Folium Ginkgo

Figure 1 A photograph of Folium Ginkgo

1. NAMES

Official Name: Folium Ginkgo

Chinese Name: 銀杏葉

Chinese Phonetic Name: Yinxingye

2. SOURCE

Folium Ginkgo is the dried leaf of *Ginkgo biloba* L. (Ginkgoaceae). The leaves are collected in autumn when they are still green, then dried immediately to obtain Folium Ginkgo.

3. DESCRIPTION

The leaves are yellowish-green to pale brownish-yellow, fan-shaped, 3.5-12 cm long, 3-15 cm wide, 2-lobed, with fine, dichotomous-parallel veins radiating out from the base, often crumpled or broken. Margins at the apical side broadly rounded, irregularly undulate, those at the leaf base side entire. Petioles 2-8 cm long. Texture light. Odour slight; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Petiole: Epidermis consists of 1 layer of cells, arranged orderly, covered with cuticle, sunken stomata occasionally visible. Collenchyma tissue present beneath the epidermis, mostly at the edges and corners of the petiole. Secretory canals are distributed in the peripheral region of the petiole. Cluster of calcium oxalate are present in the phloem. Vascular bundles collateral, in pairs (Fig. 2).

Leaf Blade: Upper epidermis consists of 1 layer of cells covered with cuticle. A secretory canal always present and is located in-between two vascular bundles. The parenchyma cells contain chloroplasts, some with cluster of calcium oxalate. Vascular bundles distributed evenly in the leaf blade, surrounded by a few fibres. Lower epidermis consists of 1 layer of cells, with sunken stomata visible (Fig. 2).

Powder

Colour yellowish-green. Cluster of calcium oxalates scattered, 23-55 µm in diameter, polychrome under the polarized microscope. Tracheids are bordered-pitted, occurring singly or bundled, 8-14 µm in diameter. Fibres dispersed singly or in bundles, 10-20 µm in diameter; Upper epidermal cells subrectangular with sinuate margin, up to 108 µm in length and 56 µm in width. Sunken stomata appear beneath epidermis (Fig. 3).

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

Standard solutions

Bilobalide standard solution

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

Ginkgolide A standard solution

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

Ginkgolide B standard solution

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

Ginkgolide C standard solution

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

Ginkgolide J standard solution

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

Developing solvent system

Prepare a mixture of cyclohexane, ethyl acetate, acetone, methanol (10:8:8:0.6, v/v).

Staining reagent

Acetic anhydride

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 (10%). Sonicate (240 W) the mixture for 30 min. Filter and transfer the solution to a 50-mL conical flask. Rinse the residue with 5 mL of methanol (2%). Filter and transfer the washings to the same 50-mL conical flask. Load the solution to a solid-phase extraction column containing ODS packing (6 mL, 1000 mg) pre-conditioned with 10 mL of methanol and 10 mL of methanol (2%). Collect the eluant in a 50-mL round-bottomed flask. Add 10 mL of methanol (50%) to the extraction column and collect the eluant in the same 50-mL round-bottomed flask. Evaporate the solvent to dryness at about 80˚C at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of methanol. Sonicate (240 W) the mixture for 5 min. Filter through a 0.45-µm nylon filter.

