remnants of rootlets on nodes on the lower part; texture fragile. Leaves simple, alternate, blade curled and shrivelled, cordate when flattened, 2.5-8 cm long, 2-6.5 cm wide, apex acuminate, margins entire; the upper surface dark yellowish-green to dark brown, the lower surface greyish-green to greyish-brown; petioles 1-3.5 cm long, bearing stipule at the base, forming a sheath. Spikes terminal, yellowish-brown. Odour fishy when rubbed; taste slightly astringent [Fig. 1 (i)-(ii)].

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Leaf: Upper and lower epidermis consist of 1 layer of cells, with non-glandular hairs on the veins. The upper epidermal cells tangentially elongated, with scattered oil cells. Beneath the epidermis, 1 layer of subsquare, relatively large parenchymatous cells. Mesophyll consists of 1 layer of palisade tissue and several layers of spongy tissues, with small clusters of calcium oxalate and starch granules, usually scattered. Vascular bundle of midrib collateral. Collenchyma arranged on the inner side of lower epidermis of midrib (Fig. 2).

Stem: Epidermal cells subsquare, arranged in 1 layer, sometimes with oil cells scattered; beneath the epidermis, 1 layer of subsquare, relatively large parenchymatous cells. Pericycle fibres arranged in a complete or interrupted ring. Collateral vascular bundles arranged in a circle. Pith large, with scattered small clusters of calcium oxalate (Fig. 2).

Powder

Colour greyish-green to brown. Oil cells subrounded or elliptical, 33-104 μm in diameter, sometimes containing yellow oil droplets. Glandular hairs, each with a 2-5 celled head containing pale brown contents, devoid of stalk, 34-168 μm, sometimes up to 214 μm long, 9-24 μm in diameter; the top cell usually shrivelled. Non-glandular hairs, each consists of 1-11 cells, 48-318 μm, sometimes up to 476 μm long, 19-94 μm in diameter at base, with linear striations on the surface. Clusters of calcium oxalate 2-25 μm in diameter, the larger ones up to 57 μm in diameter; prisms of calcium oxalate occasionally present; orange-yellow or polychromatic under the polarized microscope. Epidermal cells of leaf polygonal, with wavy striations on the surface; stomata anomocytic, with 3-4 subsidiary cells. Fibres slender, 4-37 μm in diameter. Spiral, scalariform, reticulate, and bordered-pitted vessels frequently visible. Starch granules scattered, simple granules ovoid, ellipsoid or irregular in shape, 2-29 μm, sometimes up to 39 μm in diameter; hilum pointed, cleft-like, V-shaped or stellate, striations sometimes distinct; black and cruciate in shape under the polarized microscope; compound granules rare (Fig. 3).

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

Standard solution

Quercitrin standard solution

Weigh 0.9 mg of quercitrin CRS (Fig. 4) and dissolve in 2 mL of methanol (70%).

Developing solvent system

Prepare a mixture of ethyl acetate, butan-2-one, formic acid and water (24:3.6:1.5:0.9, v/v).

Spray reagent

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

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of methanol (70%). Sonicate (140 W) the mixture for 30 min. Centrifuge at about 2500 × *g* for 10 min. Filter the supernatant.

