Psidii Guajavae Folium

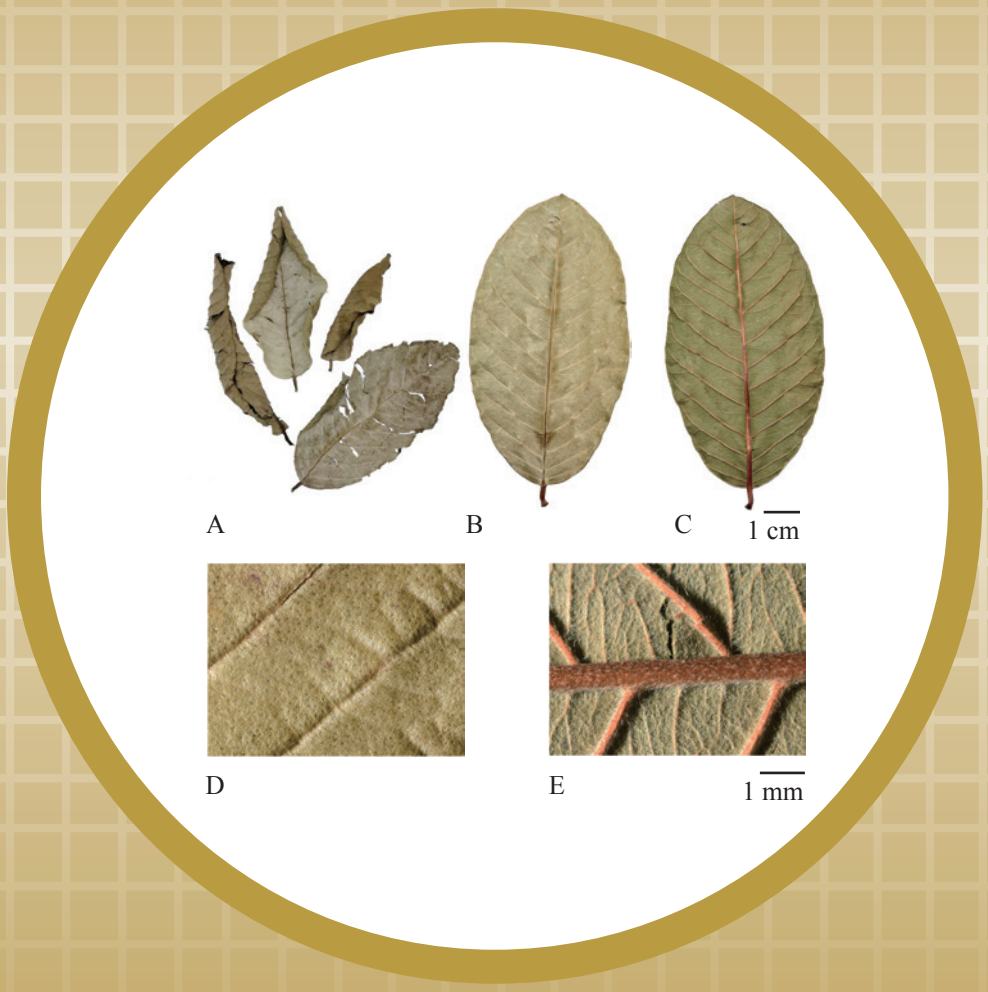

Figure 1 A photograph of Psidii Guajavae Folium

A. Psidii Guajavae Folium B. Upper surface of leaf
C. Lower surface of leaf D. Magnified image of upper surface of leaf
E. Magnified image of lower surface of leaf

1. NAMES

Official Name: Psidii Guajavae Folium

Chinese Name: 番石榴葉

Chinese Phonetic Name: Fanshiliuye

2. SOURCE

Psidii Guajavae Folium is the dried leaf of *Psidium guajava* L. (Myrtaceae). The leaf is collected in spring and summer, washed clean, then dried under the sun to obtain Psidii Guajavae Folium.