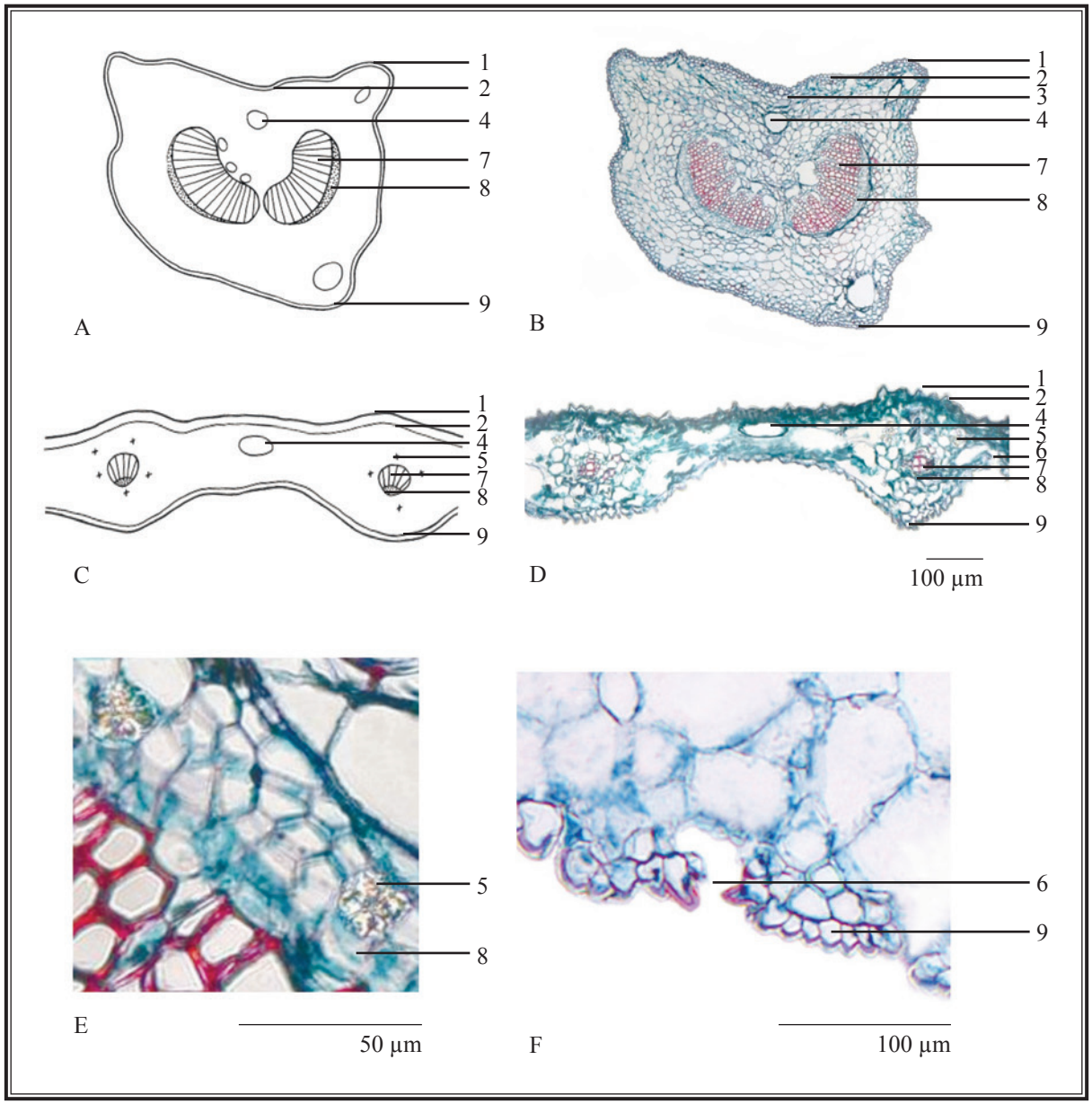


Figure 2 Microscopic features of transverse section of Folium Ginkgo

A. Sketch of petiole B. Section illustration of petiole C. Sketch of leaf blade
D. Section illustration of leaf blade E. Vascular bundle in petiole F. Stomata on the lower epidermis

1. Cuticle 2. Upper epidermis 3. Collenchyma tissue 4. Secretory canal
5. Cluster of calcium oxalate 6. Stoma 7. Xylem 8. Phloem 9. Lower epidermis

Figure 3 Microscopic features of powder of Folium Ginkgo

1. Clusters of calcium oxalate 2. Tracheids 3. Fibres 4. Upper epidermal cells
5. Stomata embedded in lower epidermis

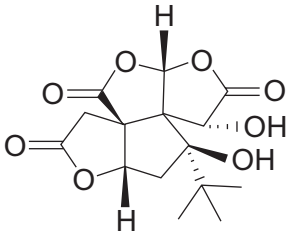
a. Features under the light microscope b. Features under the polarized microscope

Procedure

Carry out the method by using a HPTLC silica gel plate [immerse in sodium acetate solution (5%, w/v) for 20 s. Dry the plate at 70˚C for 30 min. Cool in a desiccator.], a twin trough chamber and freshly prepared developing solvent system as described above. Apply separately bilobalide standard solution, ginkgolide A standard solution, ginkgolide B standard solution, ginkgolide C standard solution and ginkgolide J standard solution (1 µL each) and the test solution (5 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. Fumigate the plate with acetic anhydride vapour for about 15 min and heat at about 140˚C for about 30 min. Examine the plate under UV light (366 nm). Calculate the R_f values by using the equation as indicated in Appendix IV(A).

