

# Equiseti Hiemalis Herba



**Figure 1** A photograph of Equiseti Hiemalis Herba

A. Equiseti Hiemalis Herba B. Magnified stem surface

C. Magnified transverse section of stem



4. IDENTIFICATION

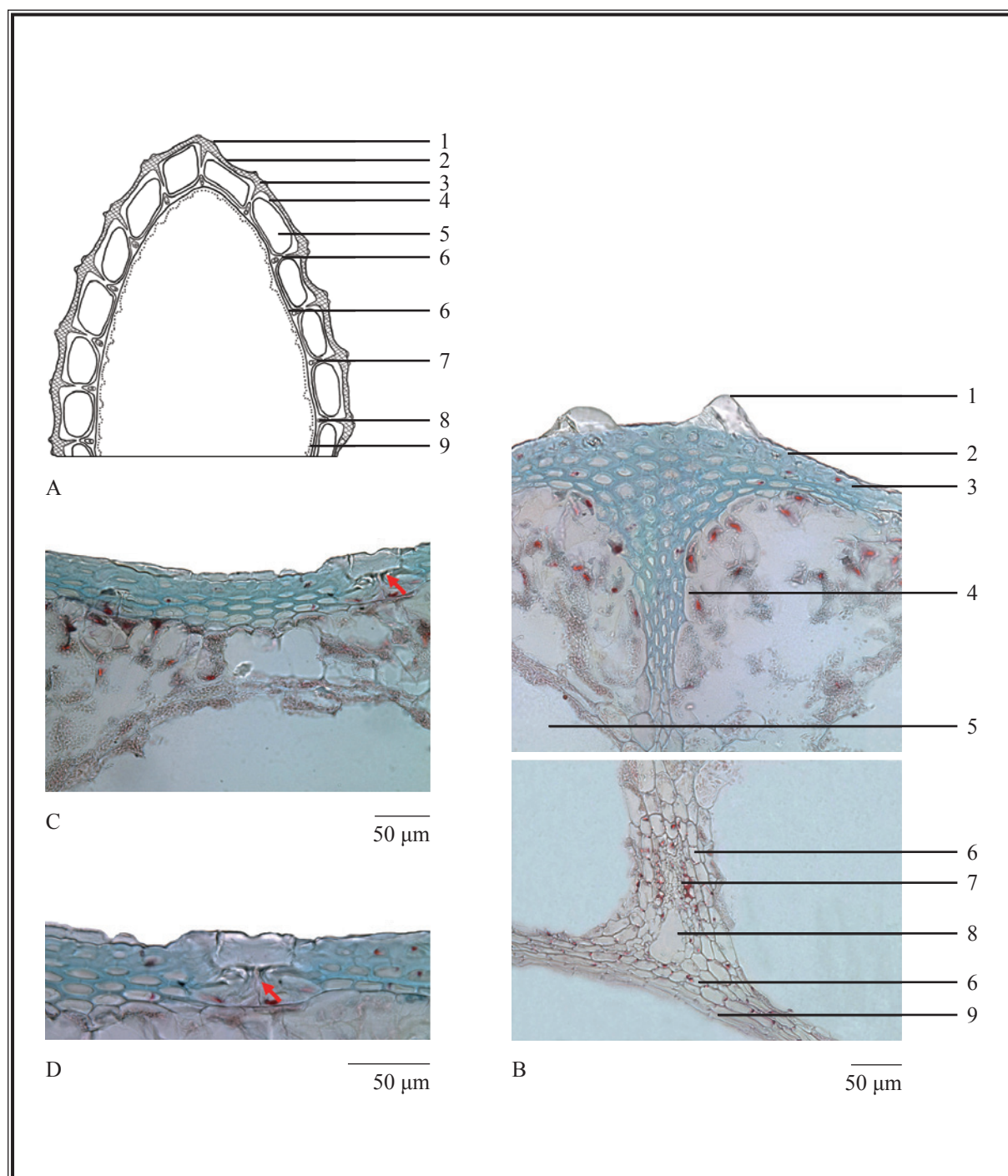
4.1 Microscopic Identification (Appendix III)

Transverse section

**Stem:** Epidermis consists of 1 layer of cells, covered with cuticle, surface with grooves and ridges. 2 transparent silica protuberances located on the each ridges; 2 sunken stomata located in each groove. Sclerenchyma occurring inside ridges and stretching into cortex parenchyma, in cuneate-shape. Sclerenchymatous cells located in grooves, 1-3 layers. Cortex consists of parenchymatous cells, cells subrounded or long cylindrical. An empty lumen located in each groove. Endodermis consists of 2 layers of cells, outer layer in a sinuous ring, and inner layer in a rounded ring, casparian dots distinct in both layers. Vascular bundles collateral, located between 2 layers of endodermis, and beneath the ridges. A centre lumen located in inner part of each vascular bundle. Pith parenchymatous cells located inside endodermis, flattened and shrunken (Fig. 2).