3. DESCRIPTION

Mostly crumpled, rolled or broken. When intact, oblong to elliptic, 3-14 cm long, 2-7 cm wide, apex rounded or acuminate, base obtuse to rounded, margin entire. The upper surface yellowish-green to pale brown, glabrous or nearly glabrous, sparsely dark brown glandular-punctate. The lower surface greyish-brown to dark green, densely covered with pubescence, principal and lateral veins slightly protuberant. Petioles 2-10 mm long. Texture leathery. Odour delicately aromatic; taste astringent (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Upper epidermis consists of 1 layer of flat polygonal cells, covered with cuticle; with unicellular non-glandular hairs on the surface. Lower epidermal cells slightly small, sparsely covered with non-glandular hairs. Hypodermis consists of 2-3 layers of collenchymatous cells, orderly arranged, with secretory glands. Palisade tissue consists of 2 layers of columnar cells, discontinue at the midrib. Spongy tissue consists of slightly small cells, with secretory glands. Vascular bundle of the midrib bicollateral, surrounded by pericycle fibres. Xylem vessels radially arranged. Phloem narrow. Clusters of calcium oxalate in rosette aggregate and prisms of calcium oxalate scattered in the mesophyll. Collenchymatous cells subrounded, located beneath the lower epidermis (Fig. 2).

Powder

Colour yellowish-brown to yellowish-green. Non-glandular hairs unicellular. Clusters of calcium oxalate numerous, in rosette aggregate, scattered or embedded in parenchymatous cells, 6-44 μm in diameter; polychromatic under the polarized microscope. Lower epidermal cells polygonal, with paracytic stomata, with 2 subsidiary cells. Secretory glands usually broken, the intact ones subrounded. Calcium oxalate prism sheath occasionally visible, Prisms of calcium oxalate scattered or embedded in parenchymatous cells, sometimes arranged in rows; polychromatic under the polarized microscope. Fibres slender, some with the walls slightly undulantly curved; white under the polarized microscope. Vessels mostly spiral, 6-40 μm in diameter (Fig. 3).