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 bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J.

(i)



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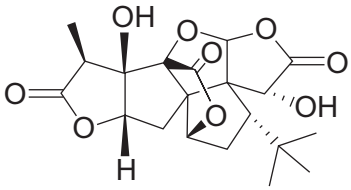

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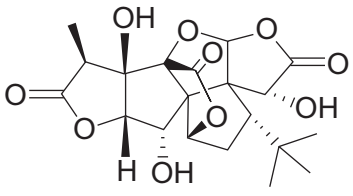

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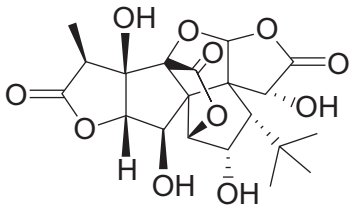
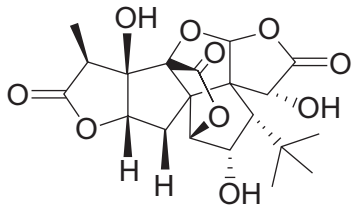
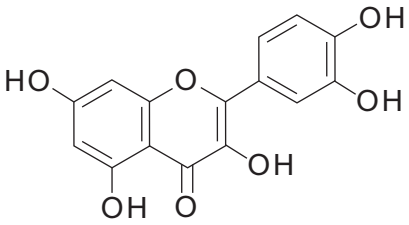


Figure 4 Chemical structures of (i) bilobalide (ii) ginkgolide A (iii) ginkgolide B (iv) ginkgolide C

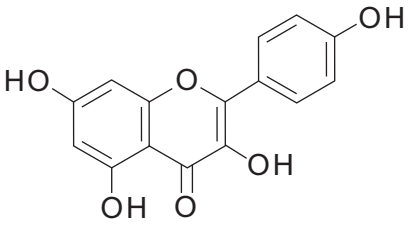
(v)



(vi)



(vii)



(viii)

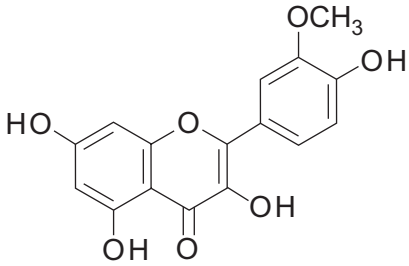


Figure 4 Chemical structures of (v) ginkgolide J (vi) quercetin (vii) kaempferol and (viii) isorhamnetin

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

Standard solutions

Bilobalide standard solution for fingerprinting, Std-FP (540 mg/L)

Weigh 5.4 mg of bilobalide CRS and dissolve in 10 mL of methanol (50%).

Ginkgolide A standard solution for fingerprinting, Std-FP (300 mg/L)

Weigh 3.0 mg of ginkgolide A CRS and dissolve in 10 mL of methanol (50%).

Ginkgolide B standard solution for fingerprinting, Std-FP (240 mg/L)

Weigh 2.4 mg of ginkgolide B CRS and dissolve in 10 mL of methanol (50%).

Ginkgolide C standard solution for fingerprinting, Std-FP (240 mg/L)

Weigh 2.4 mg of ginkgolide C CRS and dissolve in 10 mL of methanol (50%).

Ginkgolide J standard solution for fingerprinting, Std-FP (180 mg/L)

Weigh 1.8 mg of ginkgolide J CRS and dissolve in 10 mL of methanol (50%).

Reagent

Phosphate buffer solution

Weigh 1.19 g of dibasic sodium phosphate and 8.25 g of monobasic potassium phosphate and dissolve in 1000 mL of water. Adjust the pH to 5.8 with phosphoric acid.

