Fici Pumilae Receptaculum

Figure 1 A photograph of Fici Pumilae Receptaculum

A. Longitudinal section of Fici Pumilae Receptaculum (elongated-elliptical trough shape)
B. Longitudinal section of Fici Pumilae Receptaculum (obovoid-conic gourd shape)

1. NAMES

Official name: Fici Pumilae Receptaculum

Chinese name: 廣東王不留行

Chinese phonetic name: Guangdongwangbuliuxing

2. SOURCE

Fici Pumilae Receptaculum is the dried receptacle of inflorescence of *Ficus pumila* L. (Moraceae). The receptacle of inflorescence is collected in autumn before it turns dark brown and splits open, peduncle cut off, cut into 2-4 longitudinal sections, achenes removed, then dried under the sun to obtain Fici Pumilae Receptaculum.

3. DESCRIPTION

Obovoid-conic or elongated-elliptical, gourd-shaped or trough-shaped, 1.9-6.8 cm long, 1.3-4.5 cm wide, 1-14 mm thick. Externally greyish-yellow, yellowish-brown, yellowish-green or dark brown, shrunken. Internally greyish-yellow, yellowish-brown, reddish-brown or dark brown, remnants of uncleaned withered flower or elongated-spherical fruits often found. Apex truncated, with a small hole protrudes at the centre, inside filled with membranous bracteoles, outside often densely covered with fine yellowish-brown hairs. Base slightly small or handle-like, often with a short peduncle or peduncle scar. Texture hard and fragile, easily broken. Odour slight; taste bland, slightly astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

1 layer of outer epidermal cells subsquare, subrectangular or subrounded, arranged orderly, walls thin, outer walls slightly thickened and cutinized, wavelike. Underneath the outer epidermis, 1-3 layers of collenchymatous cells arranged orderly, subsquare, some containing prisms of calcium oxalate or white masses. Collenchyma densely arranged, walls of cells slightly thickened at angles. Secretory cells scattered, containing reddish-brown masses. Collateral vascular bundles scattered, phloem parenchymatous cells densely arranged; xylem vessels radially arranged. Central spongy tissue broad, loosely arranged, walls slightly thickened. Inner epidermal cells rectangular, reddish-brown, non-glandular hairs present. Remnants of fruits often found (Fig. 2).

Powder

Colour yellowish-brown to reddish-brown. Collenchymatous cells subrounded to subsquare in shape, with thick walls; thickened angles bright white under the polarized microscope. Secretory cells abundant, subrounded or elongated-rounded, filled with yellowish-brown secretions. Prisms of calcium oxalate subsquare, present in collenchymatous cells, 2-20 μm in diameter; polychromatic under the polarized microscope. Vessels small, mostly in bundles, mainly spiral type, scalariform and reticulate vessels also present, 4-32 μm in diameter. Spongy cells irregular in shape, often broken, wall slightly thickened. Non-glandular hairs unicellular or multicellular, base wide, apex acute, 72-1014 μm long, 7-40 μm in diameter (Fig. 3).

