Tamaricis Cacumen

Figure 1 A photograph of Tamaricis Cacumen

A. Tamaricis Cacumen B. Magnified image of Tamaricis Cacumen
C. Magnified image of alternated leaves
D. Magnified image of fracture of young twig

1. NAMES

Official name: Tamaricis Cacumen

Chinese name: 西河柳

Chinese phonetic name: Xiheliu

2. SOURCE

Tamaricis Cacumen is the dried young twig and leaf of *Tamarix chinensis* Lour. (Tamaricaceae). The young twig and leaf are collected in summer before flowering, then dried under the sun to obtain Tamaricis Cacumen.

3. DESCRIPTION

The upper part of young twig slender-cylindrical, 0.3-1.3 mm in diameter, externally greyish-green to yellowish-green; texture fragile, easily broken, fracture flat, yellowish-white, arranged in radial striations, with distinct ray and pith in the centre. The lower part of young twig 0.7-2.5 mm in diameter, externally yellowish-brown to reddish-brown. Leaves alternate, scaly, ovate-lanceolate, apex acute, sheath-like base. Leaves usually fallen off and the leaf-bases protruded. Odour slight, taste bland (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork contains reddish-brown pigment, the upper part of young twig consists of 1 layer of flattened cells; the lower part of young twig consists of 4-7 layers of flattened cells, with outer walls lignified. Cortex relatively broad, consisting of irregularly arranged parenchymatous cells, with cells subsquare or irregular in shape. Pericycle fibres arranged in an interrupted ring, walls of fibre markedly thickened and lignified, with surrounding cells containing crystals of calcium sulphate. Groups of stone cells occasionally exist among fibre bundles. Phloem thin. Cambium indistinct. Xylem consists of fibres and vessels. Ray 2-3 rows of cells wide, lignified. Pith relatively small, containing subrounded parenchymatous cells [Fig. 2(i) and Fig. 2(ii)].

Powder

Colour reddish-brown, brownish-green or yellowish-green. Crystals of calcium sulphate numerous, 5-28 μm in diameter, some corner angles distinct; polychromatic under the polarized microscope. Stone cells several in group, subrounded or elliptic. Leaf base fibre slender, with relatively thickened walls and thorn-like projections surface. Fibres present in bundles, 4-16 μm in diameter, walls slightly thick, lignified. Epidermal cells of leaf subsquared, subpolygonal or rectangular in surface view, slightly thickened, occasionally semilunar cuticle convex; sunken stoma with 4-6 subsidiary cells appeared in both upper and lower epidermis layer of cells. Vessels mostly reticulate and spiral vessels, 6-19 μm and 3-14 μm in diameter respectively (Fig. 3).