Test solution

Weigh 2.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of methanol (90%). Sonicate (240 W) the mixture for 30 min. Centrifuge at about 4500 × *g* for 5 min. Transfer the supernatant to a 500-mL round-bottomed flask. Repeat the extraction for two more times. Combine the extracts. Evaporate the solvent to dryness at about 50˚C at reduced pressure in a rotary evaporator. Dissolve the residue in 10 mL of phosphate buffer solution. Sonicate (240 W) the mixture for 5 min. Transfer the extract to a clean-up column filled with chromatographic siliceous earth capable of holding 20 mL of aqueous phase. Rinse the round-bottomed flask with two 5-mL portion of phosphate buffer solution. Transfer the solution to the clean-up column. After 15 min, elute with 100 mL of ethyl acetate. Collect the eluant in a 200-mL round-bottomed flask. Evaporate the solvent to dryness at about 50˚C at reduced pressure in a rotary evaporator. Dissolve the residue in 5 mL of methanol and transfer the solution to a 10-mL volumetric flask. Rinse the flask with 5 mL of water and transfer the solution to the same volumetric flask. Sonicate (240 W) the mixture for 15 min 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 an ELSD [drift tube temperature: 102˚C; nebulizer gas (N_2) flow: 2.8 L/min] 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)	Water (%, v/v)	Methanol (%, v/v)	Elution
0 – 45	75 → 52	25 → 48	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 μL of bilobalide Std-FP, ginkgolide A Std-FP, ginkgolide B Std-FP, ginkgolide C Std-FP and ginkgolide J Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J should not be more than 5.0%; the RSD of the retention times of bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J peaks should not be more than 2.0%; the column efficiencies determined from bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J peaks should not be less than 15000, 35000, 40000, 20000 and 20000 theoretical plates respectively.

The *R* values between bilobalide peak, ginkgolide A peak, ginkgolide B peak, ginkgolide C peak, ginkgolide J peak and their corresponding closest peaks in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject bilobalide Std-FP, ginkgolide A Std-FP, ginkgolide B Std-FP, ginkgolide C Std-FP, ginkgolide J Std-FP and the test solution (20 µL each) into the HPLC system and record the chromatograms. Measure the retention times of bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J peaks in the chromatograms of the corresponding Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify the peaks of bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J in the chromatogram of the test solution by comparing their retention times with those in the chromatograms of the corresponding Std-FP. The retention times of the peaks of bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J in the chromatograms of the test solution and the corresponding Std-FP should not differ by more than 2.0%.

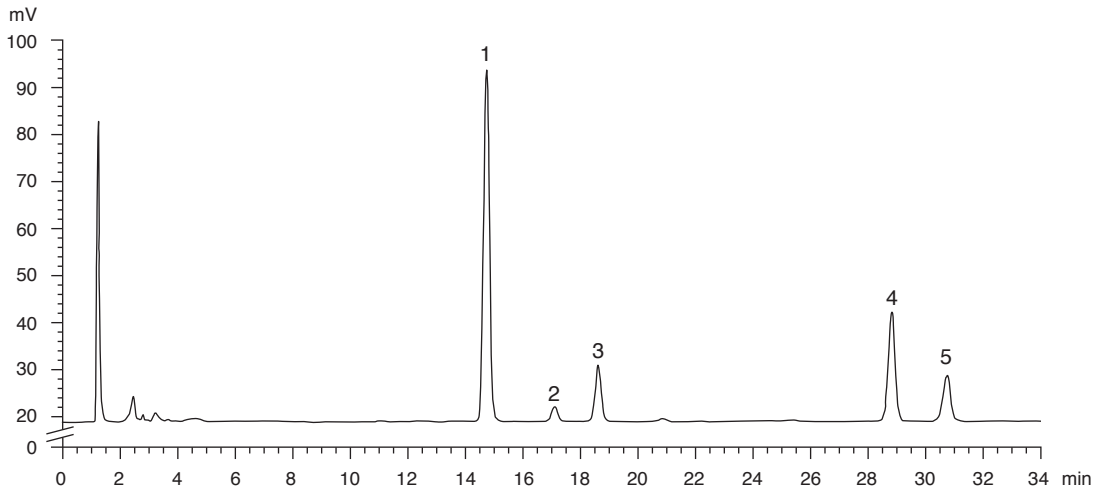


Figure 5 A reference fingerprint chromatogram of Folium Ginkgo extract

For positive identification, the sample must give the above five characteristic peaks (peak no. 1: bilobalide; peak no. 2: ginkgolide J; peak no. 3: ginkgolide C; peak no. 4: ginkgolide A; peak no. 5: ginkgolide B) with retention times of the corresponding peaks in the Std-FP chromatogram (Fig. 5).

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 XVIII)*: meet the requirements.

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

5.6 Ash *(Appendix IX)*

Total ash: not more than 12.5%.
Acid-insoluble ash: not more than 2.0%.