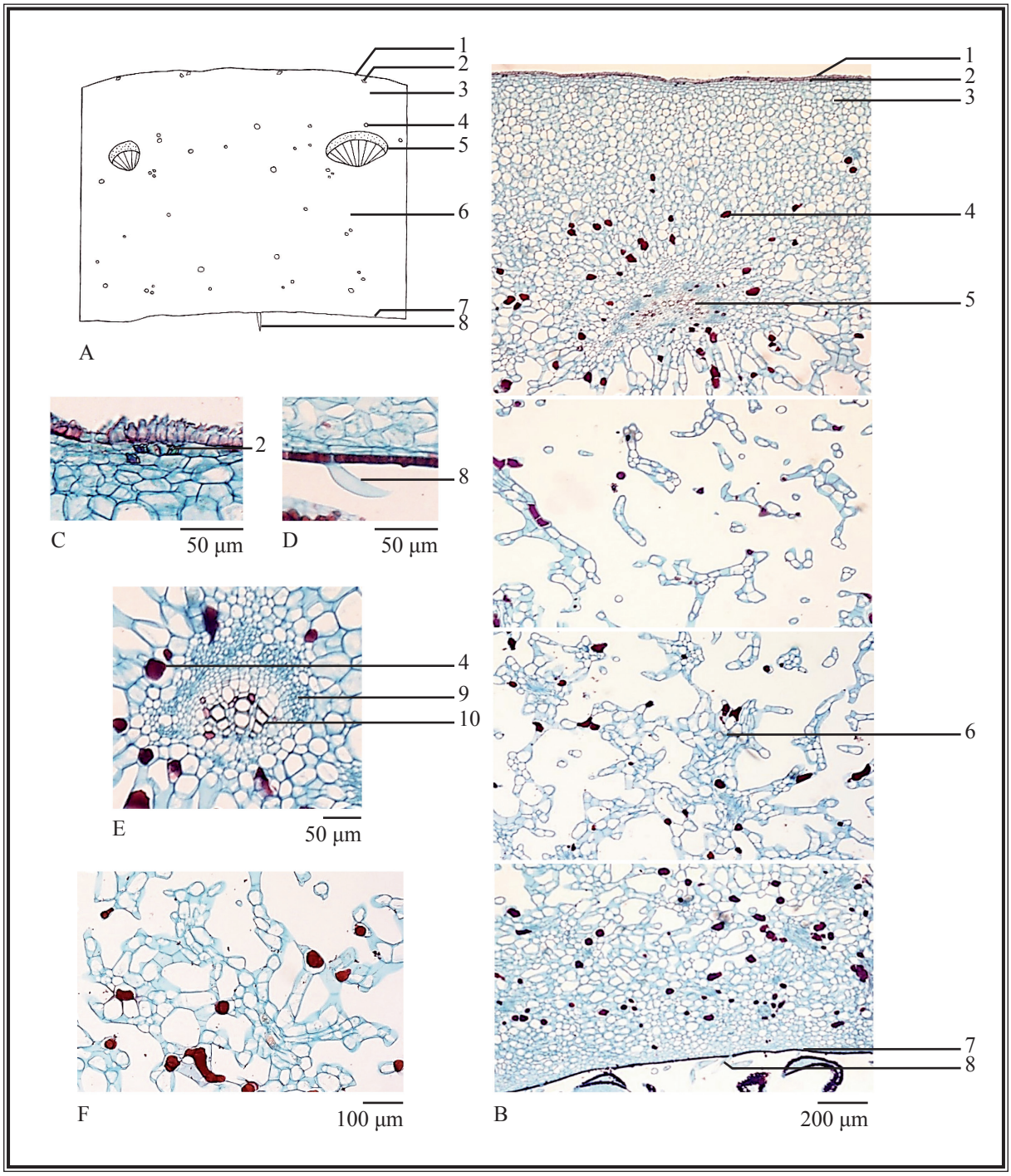


Figure 2 Microscopic features of transverse section of Fici Pumilae Receptaculum

A. Sketch B. Section illustration C. Prism of calcium oxalate
D. Non-glandular hair E. Vascular bundle F. Spongy tissue

1. Outer epidermis 2. Prism of calcium oxalate 3. Collenchyma
4. Secretory cell 5. Vascular bundle 6. Spongy tissue 7. Inner epidermis
8. Non-glandular hair 9. Phloem 10. Xylem