Powder

Colour green to greenish-brown. Epidermal cells elongated rectangular, walls thickened, stomata subrounded or elliptical, 70-85 μm in diameter, with many thickened strips in guard cells. Endodermal cells elongated rectangular, walls slightly thickened. Tracheids mainly spiral and scalariform. Epidermal cells of leaf sheath brown, elongated rectangular, walls thickened (Fig. 3).



**Figure 2** Microscopic features of transverse section of stem of *Equiseti Hiemalis Herba*

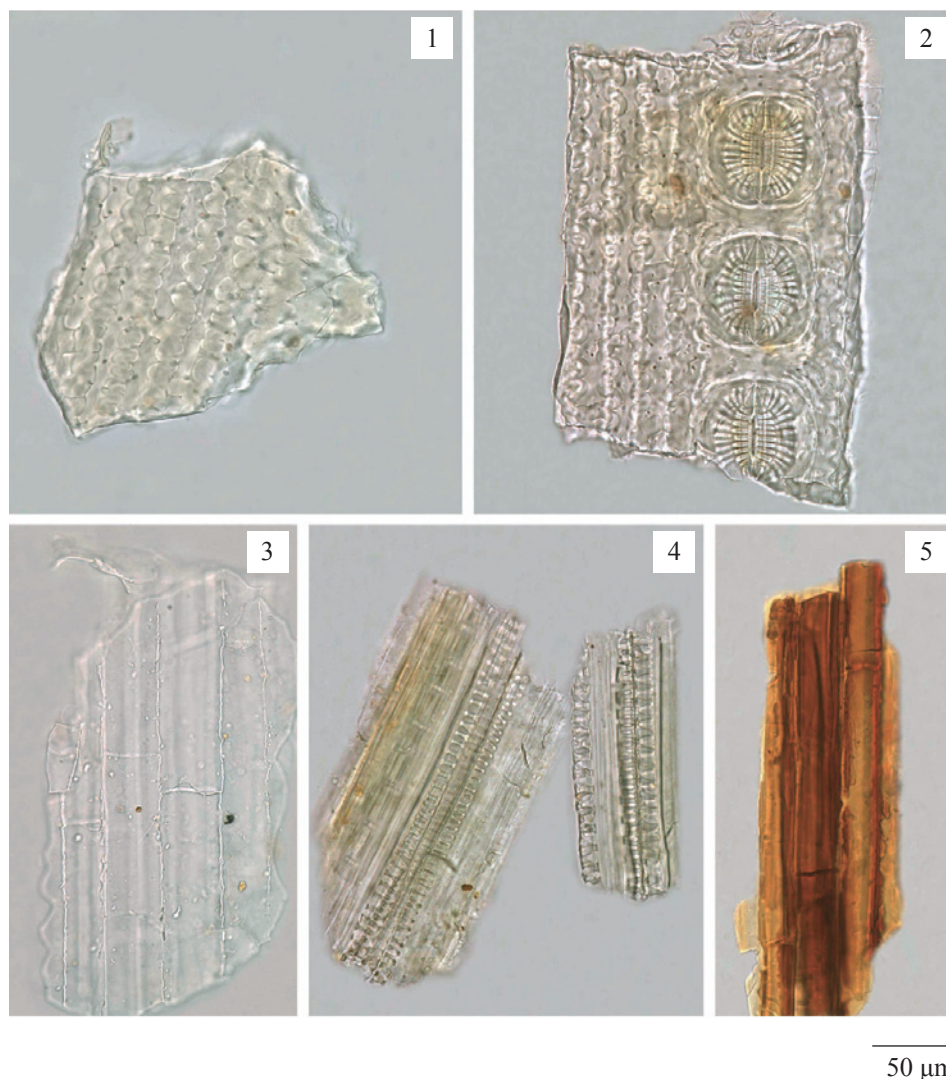
A. Sketch B. Section illustration

C. Section illustration of the part with sunken stomata D. Sunken stoma magnified

1. Silica protuberances 2. Epidermis 3. Sclerenchyma 4. Cortex 5. Lumen

6. Endodermis 7. Vascular bundles 8. Centre lumen in inner part of vascular bundle

9. Pith parenchymatous cells



**Figure 3** Microscopic features of powder of *Equiseti Hiemalis Herba* (under the light microscope)

1. Stem epidermal cells (in surface view)
2. Stem epidermal cells with sunken stomata
3. Endodermal cells
4. Tracheids
5. Leaf sheath epidermal cells

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

### Standard solution

*Kaempferol 3-β-sophoroside standard solution*

Weigh 1.0 mg of kaempferol 3-β-sophoroside CRS (Fig. 4) and dissolve in 0.5 mL of ethanol.