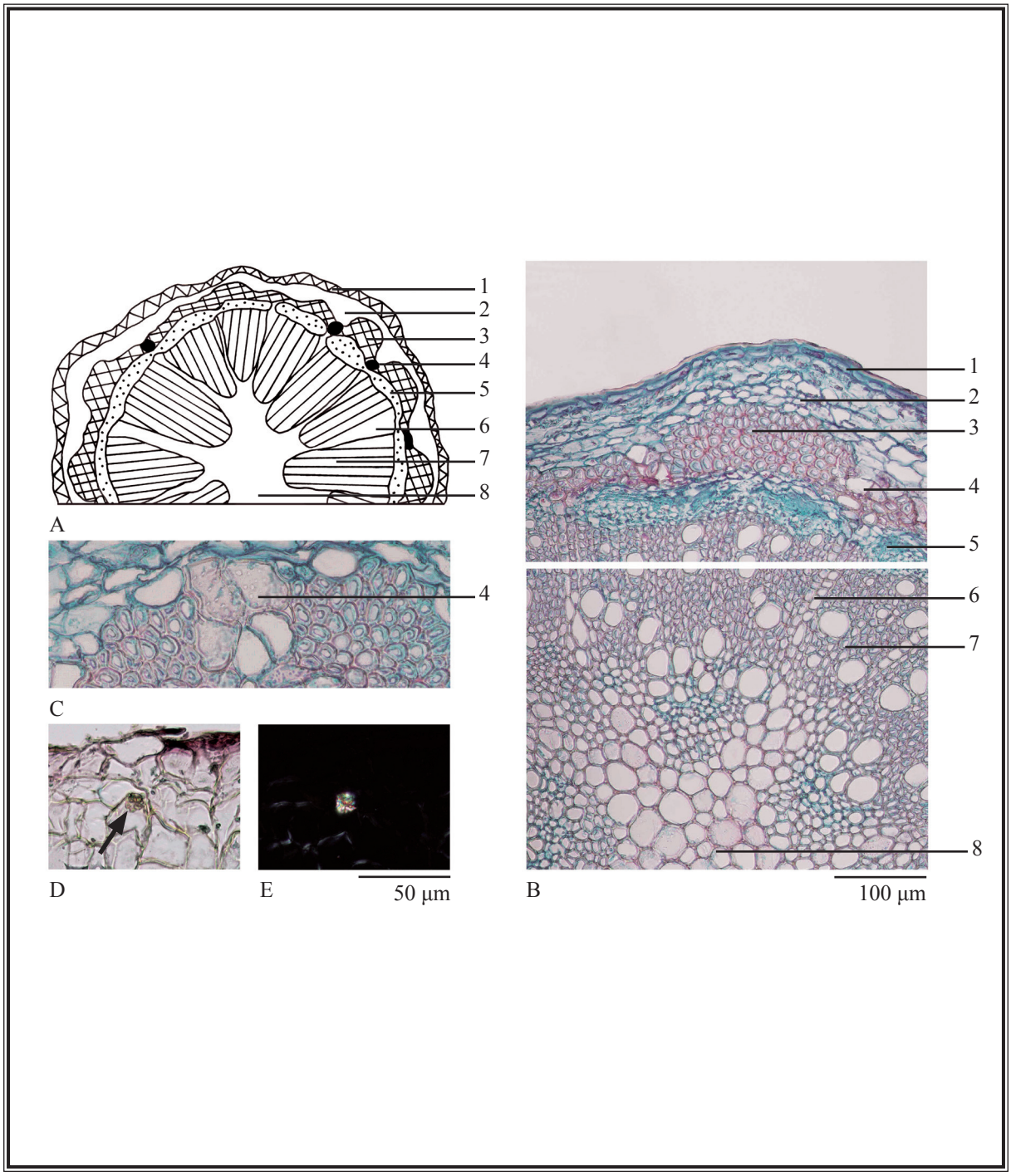


Figure 2 (i) Microscopic features of transverse section of upper part of young twig of Tamaricis Cacumen

A. Sketch B. Section illustration C. Stone cells
D. Crystals of calcium sulphate (under the light microscope ⟶)
E. Crystals of calcium sulphate (under the polarized microscope)