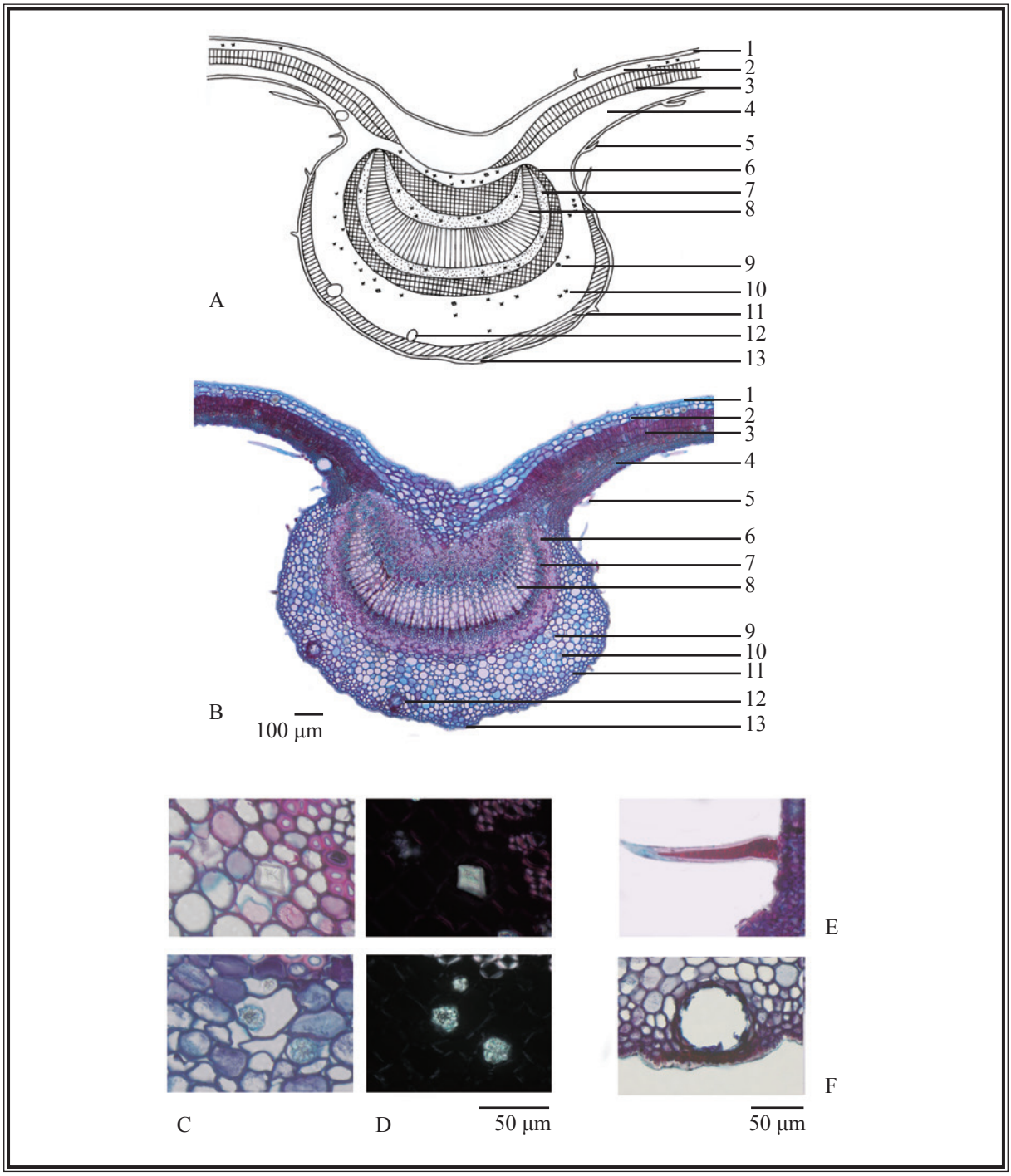

Figure 2 Microscopic features of transverse section of Psidii Guajavae Folium

A. Sketch B. Section illustration C. Prism and clusters of calcium oxalate (under the light microscope)
D. Prism and clusters of calcium oxalate (under the polarized microscope)
E. Non-glandular hair F. Secretory gland

1. Upper epidermis 2. Hypodermis 3. Palisade tissue 4. Spongy tissue
5. Non-glandular hair 6. Pericycle fiber 7. Phloem 8. Xylem 9. Prism of calcium oxalate
10. Cluster of calcium oxalate 11. Collenchyma 12. Secretory gland 13. Lower epidermis

