Sambuci Williamsii Ramulus

Figure 1 A photograph of Sambuci Williamsii Ramulus

A. Sambuci Williamsii Ramulus
B. Magnified image of surface (upper: stem, lower: branch)
C. Magnified image of cut surface (left: stem, right: branch)

1. NAMES

Official name: Sambuci Williamsii Ramulus

Chinese name: 接骨木

Chinese phonetic name: Jiegumu

2. SOURCE

Sambuci Williamsii Ramulus is the dried stem and branch of *Sambucus williamsii* Hance (Caprifoliaceae*). The stem and branch are collected all year round, cut into section when fresh, then dried under the sun to obtain Sambuci Williamsii Ramulus.

3. DESCRIPTION

Cylindrical, some cut into half, 7-83 mm in diameter. Surface brown to dark brown, with longitudinal cracks, lenticel elliptical, protruding; old stem with deep cracks, young one and branch relatively smooth. Light in weight, texture very hard. Bark narrow in cut surface, brown, easily stripped off, wood yellowish-white, pith pale brown; annual rings distinct on smooth cut surface. Odour slight; taste slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Cork consists of dozens to hundreds layers of cells, varying in thickness. Cortex narrow, cells with slightly thickened walls. Phloem with phloem fibres scattered singly or in groups. Xylem broad, consisting of vessels and fibres; xylem ray consists of 1-4 rows of cells. Pith consists of parenchymatous cells, some containing reddish-brown masses. Microcrystals of calcium oxalate scattered in parenchymatous cells of phloem and pith [Fig. 2(i) and (ii)].

Footnote: Microscopic features of transverse section have no significant differences between the stem and branch.

* Adoxaceae is used by APG IV (2016).

Powder

Colour pale brown. Microcrystals of calcium oxalate scattered or present in parenchymatous cells, prismatic or triangular; polychromatic or bright white under the polarized microscope. Xylem fibre numerous, usually in bundles, 11-47 μm in diameter, wall thickened, with sparse oblique pits; bright white or polychromatic under the polarized microscope. Phloem fibre singly scattered or in bundles, 11-52 μm in diameter, pale yellow or yellow, wall always extremely thickened, lumen linear, margin sometimes uneven; orange or bright white under the polarized microscope. Xylem parenchymatous cells square or rectangular, with distinct pit canals and pit, wall slightly thickened. Cork cells usually broken, brown or yellowish-brown, subpolygonal or subrectangular in surface view. Mainly bordered-pitted vessels, usually broken, 13-133 μm in diameter. Reddish-brown mass irregular in shape (Fig. 3).