1. Cork 2. Cortex 3. Pericycle fibre 4. Stone cell 5. Phloem 6. Ray 7. Xylem 8. Pith

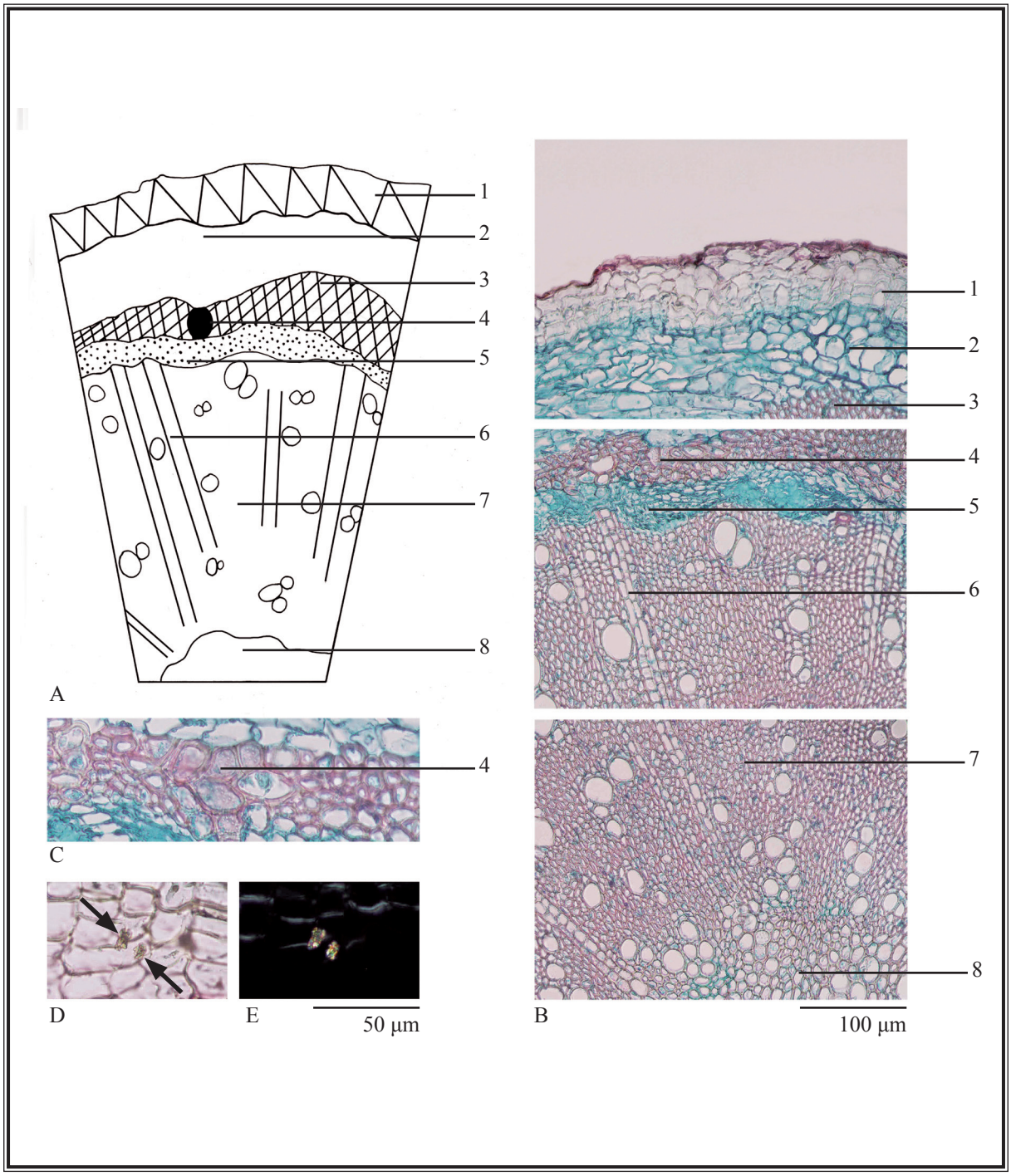


Figure 2 (ii) Microscopic features of transverse section of lower part of young twig of Tamaricis Cacumen

A. Sketch B. Section illustration C. Stone cells
D. Crystals of calcium sulphate (under the light microscope ⟶)
E. Crystals of calcium sulphate (under the polarized microscope)