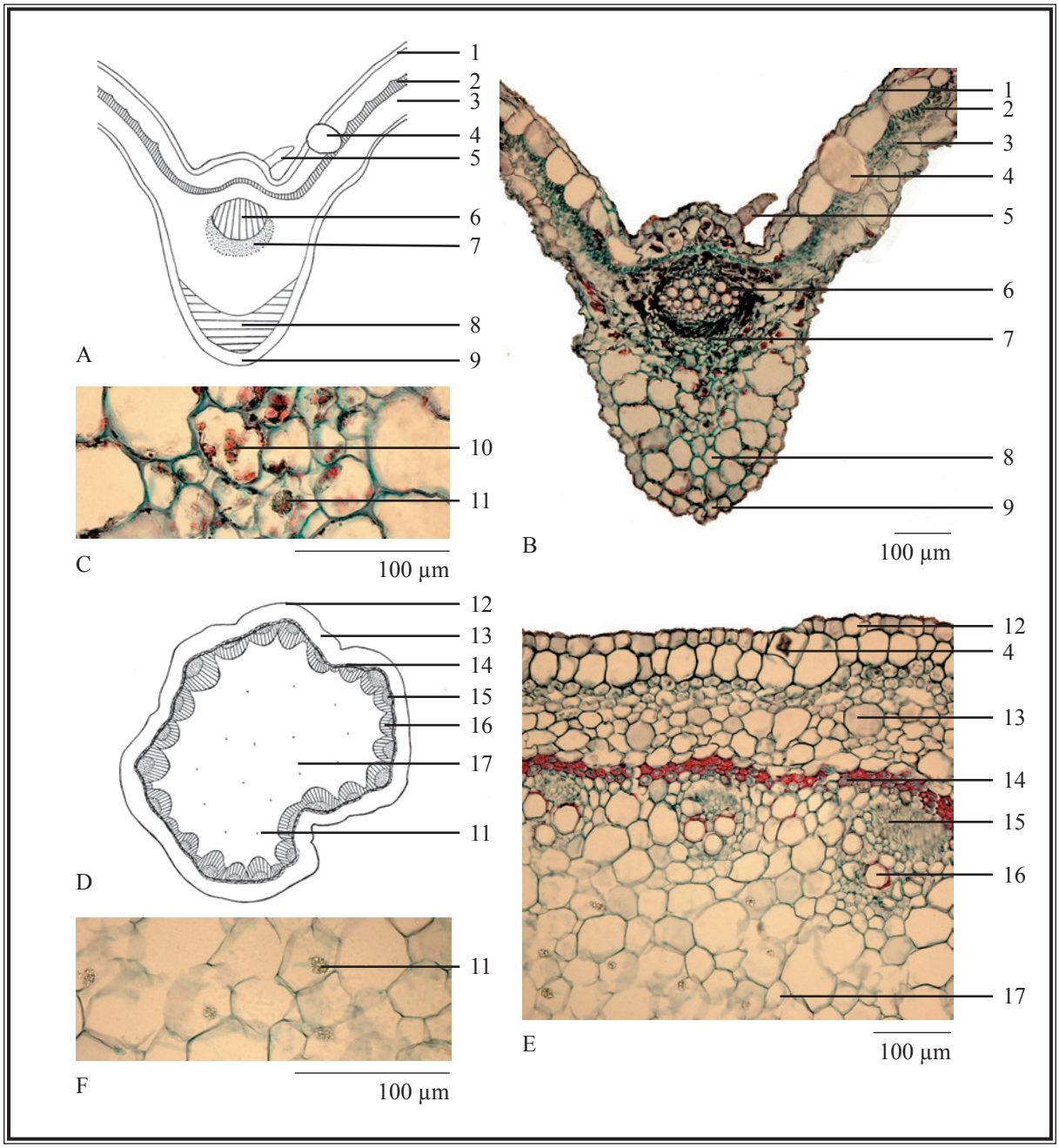


Figure 2 Microscopic features of transverse section of leaf and stem of Houttuyniae Herba

A. Sketch of leaf midvein B. Section illustration of leaf midvein
C. Starch granules and clusters of calcium oxalate scattered in mesophyll
D. Sketch of stem E. Section illustration of stem F. Clusters of calcium oxalate scattered in pith

1. Upper epidermis 2. Palisade tissue 3. Spongy tissue 4. Oil cell 5. Non-glandular hair 6. Xylem
7. Phloem 8. Collenchyma 9. Lower epidermis 10. Starch granules 11. Cluster of calcium oxalate
12. Epidermis 13. Cortex 14. Pericycle fibres 15. Phloem 16. Xylem 17. Pith