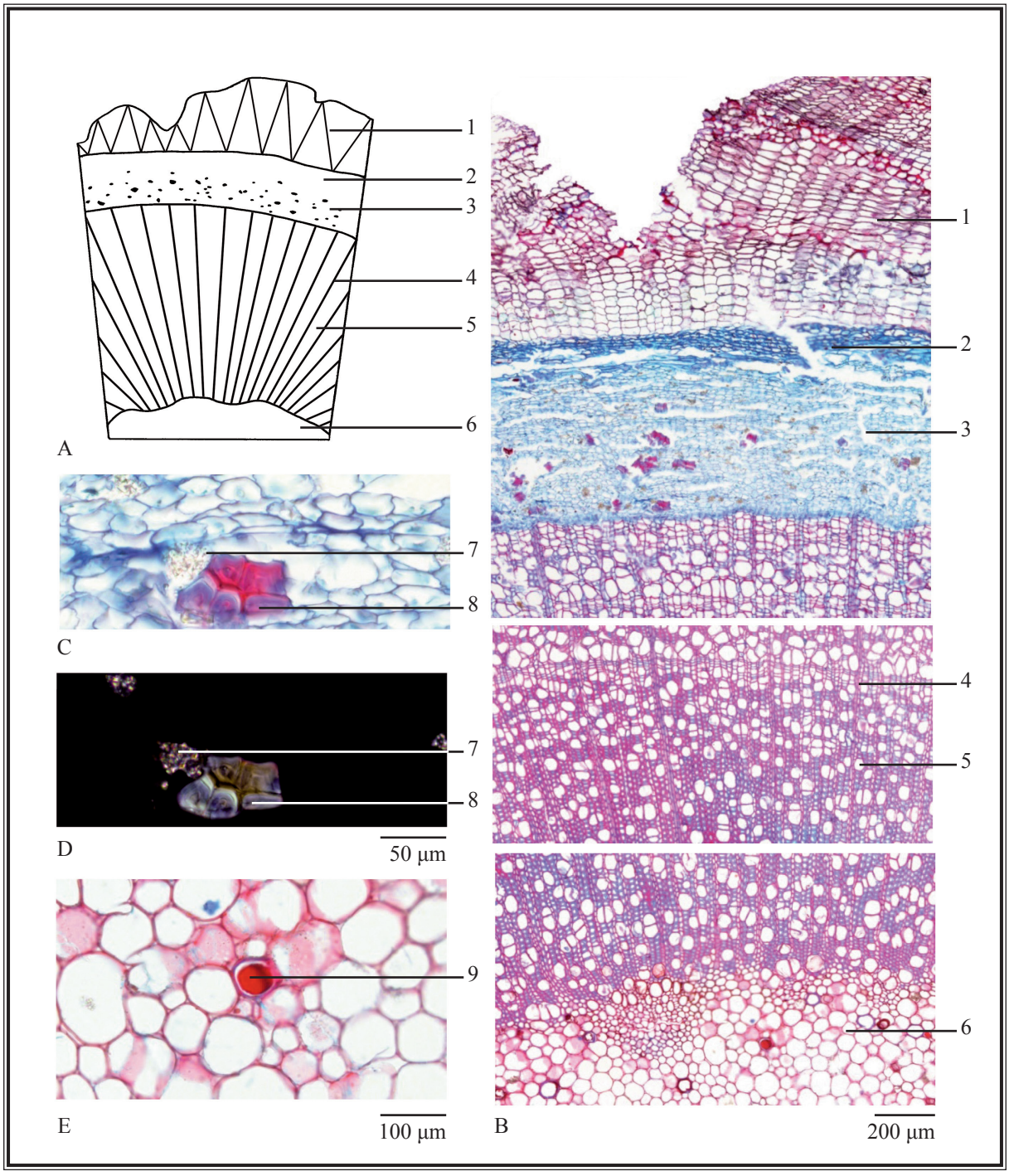


Figure 2 (i) Microscopic features of transverse section of stem of Sambuci Williamsii Ramulus