### Developing solvent system

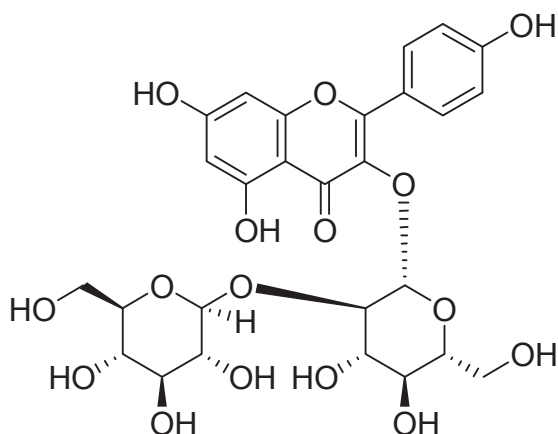
Prepare a mixture of *n*-butanol, glacial acetic acid and water (14:1:0.5, v/v).

### Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of ethanol (70%). Sonicate (270 W) the mixture for 1 h. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of ethanol (70%).

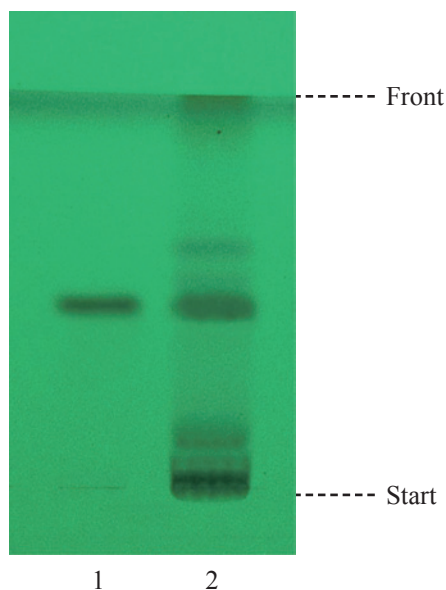
### Procedure

Carry out the method by using a HPTLC silica gel F<sub>254</sub> plate and a freshly prepared developing solvent system as described above. Apply separately kaempferol 3-β-sophoroside standard solution and the test solution (1 μL each) to the plate. Develop over a path of about 4 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Heat at about 105°C until the spots or bands become visible (about 5 min). Examine the plate under UV light (254 nm). Calculate the *R<sub>f</sub>* value by using the equation as indicated in Appendix IV (A).



**Figure 4** Chemical structure of kaempferol 3-β-sophoroside





**Figure 5** A reference HPTLC chromatogram of *Equiseti Hiemalis Herba* extract observed under UV light (254 nm)

1. Kaempferol 3- $\beta$ -sophoroside 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 kaempferol 3- $\beta$ -sophoroside (Fig. 5).

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

##### Standard solution

*Kaempferol 3- $\beta$ -sophoroside standard solution for fingerprinting, Std-FP (20 mg/L)*

Weigh 0.2 mg of kaempferol 3- $\beta$ -sophoroside CRS and dissolve in 10 mL of ethanol.

##### Test solution

Weigh 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of water. Sonicate (270 W) the mixture for 30 min. Centrifuge at about  $5000 \times g$  for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with water. Filter through a 0.45- $\mu$ m RC filter.

##### Chromatographic system

The liquid chromatograph is equipped with a DAD (265 nm) and a column (4.6  $\times$  250 mm) packed with ODS bonded silica gel (5  $\mu$ m particle size). The internal diameter of inlet column tubing is about 0.5 mm. 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% Trifluoroacetic acid (% v/v)	Acetonitrile (% v/v)	Elution
0 – 40	90 → 70	10 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of kaempferol 3-β-sophoroside Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of kaempferol 3-β-sophoroside should not be more than 5.0%; the RSD of the retention time of kaempferol 3-β-sophoroside peak should not be more than 2.0%; the column efficiency determined from kaempferol 3-β-sophoroside peak should not be less than 70000 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. 6).