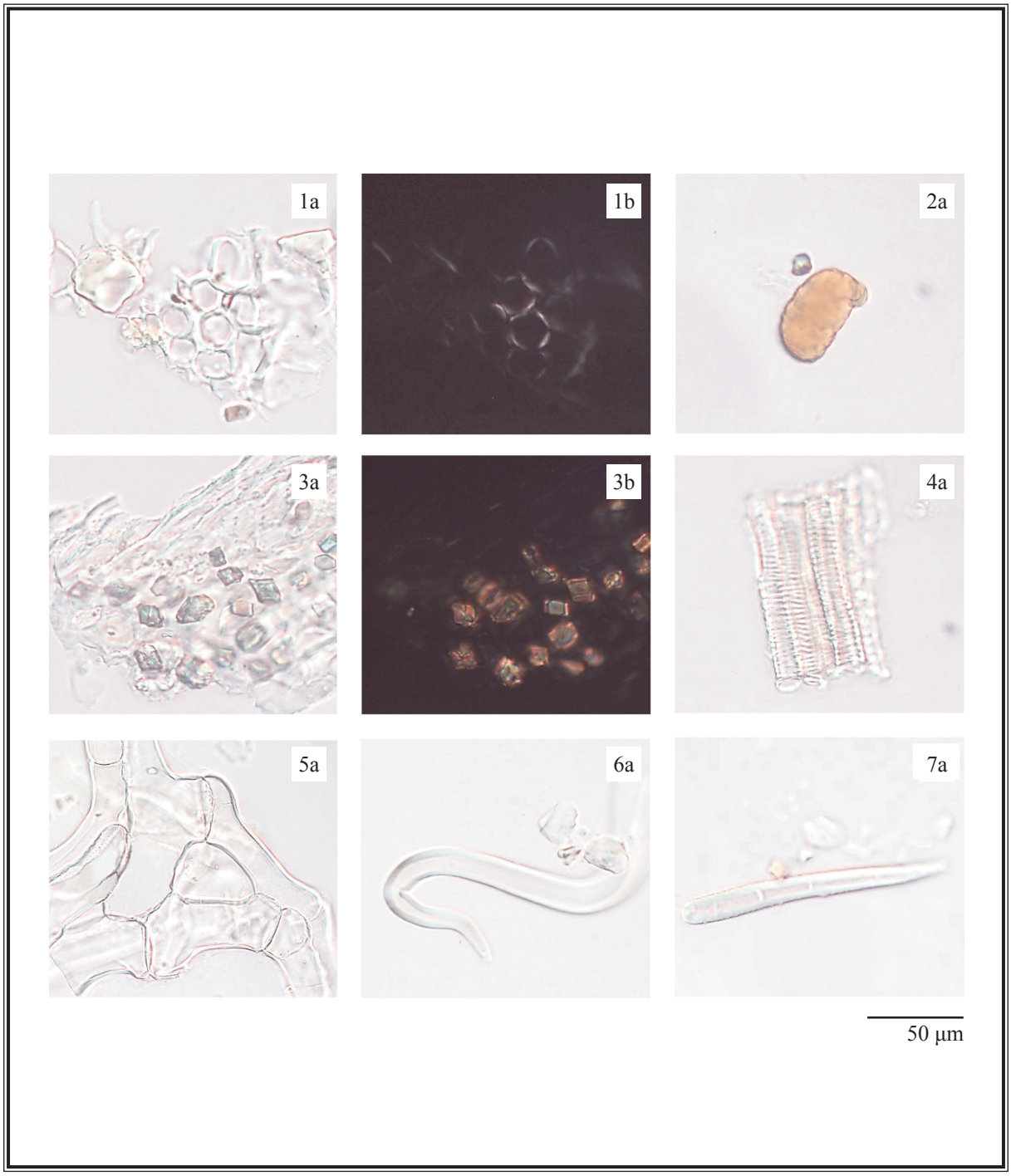


Figure 3 Microscopic features of powder of Fici Pumilae Receptaculum

1. Collenchymatous cells 2. Secretory cell 3. Prisms of calcium oxalate
4. Spiral vessels 5. Spongy cells 6. Unicellular non-glandular hair
7. Multicellular non-glandular hair

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

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

Standard solutions

Chlorogenic acid standard solution

Weigh 1.0 mg of chlorogenic acid CRS (Fig. 4) and dissolve in 1 mL of methanol.