A. Sketch B. Section illustration

C. Microcrystals of calcium oxalate and phloem fibres (under the light microscope)

D. Microcrystals of calcium oxalate and phloem fibres (under the polarized microscope)

E. Magnified image of pith

1. Cork 2. Cortex 3. Phloem 4. Xylem ray 5. Xylem 6. Pith

7. Microcrystals of calcium oxalate 8. Phloem fibre 9. Reddish-brown mass

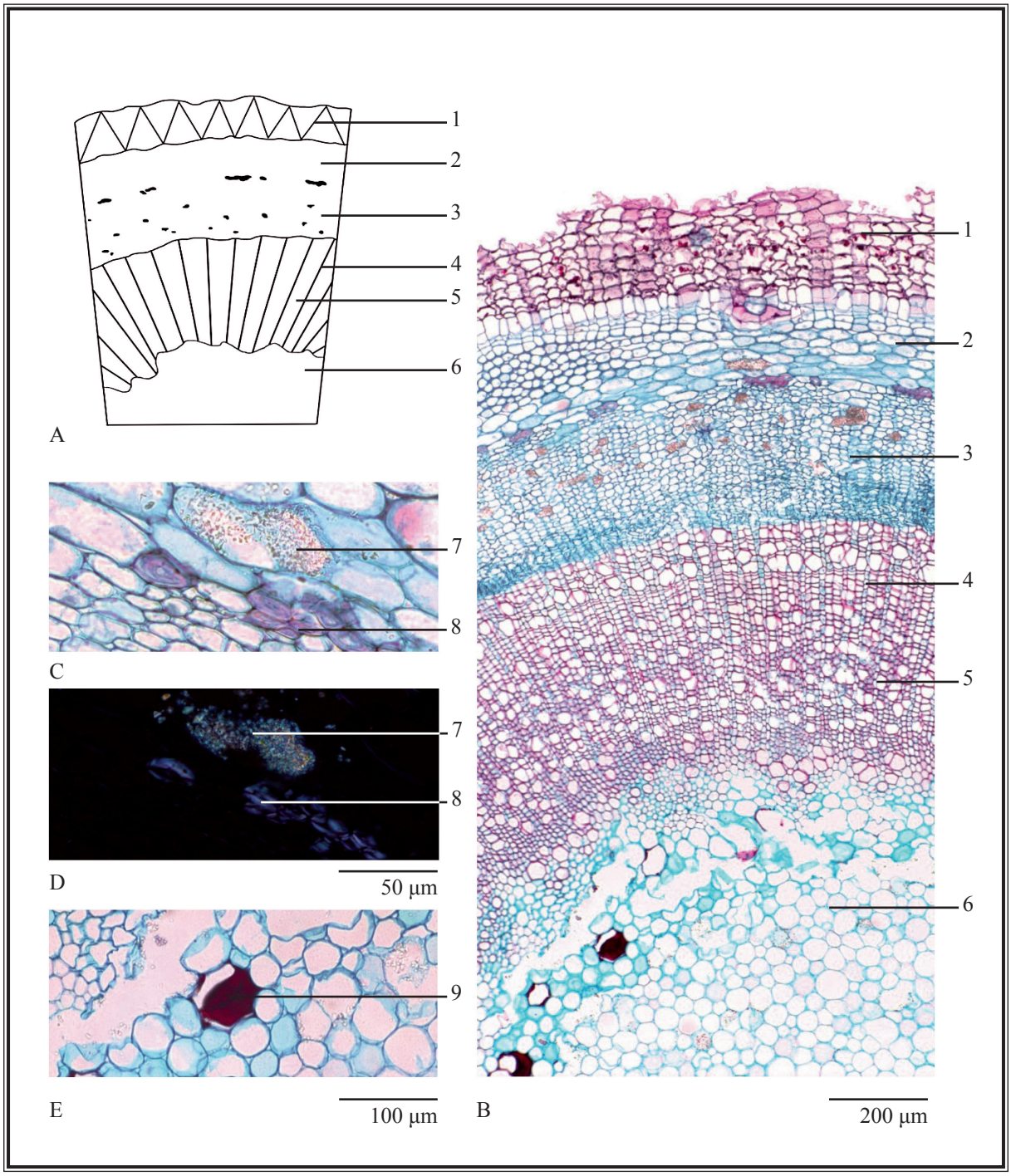


Figure 2 (ii) Microscopic features of transverse section of branch of Sambuci Williamsii Ramulus