1. Cork 2. Cortex 3. Pericycle fibre 4. Stone cell 5. Phloem 6. Ray 7. Xylem 8. Pith

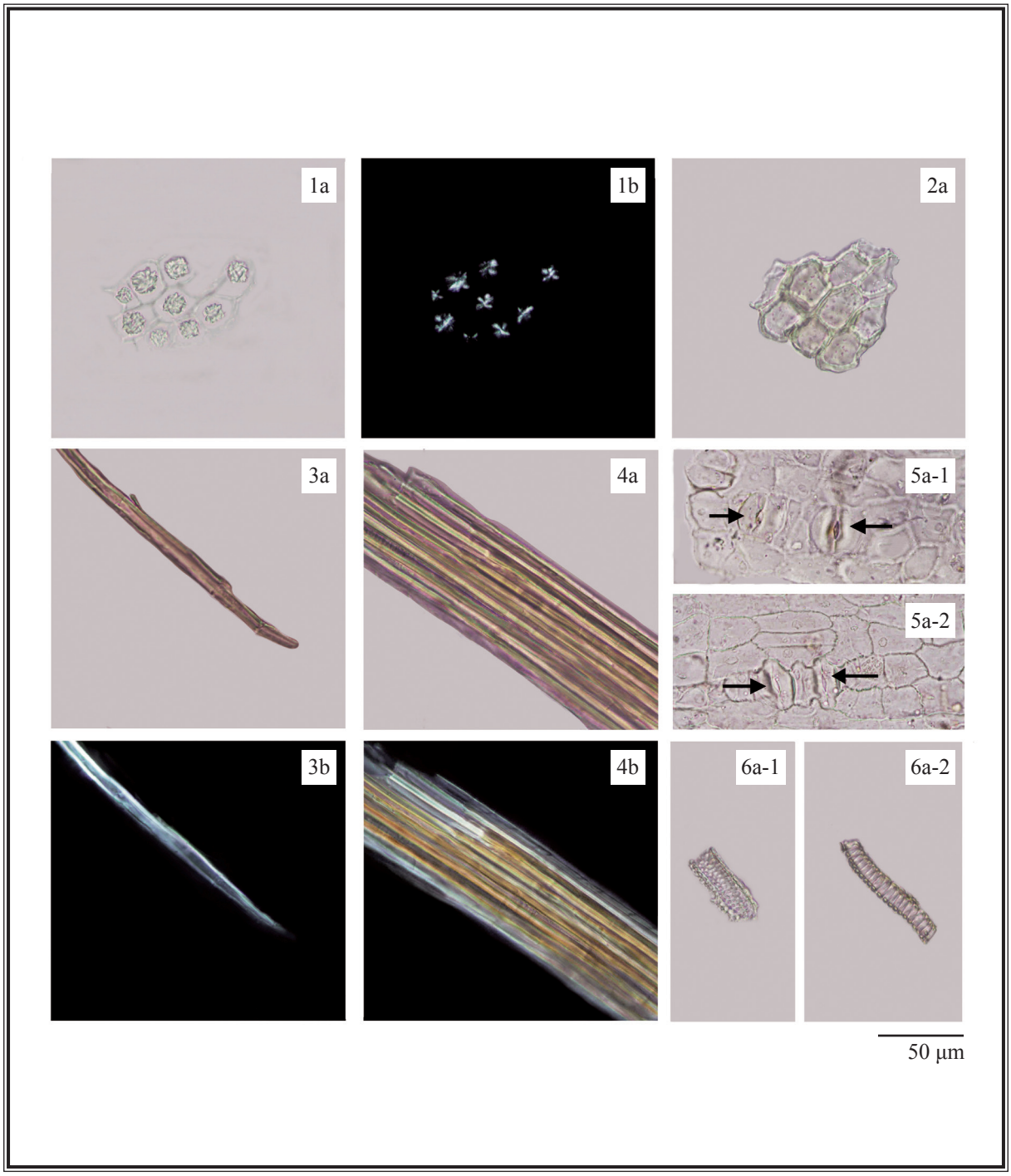


Figure 3 Microscopic features of the powder of Tamaricis Cacumen

1. Crystals of calcium sulphate 2. Stone cells 3. Leaf base fibre 4. Fibres
5. Epidermal cells of leaf (5-1 upper epidermal cells, 5-2 lower epidermal cells, anomocytic stomata ⟶)
6. Vessels (6-1 reticulate vessel, 6-2 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

Quercetin standard solution

Weigh 0.25 mg of quercetin CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, *n*-hexane, formic acid and water (11:7:2:1, v/v).

Spray reagent

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

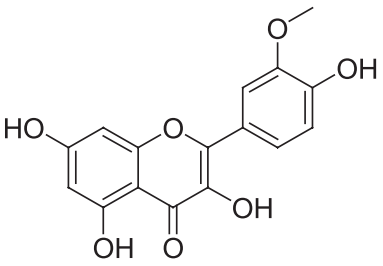
Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL round-bottomed flask, then add 10 mL of 2% hydrochloric acid in methanol. Reflux the mixture for 30 min. Cool down to room temperature. Filter through a 0.45-μm PTFE filter.

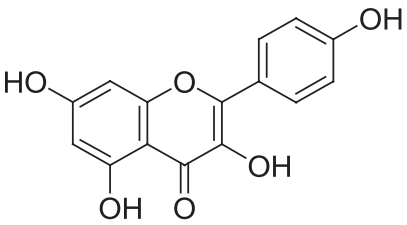
Procedure

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 quercetin standard solution (1 μL) and the test solution (2 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 2 min). Examine the plate under UV light (366 nm). Calculate the R_f value by using the equation as indicated in Appendix IV (A).