5.7 Water Content *(Appendix X)*: not more than 12.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 20.0%.
Ethanol-soluble extractives (hot extraction method): not less than 24.0%.

7. ASSAY

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

7.1 Assay of the total content of quercetin, kaempferol and isorhamnetin

Standard solution

Mixed quercetin, kaempferol and isorhamnetin standard stock solution, Std-Stock (100 mg/L for both quercetin and kaempferol and 40 mg/L for isorhamnetin)

Weigh accurately 5.0 mg of quercetin CRS (Fig. 4), 5.0 mg of kaempferol CRS (Fig. 4), 2.0 mg of isorhamnetin CRS (Fig. 4) and dissolve in 50 mL of methanol.

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

Measure accurately the volume of the mixed quercetin, kaempferol and isorhamnetin Std-Stock, dilute with methanol to produce a series of solutions of 2.5, 10, 20, 30, 40 mg/L for both quercetin and kaempferol, and 1, 4, 8, 12, 16 mg/L for isorhamnetin.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 250-mL round-bottomed flask, then add 80 mL of a mixture of ethanol, water and hydrochloric acid (50:20:8, v/v). Reflux the mixture for 1 h. Cool to room temperature. Centrifuge at about 4000 × *g* for 5 min. Transfer the supernatant to a 100-mL volumetric flask. Add 20 mL of methanol to the residue. Sonicate (240 W) the mixture for 30 min. Centrifuge at about 4000 × *g* for 5 min. Transfer the supernatant to the 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 (365 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. The mobile phase is a mixture of phosphoric acid (0.2%, v/v) and methanol (47:53, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 20 μL of the mixed quercetin, kaempferol and isorhamnetin Std-AS (10 mg/L for both quercetin and kaempferol, and 4 mg/L for isorhamnetin). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of quercetin, kaempferol and isorhamnetin should not be more than 5.0%; the RSD of the retention times of quercetin, kaempferol and isorhamnetin peaks should not be more than 2.0%; the column efficiencies determined from quercetin, kaempferol and isorhamnetin peaks should not be less than 6500, 9000 and 8500 theoretical plates respectively.

The *R* value between quercetin peak, kaempferol peak, isorhamnetin peak and their corresponding closest peaks in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

Inject a series of the mixed quercetin, kaempferol and isorhamnetin Std-AS (20 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of quercetin, kaempferol and isorhamnetin against the corresponding concentrations of the mixed quercetin, kaempferol and isorhamnetin 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 the peaks of quercetin, kaempferol and isorhamnetin in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed quercetin,

kaempferol and isorhamnetin Std-AS. The retention times of the peaks of quercetin, kaempferol and isorhamnetin in both chromatograms should not differ by more than 2.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of quercetin, kaempferol and isorhamnetin in the test solution, and calculate the percentage contents of quercetin, kaempferol and isorhamnetin in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.22% of the total content of quercetin ($C_{15}H_{10}O_7$), kaempferol ($C_{15}H_{10}O_6$) and isorhamnetin ($C_{16}H_{12}O_7$), calculated with reference to the dried substance.

7.2 Assay of the total content of bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J

Standard solution

Mixed bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J standard stock solution, Std-Stock (900 mg/L for bilobalide, 500 mg/L for ginkgolide A, 400 mg/L for ginkgolide B, 400 mg/L for ginkgolide C and 300 mg/L for ginkgolide J)

Weigh accurately 4.5 mg of bilobalide CRS, 2.5 mg of ginkgolide A CRS, 2.0 mg of ginkgolide B CRS, 2.0 mg of ginkgolide C CRS and 1.5 mg of ginkgolide J CRS and dissolve in 5 mL of methanol (50%). Sonicate (240 W) the mixture to dissolve the standards.

Mixed bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J standard solution for assay, Std-AS

Measure accurately the volume of the mixed bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J Std-Stock, dilute with methanol (50%) to produce a series of solutions of 234, 360, 540, 720, 900 mg/L for bilobalide, 130, 200, 300, 400, 500 mg/L for ginkgolide A, 104, 160, 240, 320, 400 mg/L for both ginkgolide B and ginkgolide C, and 78, 120, 180, 240, 300 mg/L for ginkgolide J.

Reagent

Phosphate buffer solution

Weigh 1.19 g of dibasic sodium phosphate and 8.25 g of monobasic potassium phosphate and dissolve in 1000 mL of water. Adjust the pH to 5.8 with phosphoric acid.