A. Sketch B. Section illustration

C. Microcrystals of calcium oxalate and phloem fibres (under the light microscope)

D. Microcrystals of calcium oxalate and phloem fibres (under the polarized microscope)

E. Magnified image of pith

1. Cork 2. Cortex 3. Phloem 4. Xylem ray 5. Xylem 6. Pith

7. Microcrystals of calcium oxalate 8. Phloem fibre 9. Reddish-brown mass

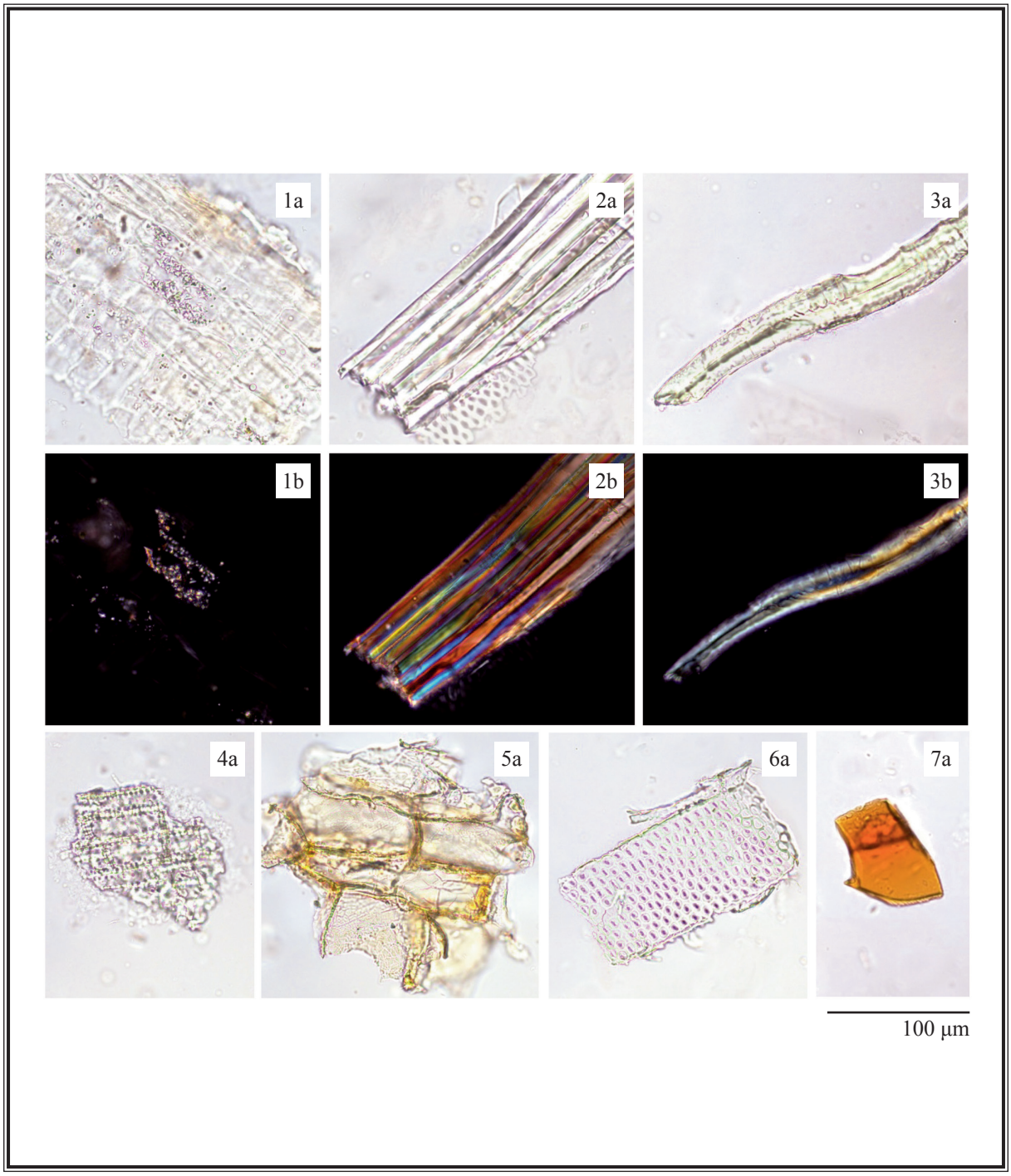


Figure 3 Microscopic features of powder of Sambuci Williamsii Ramulus

1. Microcrystals of calcium oxalate 2. Xylem fibres 3. Phloem fibre
4. Xylem parenchymatous cells 5. Cork cells 6. Bordered-pitted vessel
7. Reddish-brown mass

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

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

Standard solution

Lariciresinol standard solution

Weigh 0.6 mg of lariciresinol CRS (Fig. 4) and dissolve in 10 mL of ethanol (70%).

Developing solvent system

Prepare a mixture of dichloromethane, ethyl acetate and formic acid (24:7:1, v/v).

Spray reagent

Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

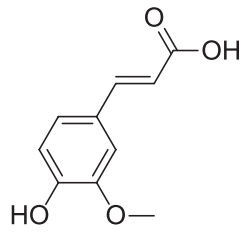
Test solution

Weigh 1.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 30 mL of ethanol (70%). Reflux the mixture for 30 min. Cool down to room temperature. Filter and transfer the filtrate to a 100-mL round-bottomed flask. Evaporate the filtrate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 5 mL of water. Transfer the extract to a 15-mL centrifuge tube. Centrifuge at about 2800 × *g* for 10 min. Pre-condition a solid phase extraction (SPE) column containing OS packing (12 mL, 2 g) with 30 mL of ethanol and then followed by 30 mL of water. Load 3 mL of the supernatant to the pre-conditioned SPE column. Add 10 mL of water to the column and discard the eluant. Add 10 mL of ethanol (30%) to the column. Collect the eluant and transfer to a 50-mL round-bottomed flask. Evaporate the eluant to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol (30%). Filter through a 0.22-μm PTFE filter.