(i)



(ii)



(iii)

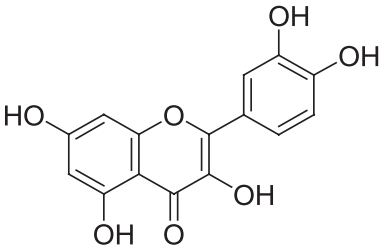


Figure 4 Chemical structures of (i) isorhamnetin (ii) kaempferol and (iii) quercetin

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

1. Quercetin 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 quercetin (Fig. 5).

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

Standard solutions

Isorhamnetin standard solution for fingerprinting, Std-FP (6 mg/L)

Weigh 0.3 mg of isorhamnetin CRS (Fig. 4) and dissolve in 50 mL of methanol.

Kaempferol standard solution for fingerprinting, Std-FP (3 mg/L)

Weigh 0.15 mg of kaempferol CRS (Fig. 4) and dissolve in 50 mL of methanol.

Quercetin standard solution for fingerprinting, Std-FP (4 mg/L)

Weigh 0.2 mg of quercetin CRS and dissolve in 50 mL of methanol.

Test solution

Weigh 0.2 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of 2% hydrochloric acid in methanol. Reflux the mixture for 30 min. Cool down to room temperature. Filter and transfer the filtrate to a 50-mL volumetric flask. Wash the residue with 3 mL of methanol. Repeat the extraction one more time. Combine the extracts 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 (364 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.5 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Methanol (%, v/v)	0.2% Phosphoric acid (%, v/v)	Elution
0 – 12	60	40	isocratic
12 – 20	60 → 65	40 → 35	linear gradient
20 – 40	65 → 70	35 → 30	linear gradient
40 – 60	70 → 74	30 → 26	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of isorhamnetin Std-FP, kaempferol Std-FP and quercetin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of isorhamnetin, kaempferol and quercetin should not be more than 5.0%; the RSD of the retention times of isorhamnetin, kaempferol and quercetin peaks should not be more than 2.0%; the column efficiencies determined from isorhamnetin, kaempferol and quercetin peaks should not be less than 20000 theoretical plates.

The *R* value between peak 1 and the closest peak; the *R* value between peak 2 and the closest peak; and 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 isorhamnetin Std-FP, kaempferol Std-FP, quercetin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention times of isorhamnetin, kaempferol and quercetin peaks in the chromatograms of isorhamnetin Std-FP, kaempferol Std-FP, quercetin Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify isorhamnetin, kaempferol and quercetin peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of isorhamnetin Std-FP, kaempferol Std-FP and quercetin Std-FP. The retention times of isorhamnetin, kaempferol and quercetin 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 four characteristic peaks of Tamaricis Cacumen extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Tamaricis Cacumen extract

Peak No.	RRT	Acceptable Range
1 (quercetin)	0.72	± 0.03
2 (marker, isorhamnetin)	1.00	-
3 (kaempferol)	1.08	± 0.03
4	1.13	± 0.03

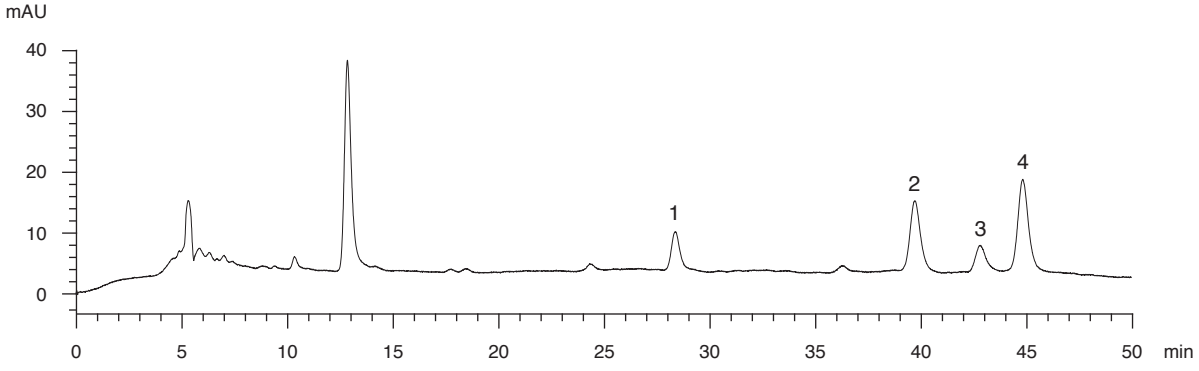


Figure 6 A reference fingerprint chromatogram of Tamaricis Cacumen extract

For positive identification, the sample must give the above four 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 2.0%.