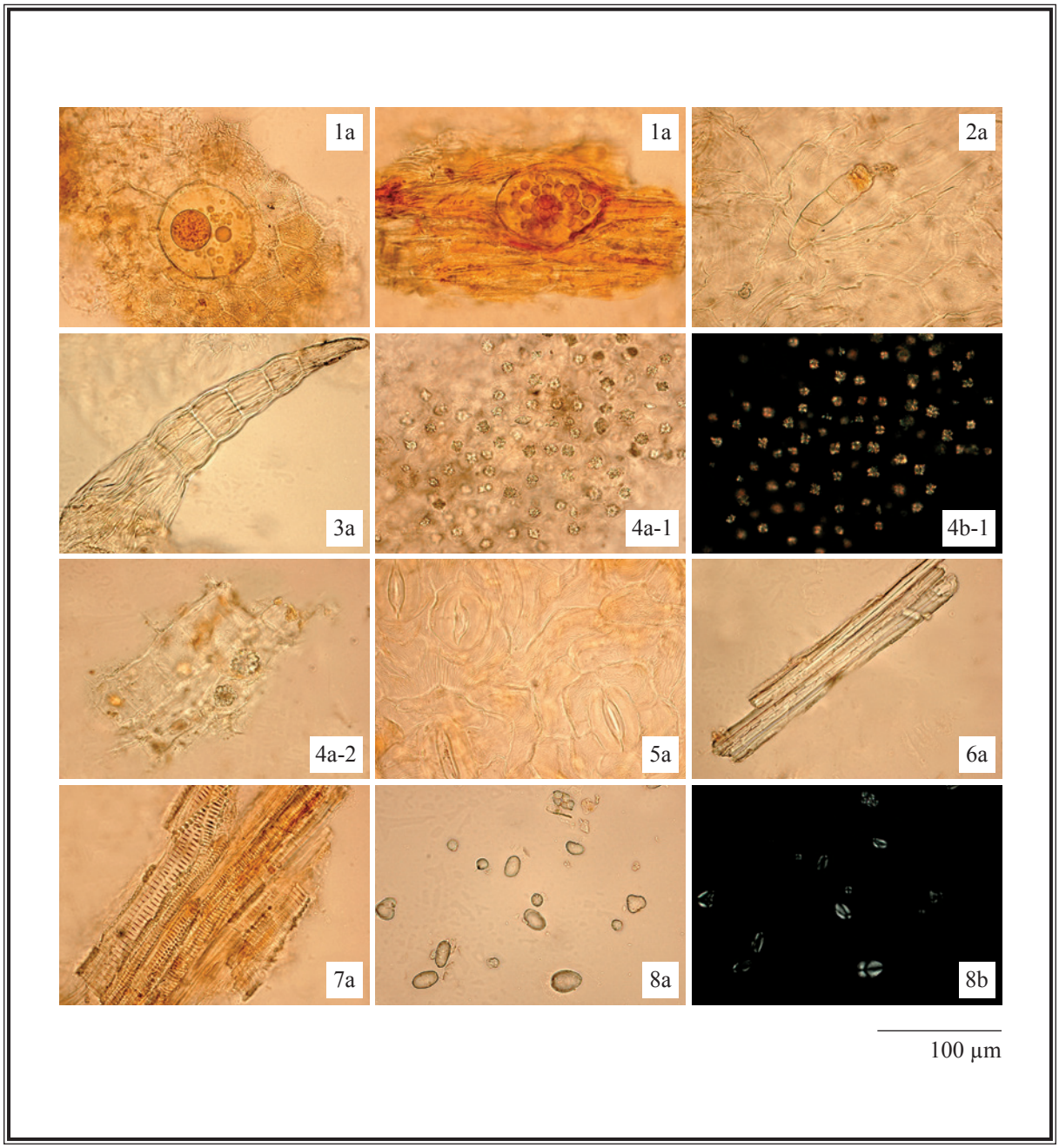


Figure 3 Microscopic features of powder of Houttuyniae Herba

1. Oil cell 2. Glandular hair 3. Non-glandular hair
4. Clusters of calcium oxalate (4-1 small ones, 4-2 large ones) 5. Epidermal cells of leaf
6. Fibres 7. Vessels 8. Starch granules

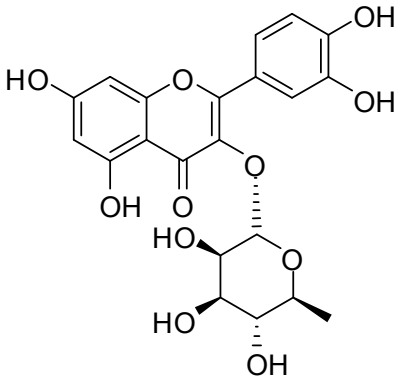
a. Features under the light microscope b. Features under the polarized microscope

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 quercitrin standard solution (1.8 µL) and the test solution (9 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 80°C until the spots or bands become visible (about 5-10 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

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 quercitrin.