Procedure

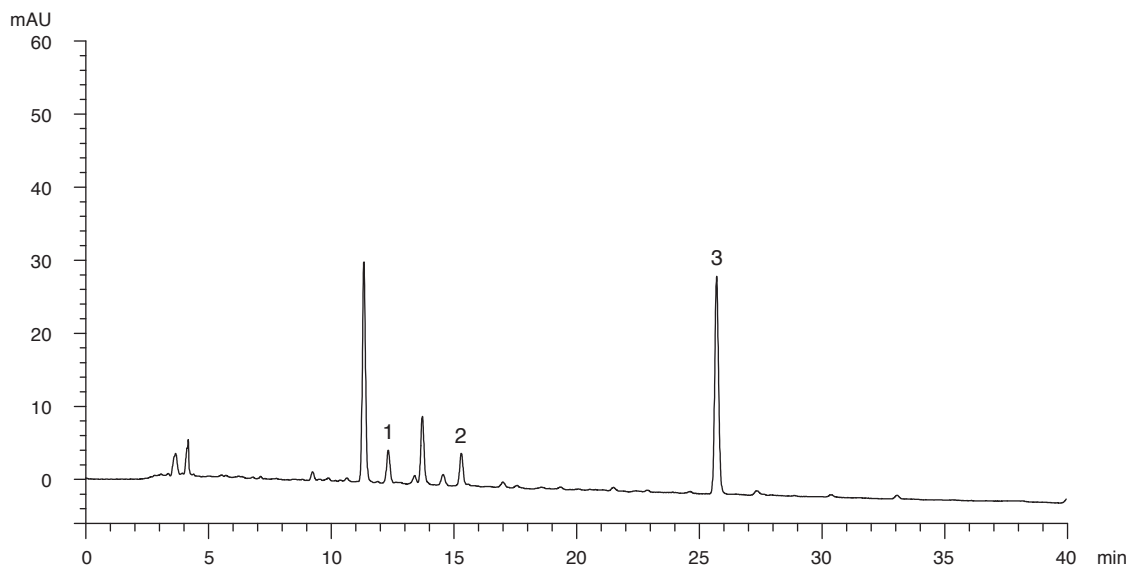
Separately inject kaempferol 3-β-sophoroside Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of kaempferol 3-β-sophoroside peak in the chromatogram of kaempferol 3-β-sophoroside Std-FP and the retention times of the three characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify kaempferol 3-β-sophoroside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of kaempferol 3-β-sophoroside Std-FP. The retention times of kaempferol 3-β-sophoroside 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 three characteristic peaks of Equiseti Hiemalis Herba extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the three characteristic peaks of Equiseti Hiemalis Herba extract

Peak No.	RRT	Acceptable Range
1	0.48	± 0.03
2	0.60	± 0.03
3 (marker, kaempferol 3-β-sophoroside)	1.00	-





**Figure 6** A reference fingerprint chromatogram of *Equiseti Hiemalis Herba* extract

For positive identification, the sample must give the above three 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 XVII*): meet the requirements.

**5.5 Foreign Matter** (*Appendix VIII*): not more than 3.0%.

**5.6 Ash** (*Appendix IX*)

Total ash: not more than 10.0%.

Acid-insoluble ash: not more than 2.5%.

**5.7 Water Content** (*Appendix X*)

Oven dried method: not more than 13.0%.

6. EXTRACTIVES (Appendix XI)

Water-soluble extractives (cold extraction method): not less than 9.0%.  
Ethanol-soluble extractives (cold extraction method): not less than 7.0%.

7. ASSAY

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

Standard solution

*Kaempferol 3-β-sophoroside standard stock solution, Std-Stock (200 mg/L)*  
Weigh accurately 5.0 mg of kaempferol 3-β-sophoroside CRS and dissolve in 25 mL of ethanol.  
*Kaempferol 3-β-sophoroside standard solution for assay, Std-AS*  
Measure accurately the volume of the kaempferol 3-β-sophoroside Std-Stock, dilute with ethanol to produce a series of solutions of 1, 2, 5, 10, 20 mg/L for kaempferol 3-β-sophoroside.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of water. Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × g for 5 min. Filter and transfer the filtrate to a 25-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with water. Filter through a 0.45-μm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a DAD (265 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The internal diameter of inlet column tubing is about 0.5 mm. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.1% Trifluoroacetic acid (% v/v)	Acetonitrile (% v/v)	Elution
0 – 40	90 → 70	10 → 30	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 µL of kaempferol 3-β-sophoroside Std-AS (5 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of kaempferol 3-β-sophoroside should not be more than 5.0%; the RSD of the retention time of kaempferol 3-β-sophoroside peak should not be more than 2.0%; the column efficiency determined from kaempferol 3-β-sophoroside peak should not be less than 70000 theoretical plates.

The *R* value between kaempferol 3-β-sophoroside peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curve

Inject a series of kaempferol 3-β-sophoroside Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of kaempferol 3-β-sophoroside against the corresponding concentrations of kaempferol 3-β-sophoroside Std-AS. Obtain the slope, y-intercept and the *r*<sup>2</sup> value from the 5-point calibration curve.

Procedure

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify kaempferol 3-β-sophoroside peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of kaempferol 3-β-sophoroside Std-AS. The retention times of kaempferol 3-β-sophoroside peaks from the two chromatograms should not differ by more than 5.0%. Measure the peak area and calculate the concentration (in milligram per litre) of kaempferol 3-β-sophoroside in the test solution, and calculate the percentage content of kaempferol 3-β-sophoroside in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.048% of kaempferol 3-β-sophoroside (C<sub>27</sub>H<sub>30</sub>O<sub>16</sub>), calculated with reference to the dried substance.