5.6 Ash *(Appendix IX)*

Total ash: not more than 15.0%.
Acid-insoluble ash: not more than 3.0%.

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 20.0%.
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 isorhamnetin, kaempferol and quercetin standard stock solution, Std-Stock (330 mg/L for isorhamnetin, 210 mg/L for kaempferol and 220 mg/L for quercetin)

Weigh accurately 3.3 mg of isorhamnetin CRS, 2.1 mg of kaempferol CRS and 2.2 mg of quercetin CRS, and dissolve in 10 mL of methanol.

Mixed isorhamnetin, kaempferol and quercetin standard solution for assay, Std-AS

Measure accurately the volume of the mixed isorhamnetin, kaempferol and quercetin Std-Stock, dilute with methanol to produce a series of solutions of 0.65, 1.3, 2.6, 5.2, 10.4 mg/L for isorhamnetin, 0.4, 0.8, 1.6, 3.2, 6.4 mg/L for kaempferol and 0.4, 0.8, 1.6, 3.2, 6.4 for quercetin.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 20 mL of 2% hydrochloric acid in methanol. Reflux the mixture for 30 min. Cool down to room temperature. Filter and transfer the filtrate to a 50-mL volumetric flask. Wash the residue with 3 mL of methanol. Repeat the extraction one more time. Combine the extracts 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 (364 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.5 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	Methanol (%, v/v)	0.2% Phosphoric acid (%, v/v)	Elution
0 – 12	60	40	isocratic
12 – 20	60 → 65	40 → 35	linear gradient
20 – 40	65 → 70	35 → 30	linear gradient
40 – 60	70 → 74	30 → 26	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed isorhamnetin, kaempferol and quercetin Std-AS (2.6 mg/L for isorhamnetin, 1.6 mg/L for kaempferol and 1.6 mg/L for quercetin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of isorhamnetin, kaempferol and quercetin should not be more than 5.0%; the RSD of the retention times of isorhamnetin, kaempferol and quercetin peaks should not be more than 2.0%; the column efficiencies determined from isorhamnetin, kaempferol and quercetin peaks should not be less than 20000 theoretical plates.

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

Limits

The sample contains not less than 0.12% of the total content of isorhamnetin ($C_{16}H_{12}O_7$), kaempferol ($C_{15}H_{10}O_6$) and quercetin ($C_{15}H_{10}O_7$), calculated with reference to the dried substance.

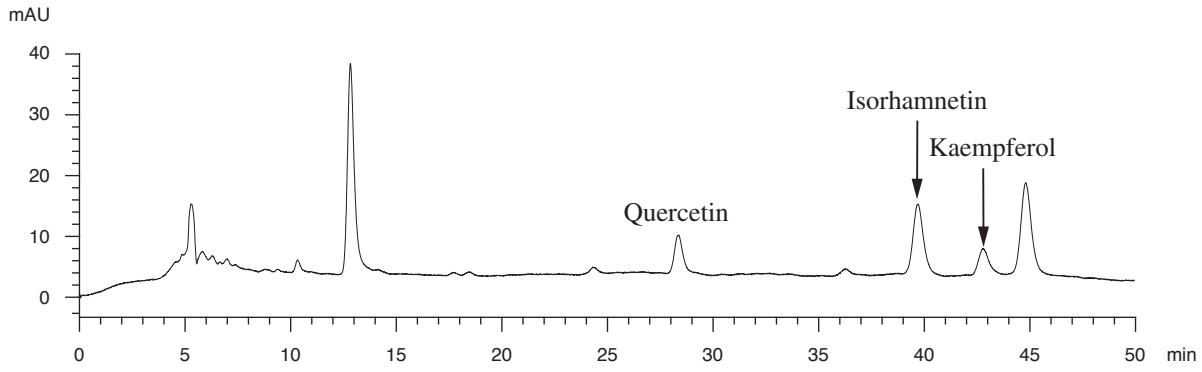


Figure 7 A reference assay chromatogram of Tamaricis Cacumen extract