(i)



(ii)

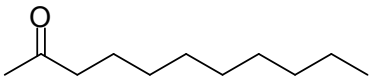


Figure 4 Chemical structures of (i) quercitrin and (ii) methylnonylketone

4.3 Chromatographic Fingerprinting *(Appendix XII)*

(I) Gas chromatography with flame ionization detector (GC/FID)

Standard solution

Methylnonylketone standard solution for fingerprinting, Std-FP (160 mg/L)

Weigh 1.6 mg of methylnonylketone CRS (Fig. 4) and dissolve in 10 mL of ethyl acetate.

Test solution

Prepare 20.0 g of test sample by cutting the sample into pieces of less than 3 mm in length and place it in a 1000-mL round-bottomed flask, then add 500 mL of water and a few glass

beads, shake and mix well. Connect the round-bottomed flask to a volatile oil determination tube and then connect the volatile oil determination tube to a reflux condenser. Add 3 mL of water and then 2 mL of ethyl acetate through the top of reflux condenser until the graduated tube of volatile oil determination tube is filled. Heat the flask by reflux for 3 h. Allow to stand for a while. Open the stopcork at the lower part of volatile oil determination tube and run off the water layer slowly until the ethyl acetate layer is 5 mm above the zero mark. Transfer the ethyl acetate layer to a 10-mL centrifuge tube, then add 0.5 g of anhydrous sodium sulphate. Centrifuge at about 4000 × *g* for 5 min. Transfer 1 mL of the supernatant to a 10-mL volumetric flask. Make up to the mark with ethyl acetate. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The gas chromatograph is equipped with a FID and a capillary column (DB-5, 0.25 mm × 30 m) of which the internal wall is covered with (5%-phenyl)-methylpolysiloxane in a layer about 0.25 μm thick. The injection temperature is at 250°C. The detector temperature is at 300°C. The splitless injection mode is used. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Temperature (°C)	Rate (°C /min)
0 – 10	60	-
10 – 30	60 → 140	4
30 – 50	140 → 200	3
50 – 57	200 → 270	10
57 – 60	270	-

System suitability requirements

Perform at least five replicate injections, each using 1 μL of methylnonylketone Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of methylnonylketone should not be more than 5.0%; the RSD of the retention time of methylnonylketone peak should not be more than 2.0%; the column efficiency determined from methylnonylketone peak should not be less than 50000 theoretical plates.

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

Procedure

Separately inject methylnonylketone Std-FP and the test solution (1 μL each) into the GC system and record the chromatograms. Measure the retention time of methylnonylketone peak in the chromatogram of methylnonylketone Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify methylnonylketone peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of methylnonylketone Std-FP. The retention times of methylnonylketone 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 five characteristic peaks of Houttuyniae Herba extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Houttuyniae Herba extract

Peak No.	RRT	Acceptable Range
1	0.38	± 0.03
2	0.47	± 0.03
3	0.50	± 0.03
4	0.99	± 0.03
5 (marker, methylnonylketone)	1.00	-