Test solution

Weigh accurately 2.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 40 mL of methanol (90%). Sonicate (240 W) the mixture for 30 min. Centrifuge at about 4500 × *g* for 5 min. Transfer the supernatant to a 500-mL round-bottomed flask. Repeat the extraction for two more times. Combine the extracts. Evaporate the solvent to dryness at about

50˚C at reduced pressure in a rotary evaporator. Dissolve the residue in 10 mL of phosphate buffer solution. Sonicate (240 W) the mixture for 5 min. Transfer the extract to a clean-up column filled with chromatographic siliceous earth capable of holding 20 mL of aqueous phase. Rinse the round-bottomed flask with two 5-mL portion of phosphate buffer solution. Transfer the solution to the clean-up column. After 15 min, elute with 100 mL of ethyl acetate. Collect the eluant in a 200-mL round-bottomed flask. Evaporate the solvent to dryness at about 50˚C at reduced pressure in a rotary evaporator. Dissolve the residue in 5 mL of methanol and transfer the solution to a 10-mL volumetric flask. Rinse the flask with 5 mL of water and transfer the solution to the same volumetric flask. Sonicate (240 W) the mixture for 15 min 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 an ELSD [drift tube temperature: 102˚C; nebulizer gas (N_2) flow: 2.8 L/min] 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 2) –

Table 2 Chromatographic system conditions

Time (min)	Water (%, v/v)	Methanol (%, v/v)	Elution
0 – 10	75 → 52	25 → 48	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 µL of the mixed bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J Std-AS (540 mg/L for bilobalide, 300 mg/L for ginkgolide A, 240 mg/L for both ginkgolide B and ginkgolide C, and 180 mg/L ginkgolide J). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J should not be more than 5.0%; the RSD of the retention times of bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J peaks should not be more than 2.0%; the column efficiencies determined from bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J peaks should not be less than 15000, 35000, 40000, 20000 and 20000 theoretical plates respectively.

The *R* values between bilobalide peak, ginkgolide A peak, ginkgolide B peak, ginkgolide C peak, ginkgolide J peak and their corresponding closest peaks in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

Inject a series of the mixed bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the natural logarithm of peak areas of bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J against the natural logarithm of the corresponding concentrations of the mixed bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J 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 the peaks of bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J Std-AS. The retention times of the peaks of bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J in both chromatograms should not differ by more than 2.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J in the test solution by using the following equation –

$$\text{Concentration of bilobalide, ginkgolide A, ginkgolide B, ginkgolide C or ginkgolide J in the test solution} = e^{[\text{Ln (A)}-I]/m}$$

Where A = the peak area of bilobalide, ginkgolide A, ginkgolide B, ginkgolide C or ginkgolide J in the test solution,

I = the y-intercept of the 5-point calibration curve of bilobalide, ginkgolide A, ginkgolide B, ginkgolide C or ginkgolide J,

m = the slope of the 5-point calibration curve of bilobalide, ginkgolide A, ginkgolide B, ginkgolide C or ginkgolide J.

Calculate the percentage contents of bilobalide, ginkgolide A, ginkgolide B, ginkgolide C and ginkgolide J in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.32% of the total content of bilobalide ($C_{15}H_{18}O_8$), ginkgolide A ($C_{20}H_{24}O_9$), ginkgolide B ($C_{20}H_{24}O_{10}$), ginkgolide C ($C_{20}H_{24}O_{11}$) and ginkgolide J ($C_{20}H_{24}O_{10}$), calculated with reference to the dried substance.

Supplementary Information

Add the TLC Identification & HPLC Fingerprinting to read:

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

Standard solution

Rutin standard solution

Weigh 1.5 mg of rutin CRS (Fig. 1) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of ethyl acetate, water, formic acid and glacial acetic acid (67.5:17.5:7.5:7.5, v/v).

Spray reagents

Spray reagent 1

Weigh 0.1 g of 2-aminoethyl diphenylborinate and dissolve in 10 mL of methanol.

Spray reagent 2

Weigh 0.5 g of polyethylene glycol 400 and dissolve in 10 mL of ethanol.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of methanol. Sonicate (240 W) the mixture for 30 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate, a twin trough chamber and freshly prepared developing solvent system as described above. Apply separately rutin standard solution (2 µL) and the test solution (10 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 20 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 8.5 cm. After the development, remove the plate from the chamber, mark the solvent front and heat at about 105º C (about 3 min). Spray the plate evenly with the spray reagent 1 and the spray reagent 2. Dry the plate in air until the spots or bands become visible (about 30 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 rutin.