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 lariciresinol standard solution (15 μL) and the test solution (25 μL) to the plate. Develop over a path of about 8.5 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 until the spots or bands become visible (about 3 – 5 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

(i)



(ii)

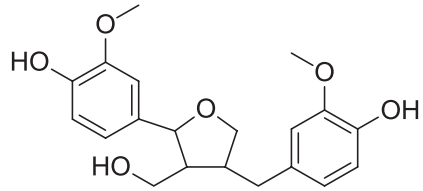


Figure 4 Chemical structures of (i) (*E*)-ferulic acid and (ii) lariciresinol

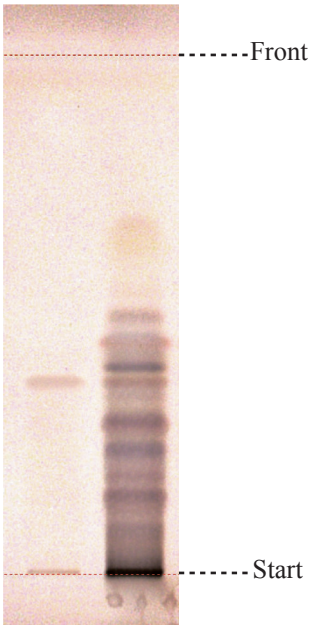


Figure 5 A reference HPTLC chromatogram of Sambuci Williamsii Ramulus extract observed under visible light after staining

1. Lariciresinol 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 lariciresinol (Fig. 5).

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

Standard solutions

(E)-Ferulic acid standard solution for fingerprinting, Std-FP (50 mg/L)

Weigh 0.5 mg of (*E*)-ferulic acid CRS (Fig. 4) and dissolve in 10 mL of ethanol (70%).

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

Weigh 1.5 mg of lariciresinol CRS and dissolve in 100 mL of ethanol (70%).

Test solution

Weigh 1.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 30 mL of ethanol (70%). Reflux the mixture for 30 min. Cool down to room temperature. Transfer the extract to a 50-mL centrifuge tube. Centrifuge at about 2800 × *g* for 10 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Repeat the extraction two more times. Combine the filtrates. Evaporate the combined filtrates to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in ethanol (70%). Transfer the extract to a 5-mL volumetric flask and make up to the mark with ethanol (70%). Filter through a 0.22-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (280 nm) and a column (2.1 × 100 mm) packed with OS bonded silica gel (1.7 μm particle size). The column temperature is maintained at 40ºC during the separation. The flow rate is about 0.35 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.1% Formic acid (%, v/v)	Acetonitrile : Isopropanol : Formic acid (9:1:0.01, v/v) (%, v/v)	Elution
0 – 2	88	12	isocratic
2 – 4	88 → 85	12 → 15	linear gradient
4 – 8	85 → 78	15 → 22	linear gradient
8 – 15	78 → 75	22 → 25	linear gradient
15 – 20	75 → 65	25 → 35	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 2 µL of (*E*)-ferulic acid Std-FP and lariciresinol Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of (*E*)-ferulic acid and lariciresinol should not be more than 5.0%; the RSD of the retention times of (*E*)-ferulic acid and lariciresinol peaks should not be more than 2.0%; the column efficiencies determined from (*E*)-ferulic acid and lariciresinol peaks should not be less than 3000 and 40000 theoretical plates respectively.