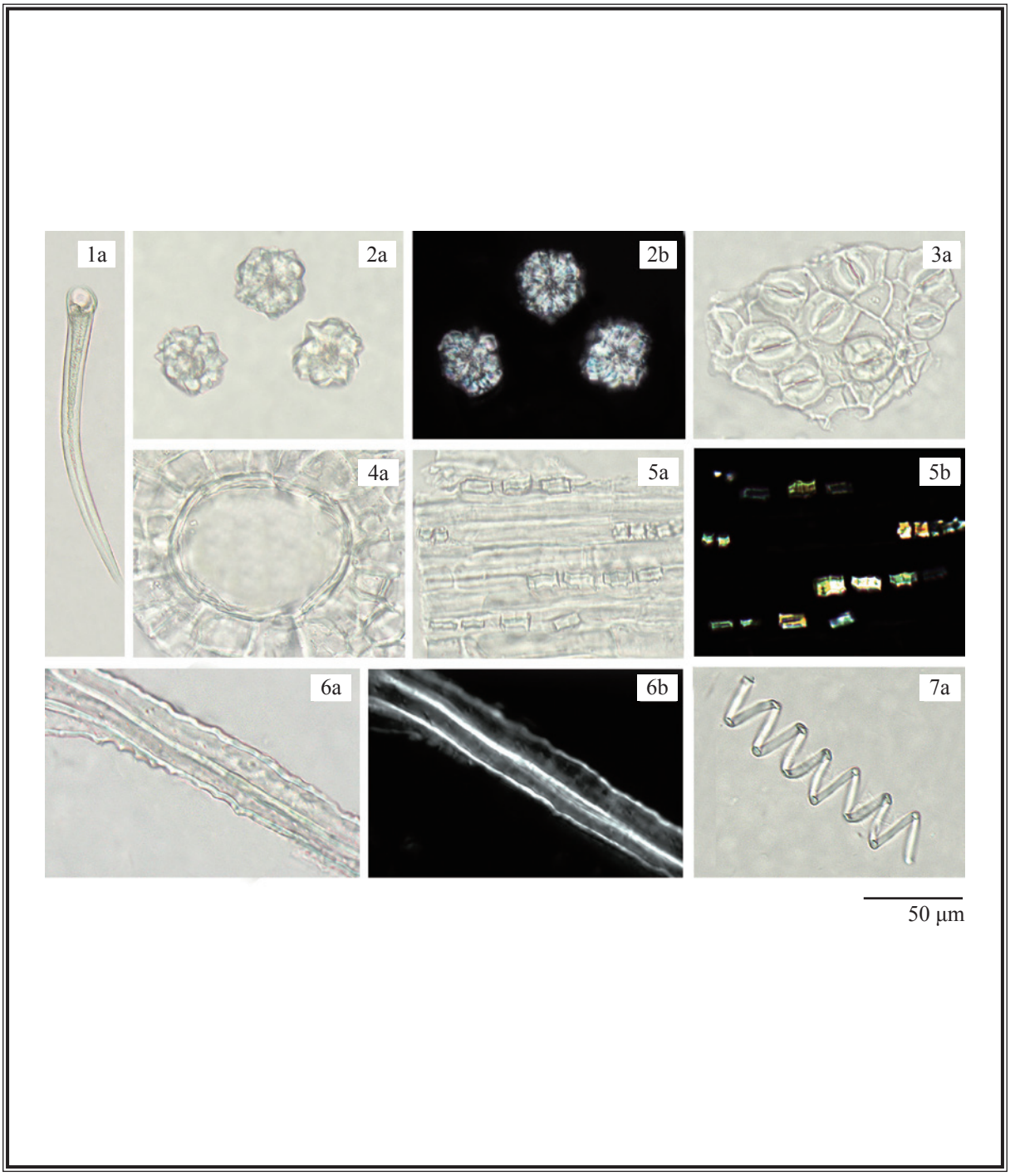

Figure 3 Microscopic features of powder of Psidii Guajavae Folium

1. Non-glandular hair 2. Clusters of calcium oxalate 3. Lower epidermis cells with paracytic stomata
4. Secretory gland 5. Calcium oxalate prisms sheath 6. Pericycle fibres 7. Spiral vessel

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

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

Standard solutions

Guaijaverin standard solution

Weigh 1.0 mg of guaijaverin CRS (Fig. 4) and dissolve in 10 mL of ethanol (50%).

Hyperoside standard solution

Weigh 1.0 mg of hyperoside CRS (Fig. 4) and dissolve in 10 mL of ethanol (50%).

Developing solvent system

Prepare a mixture of dichloromethane, ethyl acetate, formic acid, ethanol and water (6.5:3.5:3:1:0.5, v/v).

Spray reagent

Weigh 1 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 conical flask, then add 10 mL of ethanol (50%). Sonicate (350 W) the mixture for 30 min. 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 guaijaverin standard solution, hyperoside standard solution and the test solution (2 μL 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 7 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 values by using the equation as indicated in Appendix IV (A).

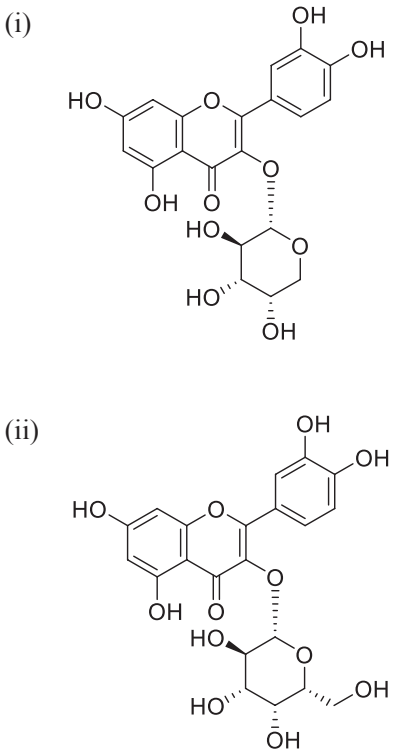

Figure 4 Chemical structures of (i) guaijaverin and (ii) hyperoside

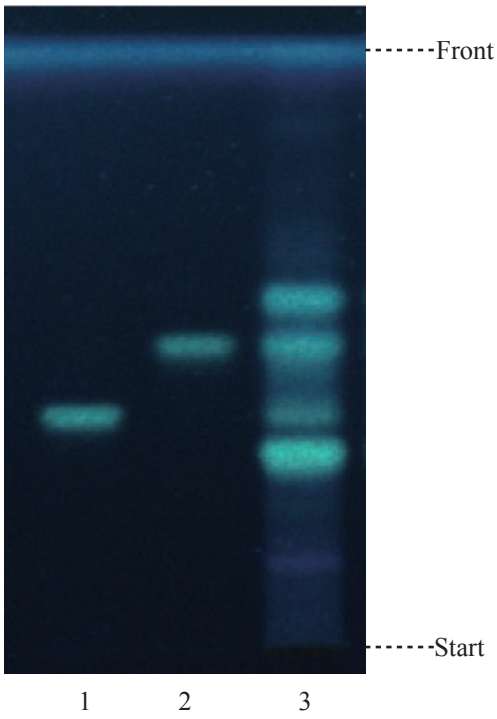

Figure 5 A reference HPTLC chromatogram of Psidii Guajavae Folium extract observed under UV light (366 nm) after staining

1. Hyperoside standard solution 2. Guaijaverin 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 guaijaverin and hyperoside (Fig. 5).