Rutin standard solution

Weigh 1.0 mg of rutin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, acetone, water and formic acid (20:3:3:1.5, v/v).

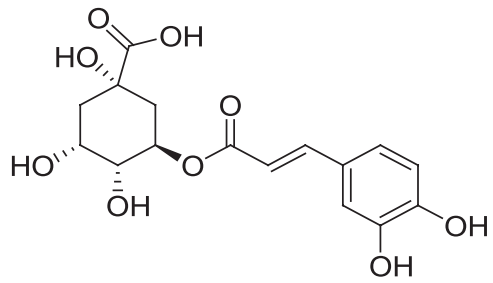
Test solution

Weigh 2.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (50%). Sonicate (240 W) the mixture for 1 h. Centrifuge at about 6000 × *g* for 10 min. Transfer the supernatant to a 50-mL round-bottomed flask. Evaporate the supernatant to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol (50%). Transfer the extract to a 5-mL volumetric flask and make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

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 chlorogenic acid standard solution (2 μL), rutin standard solution (2 μL) and the test solution (6 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 7 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (254 nm). Calculate the R_f values by using the equation as indicated in Appendix IV (A).

(i)



(ii)

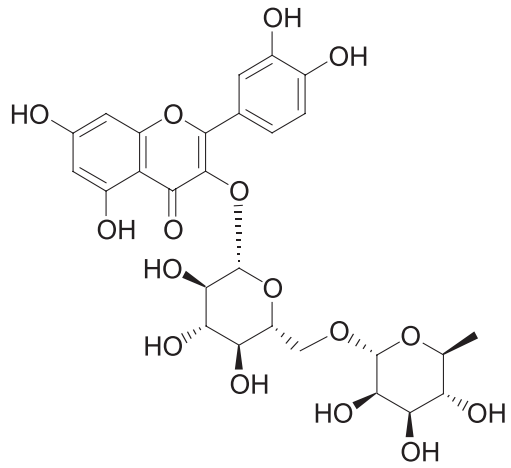


Figure 4 Chemical structures of (i) chlorogenic acid and (ii) rutin

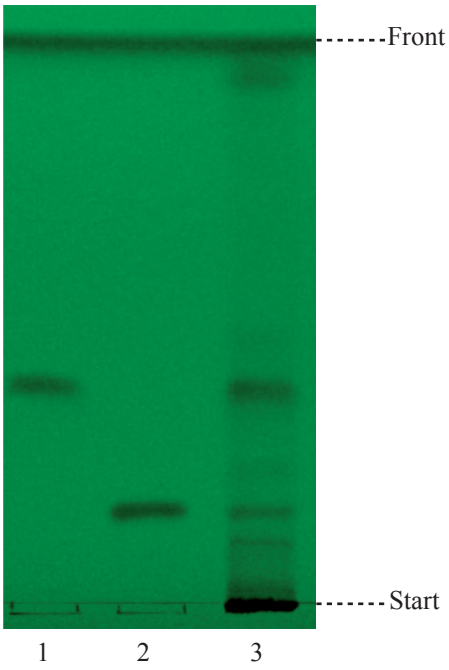


Figure 5 A reference HPTLC chromatogram of Fici Pumilae Receptaculum extract observed under UV light (254 nm)

1. Chlorogenic acid standard solution 2. Rutin 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 chlorogenic acid and rutin (Fig. 5).

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

Standard solutions

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

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

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

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

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol. Sonicate (240 W) the mixture for 1 h. Centrifuge at about 6000 × *g* for 10 min. Transfer the supernatant to a 50-mL round-bottomed flask. Evaporate the supernatant to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in methanol. Transfer the extract to a 5-mL volumetric flask and make up to the mark with methanol. Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (254 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size, 120 Å pore size and 11% carbon loading). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.2% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 10	98 → 95	2 → 5	linear gradient
10 – 20	95 → 90	5 → 10	linear gradient
20 – 40	90 → 87	10 → 13	linear gradient
40 – 55	87 → 80	13 → 20	linear gradient
55 – 60	80 → 70	20 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 5 μL of chlorogenic acid Std-FP and rutin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of chlorogenic acid and rutin should not be more than 5.0%; the RSD of the retention times of chlorogenic acid and rutin peaks should not be more than 2.0%; the column efficiencies determined from chlorogenic acid and rutin peaks should not be less than 30000 and 140000 theoretical plates respectively.