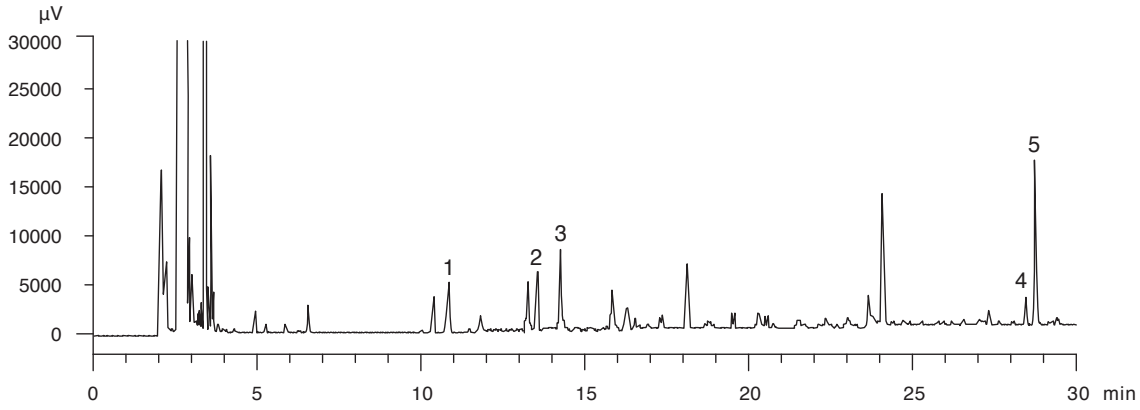


Figure 5 A reference GC fingerprint chromatogram of Houttuyniae Herba extract

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

(II) High performance liquid chromatography with diode array detector (HPLC/DAD)

Standard solution

Quercitrin standard solution for fingerprinting, Std-FP (20 mg/L)

Weigh 0.2 mg of quercitrin CRS and dissolve in 10 mL of methanol (70%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of methanol (70%). Sonicate (270 W) the mixture for 10 min. Centrifuge at about 3500 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with methanol (70%). Centrifuge at about 3500 × *g* for 10 min. 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 (254 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 0.8 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.2% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	95 → 80	5 → 20	linear gradient
20 – 40	80	20	isocratic
40 – 60	80 → 65	20 → 35	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of quercitrin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of quercitrin should not be more than 5.0%; the RSD of the retention time of quercitrin peak should not be more than 2.0%; the column efficiency determined from quercitrin peak should not be less than 50000 theoretical plates.

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

Procedure

Separately inject quercitrin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of quercitrin peak in the chromatogram of quercitrin Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify quercitrin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of quercitrin Std-FP. The retention times of quercitrin 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 Houttuyniae Herba extract are listed in Table 4.

Table 4 The RRTs and acceptable ranges of the three characteristic peaks of Houttuyniae Herba extract

Peak No.	RRT	Acceptable Range
1 (hyperoside)	0.81	± 0.05
2	0.83	± 0.06
3 (marker, quercitrin)	1.00	-

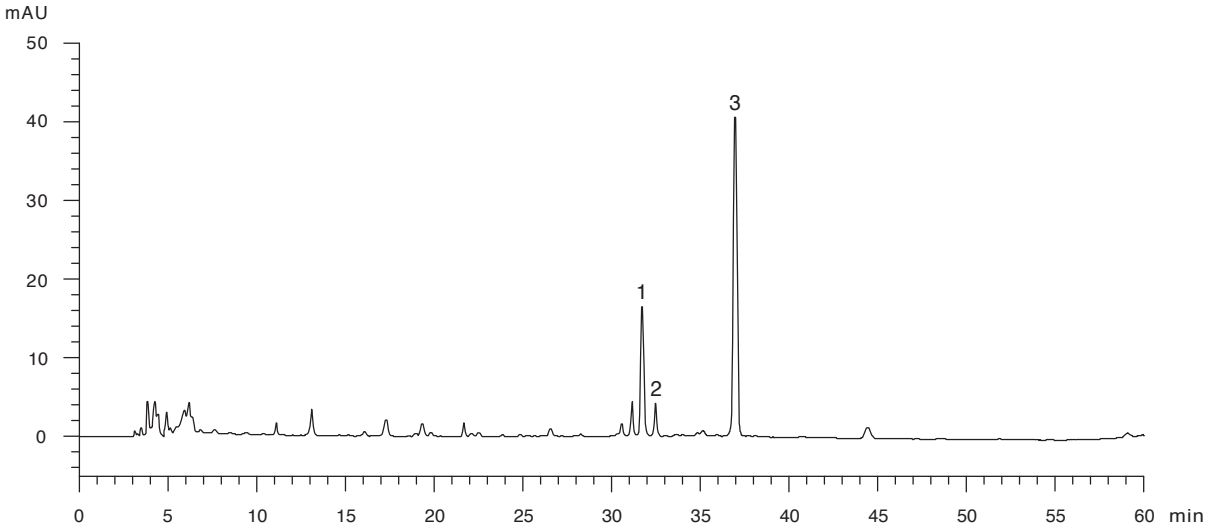


Figure 6 A reference HPLC fingerprint chromatogram of Houttuyniae Herba extract

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 HPLC 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 XVIII)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 12.5%.
Acid-insoluble ash: not more than 2.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 14.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 13.0%.
Ethanol-soluble extractives (cold extraction method): not less than 6.0%.