The *R* value between peak 1 and the closest peak; and the *R* value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure

Separately inject (*E*)-ferulic acid Std-FP, lariciresinol Std-FP and the test solution (2 µL each) into the UHPLC system and record the chromatograms. Measure the retention times of (*E*)-ferulic acid and lariciresinol peaks in the chromatograms of (*E*)-ferulic acid Std-FP, lariciresinol Std-FP and the retention times of the five characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify (*E*)-ferulic acid and lariciresinol peaks in the chromatogram of the test solution by comparing its retention time with that in the chromatograms of (*E*)-ferulic acid Std-FP and lariciresinol Std-FP. The retention times of (*E*)-ferulic acid and lariciresinol 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 Sambuci Williamsii Ramulus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the five characteristic peaks of Sambuci Williamsii Ramulus extract

Peak No.	RRT	Acceptable Range
1 [(*E*)-ferulic acid]	0.48	± 0.06
2 (marker, lariciresinol)	1.00	-
3	1.05	± 0.03
4	1.07	± 0.03
5	1.09	± 0.03

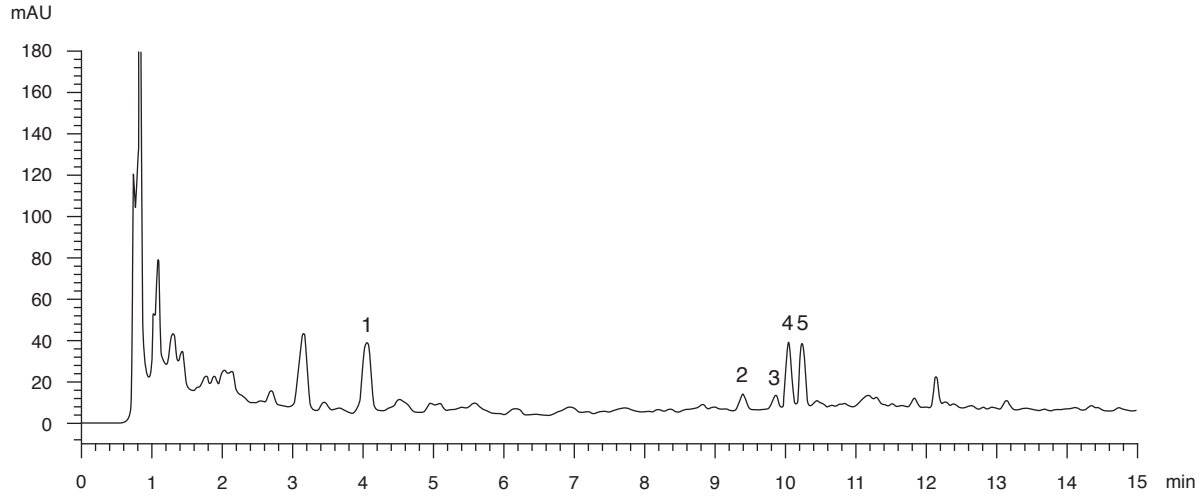


Figure 6 A reference fingerprint chromatogram of Sambuci Williamsii Ramulus extract

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 3.5%.
Acid-insoluble ash: not more than 0.5%.

5.7 Water Content *(Appendix X)*

Oven dried method: not more than 9.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 2.0%.
Ethanol-soluble extractives (cold extraction method): not less than 3.0%

7. ASSAY

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

Standard solution

Mixed (E)-ferulic acid and lariciresinol standard stock solution, Std-Stock [800 mg/L for (E)-ferulic acid and 240 mg/L for lariciresinol]

Weigh accurately 4.0 mg of (*E*)-ferulic acid CRS and 1.2 mg of lariciresinol CRS, and dissolve in 5 mL of ethanol (70%).

Mixed (E)-ferulic acid and lariciresinol standard solution for assay, Std-AS

Measure accurately the volume of the mixed (*E*)-ferulic acid and lariciresinol Std-Stock, dilute with ethanol (70%) to produce a series of solutions of 4, 6, 25, 50, 100 mg/L for (*E*)-ferulic acid and 3, 7.5, 15, 30, 60 mg/L for lariciresinol.