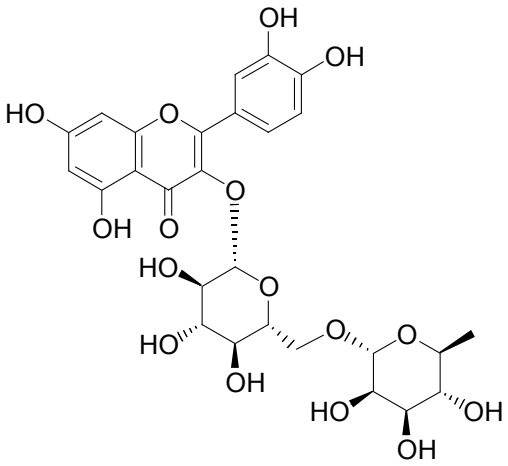


Figure 1 Chemical structure of rutin

2. High-Performance Liquid Chromatographic Fingerprinting *(Appendix XII)*

Standard solution

Rutin standard solution for fingerprinting, Std-FP (60 mg/L)

Weigh 0.6 mg of rutin CRS and dissolve in 10 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 (60%). Sonicate (240 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 (360 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)	0.1% Formic acid (%, v/v)	Formic acid : Acetonitrile (0.1:99.9, v/v) (%, v/v)	Elution
0 – 40	90 → 74	10 → 26	linear gradient
40 – 60	74 → 58	26 → 42	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of rutin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of rutin should not be more than 5.0%; the RSD of the retention time of rutin peak should not be more than 2.0%; the column efficiency determined from rutin peak should not be less than 40000 theoretical plates.

The *R* value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 2).

Procedure

Separately inject rutin Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of rutin peak in the chromatogram of rutin Std-FP and the retention times of the eight characteristic peaks (Fig. 2) in the chromatogram of the test solution. Identify rutin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of rutin Std-FP. The retention times of rutin 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 eight characteristic peaks of Folium Ginkgo extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the eight characteristic peaks of Folium Ginkgo extract

Peak No.	RRT	Acceptable Range
1	0.26	± 0.03
2	0.79	± 0.03
3 (marker, rutin)	1.00	-
4	1.03	± 0.03
5	1.17	± 0.03
6	1.20	± 0.03
7	1.49	± 0.03
8	1.66	± 0.03

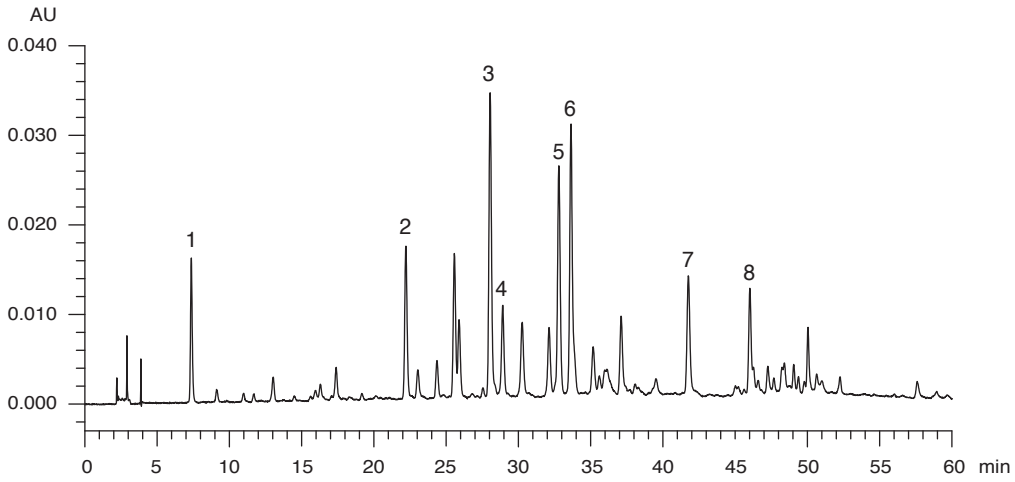


Figure 2 A reference fingerprint chromatogram of Folium Ginkgo extract

For positive identification, the sample must give the above eight characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 2).