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

Standard solutions

Guaijaverin standard solution for fingerprinting, Std-FP (15 mg/L)

Weigh 0.3 mg of guaijaverin CRS and dissolve in 20 mL of ethanol (50%).

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

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

Test solution

Weigh 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (50%). Sonicate (120 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. 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 flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	2.5 mM Sodium acetate solution (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	86	14	isocratic
5 – 60	86 → 82	14 → 18	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of guaijaverin Std-FP and hyperoside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of guaijaverin and hyperoside should not be more than 5.0%; the RSD of the retention times of guaijaverin and hyperoside peaks should not be more than 2.0%; the column efficiencies determined from guaijaverin and hyperoside peaks should not be less than 25000 theoretical plates.

The *R* value between peak 2 and the closest peak; and the *R* value between peak 5 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

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

Table 2 The RRTs and acceptable ranges of the six characteristic peaks of Psidii Guajavae Folium extract

Peak No.	RRT	Acceptable Range
1 (ellagic acid)	0.91	± 0.03
2 (marker, hyperoside)	1.00	-
3 (isoquercitrin)	1.06	± 0.03
4 (reynoutrin)	1.23	± 0.03
5 (guaijaverin)	1.32	± 0.03
6 (avicularin)	1.43	± 0.03

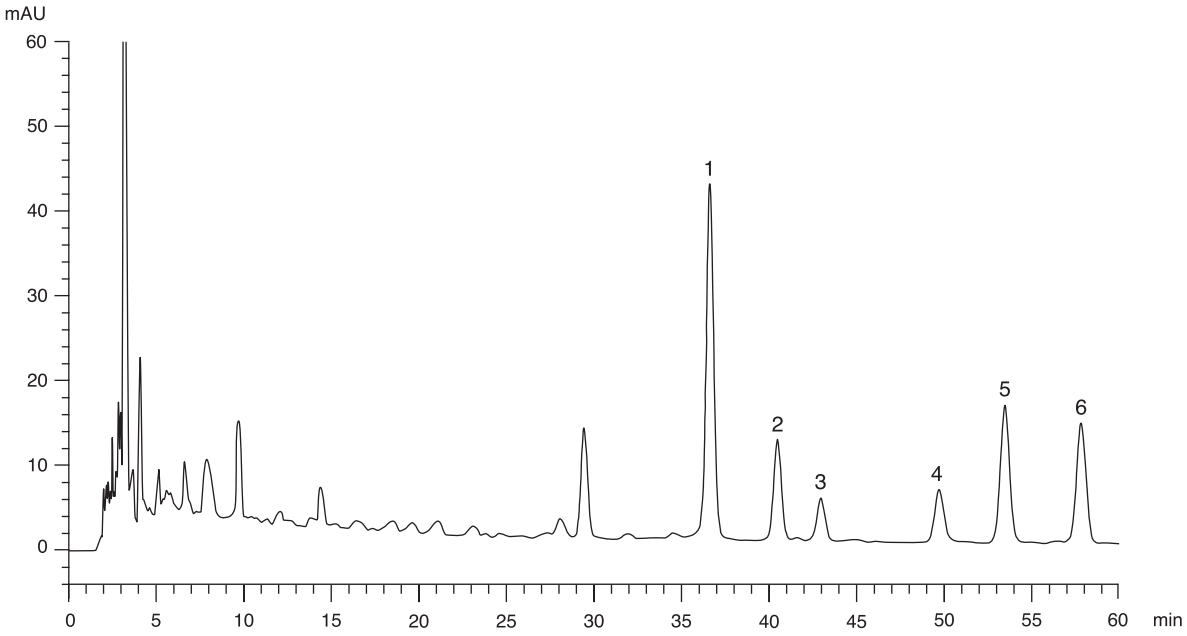

Figure 6 A reference fingerprint chromatogram of Psidii Guajavae Folium extract

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 5.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 6.0%.

Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 11.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 10.0%.
Ethanol-soluble extractives (cold extraction method): not less than 22.0%.

7. ASSAY

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

Standard solution

Mixed guaijaverin and hyperoside standard stock solution, Std-Stock (100 mg/L each)

Weigh accurately 1.0 mg of guaijaverin CRS and 1.0 mg of hyperoside CRS, and dissolve in 10 mL of ethanol (50%).

Mixed guaijaverin and hyperoside standard solution for assay, Std-AS

Measure accurately the volume of the mixed guaijaverin and hyperoside Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 0.5, 1, 5, 10, 25 mg/L for both guaijaverin and hyperoside.

Test solution

Weigh accurately 0.1 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol (50%). Sonicate (120 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 10 min. Transfer the supernatant to a 25-mL volumetric flask. Repeat the extraction for two more times with 10 mL of ethanol (50%) and 5 mL of ethanol (50%) respectively. 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 (254 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)	2.5 mM Sodium acetate solution (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 5	86	14	isocratic
5 – 60	86 → 82	14 → 18	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 µL of the mixed guaijaverin and hyperoside Std-AS (5 mg/L each). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of guaijaverin and hyperoside should not be more than 5.0%; the RSD of the retention times of guaijaverin and hyperoside peaks should not be more than 2.0%; the column efficiencies determined from guaijaverin and hyperoside peaks should not be less than 30000 and 20000 theoretical plates respectively.

The *R* value between guaijaverin peak and the closest peak; and the *R* value between hyperoside 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 guaijaverin and hyperoside Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of guaijaverin and hyperoside against the corresponding concentrations of the mixed guaijaverin and hyperoside Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify guaijaverin and hyperoside peaks (Fig. 7) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed guaijaverin and hyperoside Std-AS. The retention times of guaijaverin and hyperoside 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 guaijaverin and hyperoside in the test solution, and calculate the percentage contents of guaijaverin and hyperoside in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.16% of guaijaverin ($C_{20}H_{18}O_{11}$) and not less than 0.10% of hyperoside ($C_{21}H_{20}O_{12}$), calculated with reference to the dried substance.

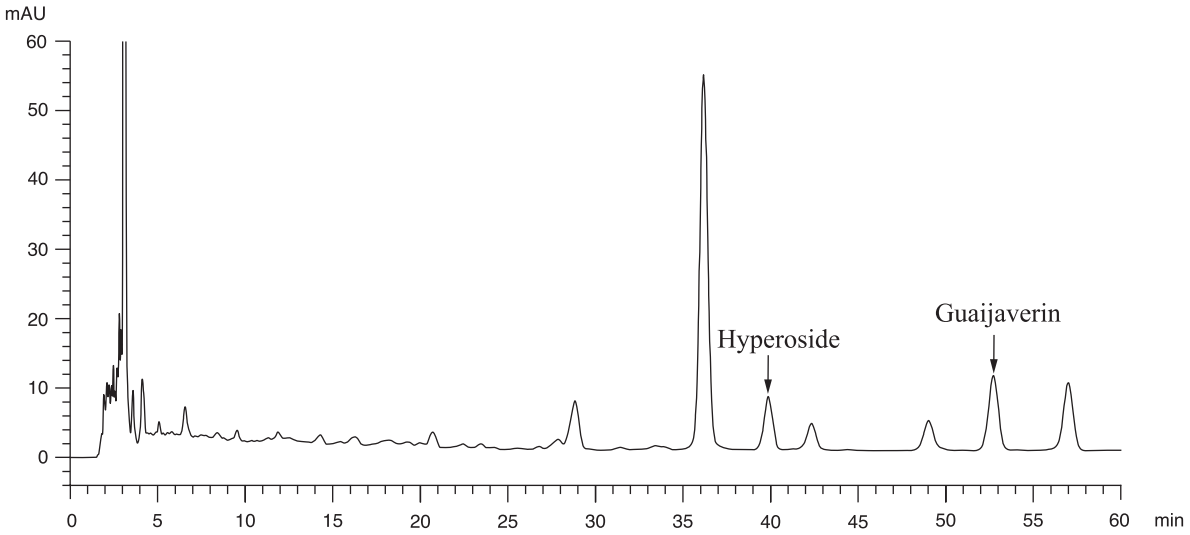

Figure 7 A reference assay chromatogram of Psidii Guajavae Folium extract