Test solution

Weigh accurately 1.0 g of the powdered sample and place it in a 100-mL round-bottomed flask, then add 30 mL of ethanol (70%). Reflux the mixture for 30 min. Cool down to room temperature. Transfer the extract to a 50-mL centrifuge tube. Centrifuge at about 2800 × *g* for 10 min. Filter and transfer the filtrate to a 250-mL round-bottomed flask. Repeat the extraction two more times. Combine the filtrates. Evaporate the combined filtrates to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in ethanol (70%). Transfer the extract to a 5-mL volumetric flask and make up to the mark with ethanol (70%). Filter through a 0.22-μm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (280 nm) and a column (2.1 × 100 mm) packed with OS bonded silica gel (1.7 µm particle size). The column temperature is maintained at 40ºC during the separation. The flow rate is about 0.35 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Formic acid (%, v/v)	Acetonitrile : Isopropanol : Formic acid (9:1:0.01, v/v) (%, v/v)	Elution
0 – 2	88	12	isocratic
2 – 4	88 → 85	12 → 15	linear gradient
4 – 8	85 → 78	15 → 22	linear gradient
8 – 15	78 → 75	22 → 25	linear gradient
15 – 20	75 → 65	25 → 35	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 2 µL of the mixed (*E*)-ferulic acid and lariciresinol Std-AS [25 mg/L for (*E*)-ferulic acid and 15 mg/L for lariciresinol]. The requirements of the system suitability parameters are as follows: the RSD of the peak areas of (*E*)-ferulic acid and lariciresinol should not be more than 5.0%; the RSD of the retention times of (*E*)-ferulic acid and lariciresinol peaks should not be more than 2.0%; the column efficiencies determined from (*E*)-ferulic acid and lariciresinol peaks should not be less than 3000 and 40000 theoretical plates respectively.

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

Procedure

Inject 2 µL of the test solution into the UHPLC system and record the chromatogram. Identify (*E*)-ferulic acid and lariciresinol peaks (Fig. 7) in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed (*E*)-ferulic acid and lariciresinol Std-AS. The retention times of (*E*)-ferulic acid and lariciresinol 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 (*E*)-ferulic acid and lariciresinol in the test solution, and calculate the percentage contents of (*E*)-ferulic acid and lariciresinol in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.014% of the total content of (*E*)-ferulic acid ($C_{10}H_{10}O_4$) and lariciresinol ($C_{20}H_{24}O_6$), calculated with reference to the dried substance.

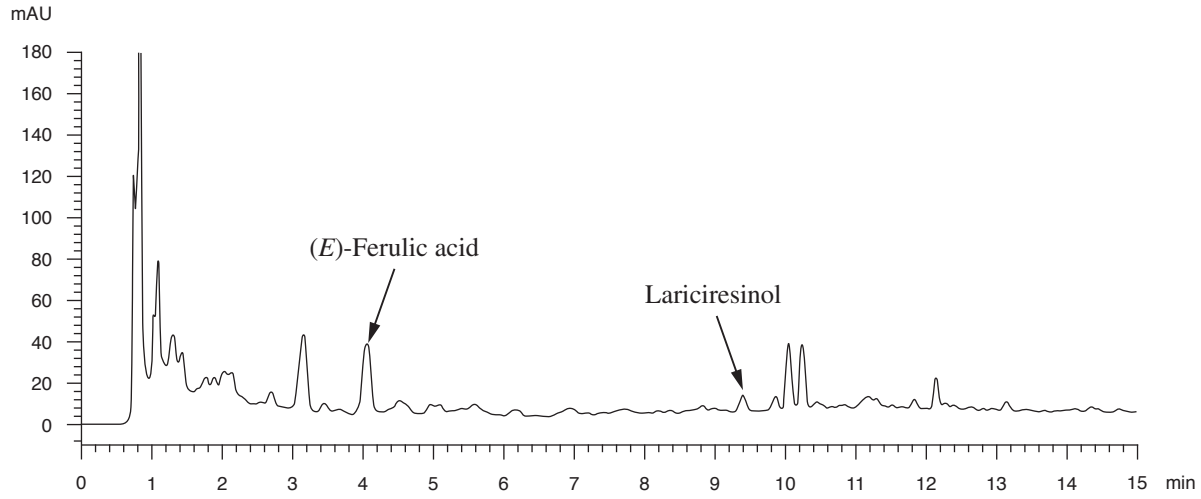


Figure 7 A reference assay chromatogram of Sambuci Williamsii Ramulus extract