The *R* value between peak 3 and the closest peak; and the *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 6).

Procedure

Separately inject chlorogenic acid Std-FP, rutin Std-FP and the test solution (5 μL each) into the HPLC system and record the chromatograms. Measure the retention times of chlorogenic acid and rutin peaks in the chromatograms of chlorogenic acid Std-FP, rutin Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify chlorogenic acid and rutin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of chlorogenic acid Std-FP and rutin Std-FP. The retention times of chlorogenic acid and rutin 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 five characteristic peaks of Fici Pumilae Receptaculum extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Fici Pumilae Receptaculum extract

Peak No.	RRT	Acceptable Range
1	0.57	± 0.03
2	0.81	± 0.03
3 (marker, chlorogenic acid)	1.00	-
4 (rutin)	2.10	± 0.06
5	2.14	± 0.06

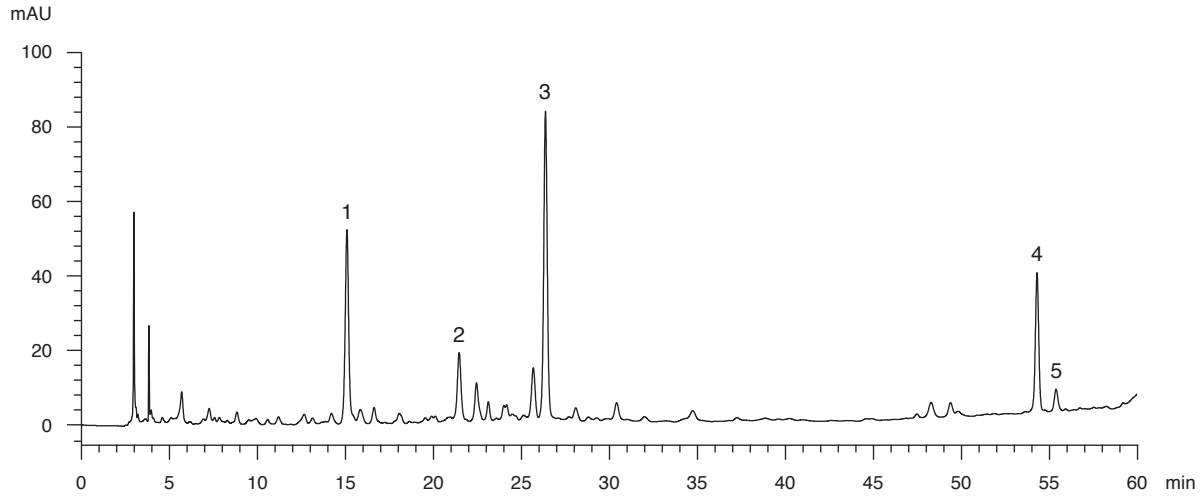


Figure 6 A reference fingerprint chromatogram of Fici Pumilae Receptaculum extract

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 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 7.5%.
Acid-insoluble ash: not more than 1.5%.

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 15.0%.
Ethanol-soluble extractives (cold extraction method): not less than 14.0%.

7. ASSAY

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

Standard solution

Mixed chlorogenic acid and rutin standard stock solution, Std-Stock (100 mg/L for chlorogenic acid and 20 mg/L for rutin)

Weigh accurately 1.0 mg of chlorogenic acid CRS and 0.2 mg of rutin CRS, and dissolve in 10 mL of methanol.