7. ASSAY

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

Standard solution

Quercitrin standard stock solution, Std-Stock (450 mg/L)

Weigh accurately 4.5 mg of quercitrin CRS and dissolve in 10 mL of methanol (70%).

Quercitrin standard solution for assay, Std-AS

Measure accurately the volume of the quercitrin Std-Stock, dilute with methanol (70%) to produce a series of solutions of 4.5, 9, 22.5, 45, 90 mg/L for quercitrin.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of methanol (70%). Sonicate (270 W) the mixture for 10 min. Centrifuge at about 3500 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Wash the residue with methanol (70%). Centrifuge at about 3500 × *g* for 10 min. 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 (254 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 0.8 mL/min. Programme the chromatographic system as follows (Table 5) –

Table 5 Chromatographic system conditions

Time (min)	0.2% Acetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	95 → 80	5 → 20	linear gradient
20 – 40	80	20	isocratic
40 – 60	80 → 65	20 → 35	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of quercitrin Std-AS (45 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of quercitrin should not be more than 5.0%; the RSD of the retention time of quercitrin peak should not be more than 2.0%; the column efficiency determined from quercitrin peak should not be less than 7000 theoretical plates.

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

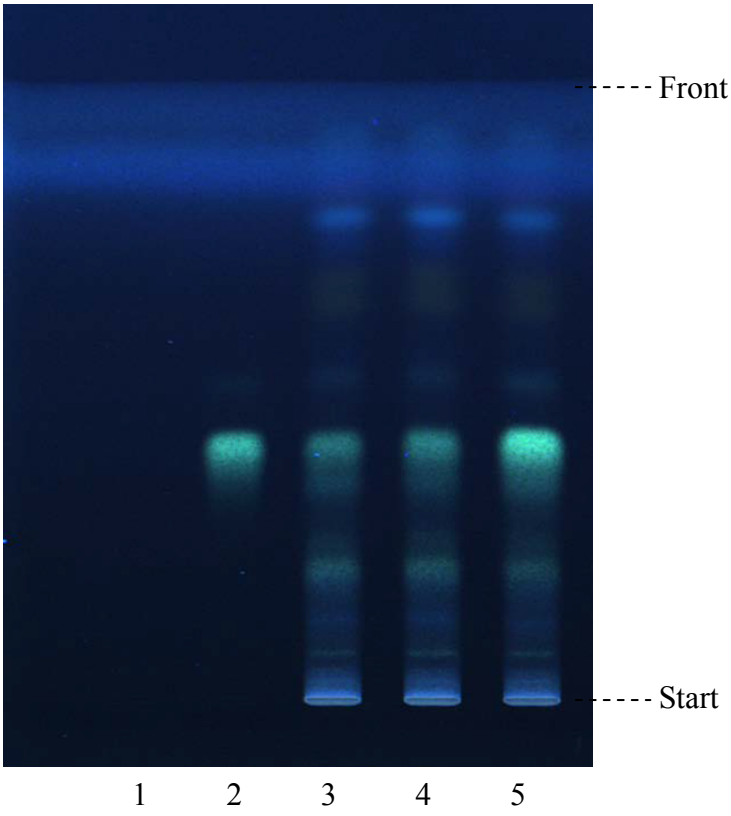
Procedure

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

Limits

The sample contains not less than 0.17% of quercitrin ($C_{21}H_{20}O_{11}$), calculated with reference to the dried substance.

Houttuyniae Herba　(魚腥草)



Lane	Sample	Results
1	Blank (70% methanol)	Negative
2	Standard (Quercitrin)	Quercitrin positive
3	Sample (Houttuyniae Herba)	Quercitrin positive
4	Sample duplicate (Houttuyniae Herba)	Quercitrin positive
5	Spiked sample (Sample plus quercitrin)	Quercitrin positive

Figure 1　TLC results of Houttuyniae Herba extract observed under UV light (366 nm) after staining