Mixed chlorogenic acid and rutin standard solution for assay, Std-AS

Measure accurately the volume of the mixed chlorogenic acid and rutin Std-Stock, dilute with methanol to produce a series of solutions of 0.2, 1, 10, 20, 80 mg/L for chlorogenic acid and 0.2, 1, 2, 10, 20 mg/L for rutin.

Test solution

Weigh accurately 0.25 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of methanol (50%). Sonicate (240 W) the mixture for 30 min. Centrifuge at about 5000 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction two more times each with 5 mL of methanol (50%). Combine the supernatants and make up to the mark with methanol (50%). Filter through a 0.45-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (340 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size, 100 Å pore size and 15.5% carbon loading). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.2% Phosphoric acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	90 → 89	10 → 11	linear gradient
5 – 13	89 → 87	11 → 13	linear gradient
13 – 17	87 → 80	13 → 20	linear gradient
17 – 30	80 → 65	20 → 35	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed chlorogenic acid and rutin Std-AS (10 mg/L for chlorogenic acid and 2 mg/L for rutin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of chlorogenic acid and rutin should not be more than 5.0%; the RSD of the retention times of chlorogenic acid and rutin peaks should not be more than 2.0%; the column efficiencies determined from chlorogenic acid and rutin peaks should not be less than 4000 and 70000 theoretical plates respectively.

The *R* value between chlorogenic acid peak and the closest peak; and the *R* value between rutin 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 chlorogenic acid and rutin Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of chlorogenic acid and rutin against the corresponding concentrations of the mixed chlorogenic acid and rutin 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 chlorogenic acid and rutin peaks (Fig. 7) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed chlorogenic acid and rutin Std-AS. The retention times of chlorogenic acid and rutin 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 chlorogenic acid and rutin in the test solution, and calculate the percentage contents of chlorogenic acid and rutin in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.11% of chlorogenic acid ($C_{16}H_{18}O_9$) and not less than 0.022% of rutin ($C_{27}H_{30}O_{16}$), calculated with reference to the dried substance.

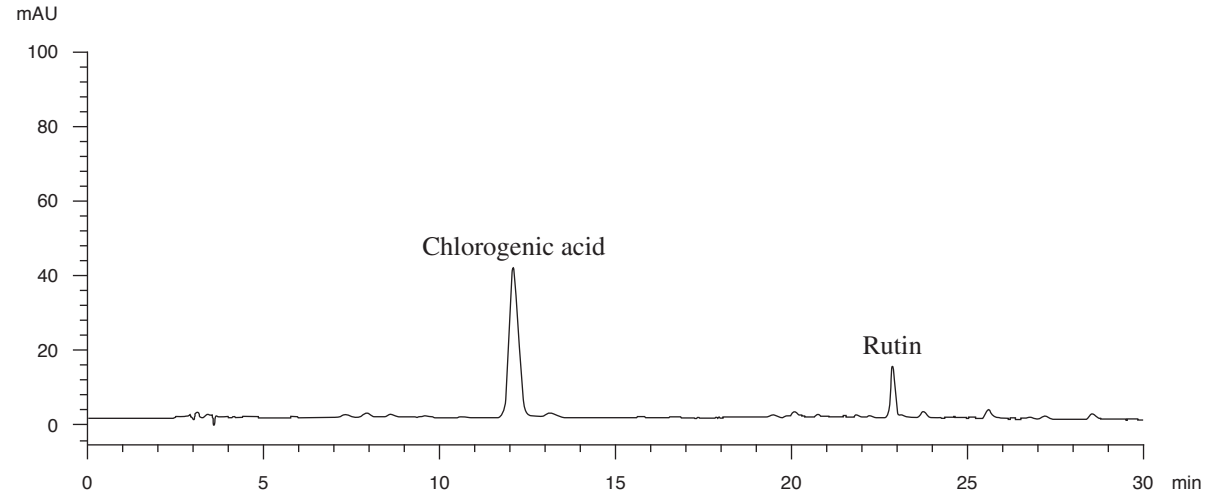


Figure 7 A reference assay chromatogram of Fici Pumilae Receptaculum extract