

Corni Fructus



Figure 1 A photograph of Corni Fructus

1. NAMES

Official Name: Corni Fructus

Chinese Name: 山茱萸

Chinese Phonetic Name: Shanzhuyu

2. SOURCE

Corni Fructus is the dried ripe sarcocarp of *Cornus officinalis* Sieb. et Zucc. (Cornaceae). The fruit is collected in late autumn and early winter when the pericarp turns red. It is dipped in boiling water for several minutes, then the core is removed, and the seedless fruit is dried to obtain Corni Fructus.

3. DESCRIPTION

Irregular pieces or sacciform, 0.9-2 cm long and 0.5-1.2 cm wide. Externally dark red, cracked and shrivelled. Sometimes with a rounded scar of persistent calyx at the apex and a fruit stalk scar at the base. Texture soft. Odour slight; taste sour, astringent and slightly bitter (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

Exocarp consists of 1 layer of slightly flat cells, covered by relatively thick cuticle. Mesocarp broad, consisting of numerous rows of parenchymatous cells varying in size, some cells contain dark brown pigment masses. Vascular bundles 8, arranged in an interrupted ring on the inner side. Fibres occasionally present. Clusters of calcium oxalate scattered in parenchymatous cells (Fig. 2).

Powder

Colour red to pale reddish-brown. Exocarp cells colourless to pale orange-yellow, polygonal or subrectangular in surface view, 7-34 μm in diameter, anticlinal walls slightly bead-like thickened, outer periclinal walls granularly cutinized and thickened; rectangular in lateral view, with relatively thick cuticle. Mesocarp cells pale orange-brown, mostly shrunken, with indistinct boundaries. Inulin masses with fan-shaped striations, 23-105 μm in diameter; orange to polychromatic under the

polarized microscope. Clusters of calcium oxalate 4-38 μm in diameter; polychromatic under the polarized microscope. Stone cells single or in groups; single cells ovoid, subsquare, rectangular or irregular in shape, 18-106 μm long, sometimes up to 176 μm , 11-63 μm in diameter, usually with distinct pits and a large lumen, sometimes with distinct striations and a small lumen. Fibres 9-40 μm in diameter. Mainly spiral vessels, 5-28 μm in diameter (Fig. 3).

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

Standard solutions

Loganin standard solution

Weigh 0.5 mg of loganin CRS (Fig. 4) and dissolve in 1 mL of ethanol.

Morroniside standard solution

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

Developing solvent system

Prepare a mixture of ethyl acetate, water and formic acid (6:1:1, v/v).

Spray reagent

Add slowly 1 mL of sulphuric acid to 50 mL of glacial acetic acid and then add 0.5 mL of 4-methoxybenzaldehyde. Freshly prepare the reagent.

Test solution

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 10 mL of ethanol. Sonicate (140 W) the mixture for 5 min. Centrifuge at about $2500 \times g$ for 10 min. Filter the supernatant.

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 loganin standard solution (1 μL), morroniside standard solution (0.5 μL) and the test solution (0.8 μL) to the plate. Develop over a path of about 6 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 90°C until the spots or bands become visible (about 5-7 min). Examine the plate under visible light. 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 loganin and morroniside.

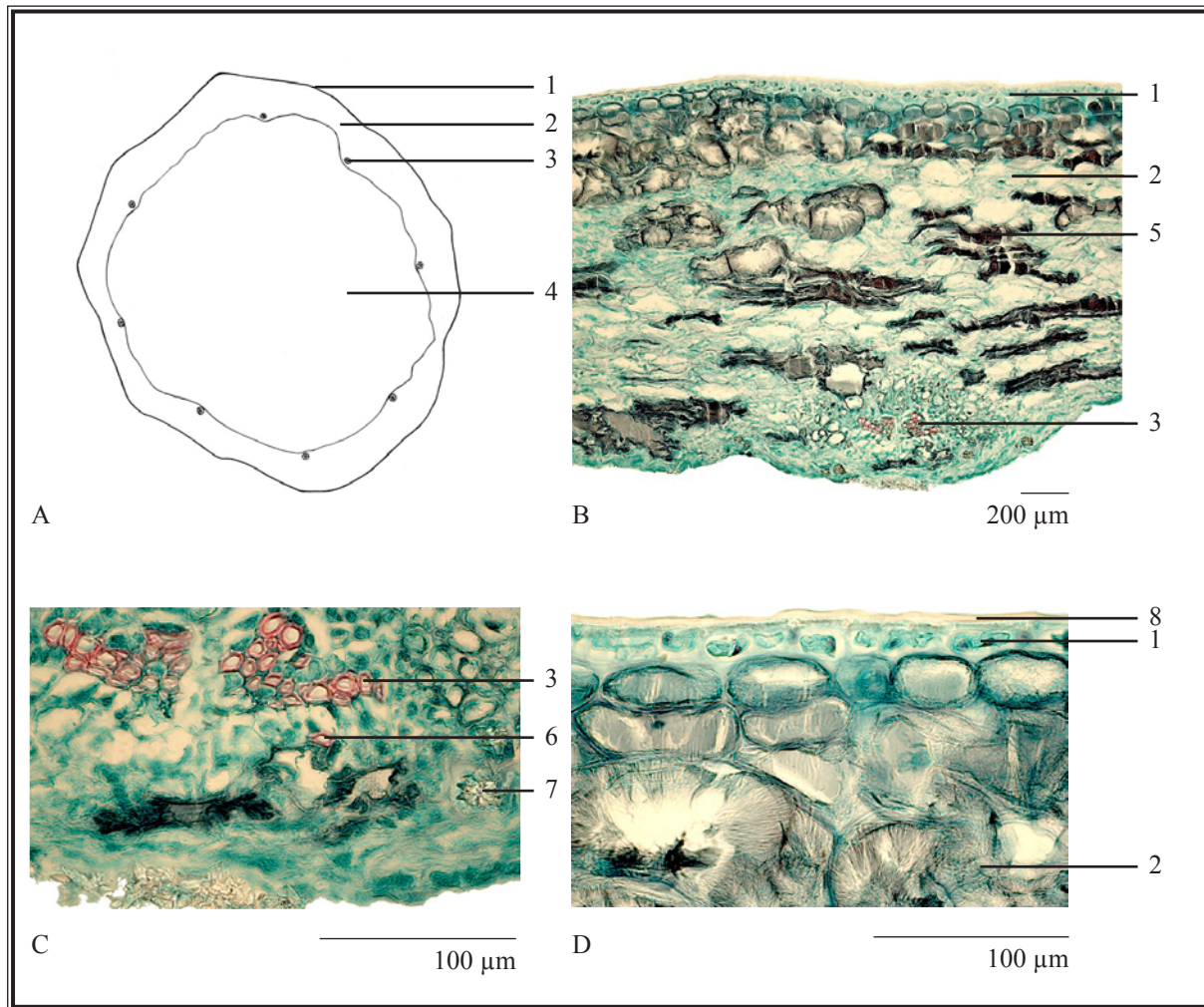


Figure 2 Microscopic features of transverse section of Corni Fructus

A. Sketch B. Section illustration

C. Vascular bundles, fibres and clusters of calcium oxalate scattered in parenchyma

D. Exocarp covered by cuticle

1. Exocarp 2. Mesocarp 3. Vascular bundle 4. Hollow cavity 5. Pigment masses 6. Fibre

7. Cluster of calcium oxalate 8. Cuticle

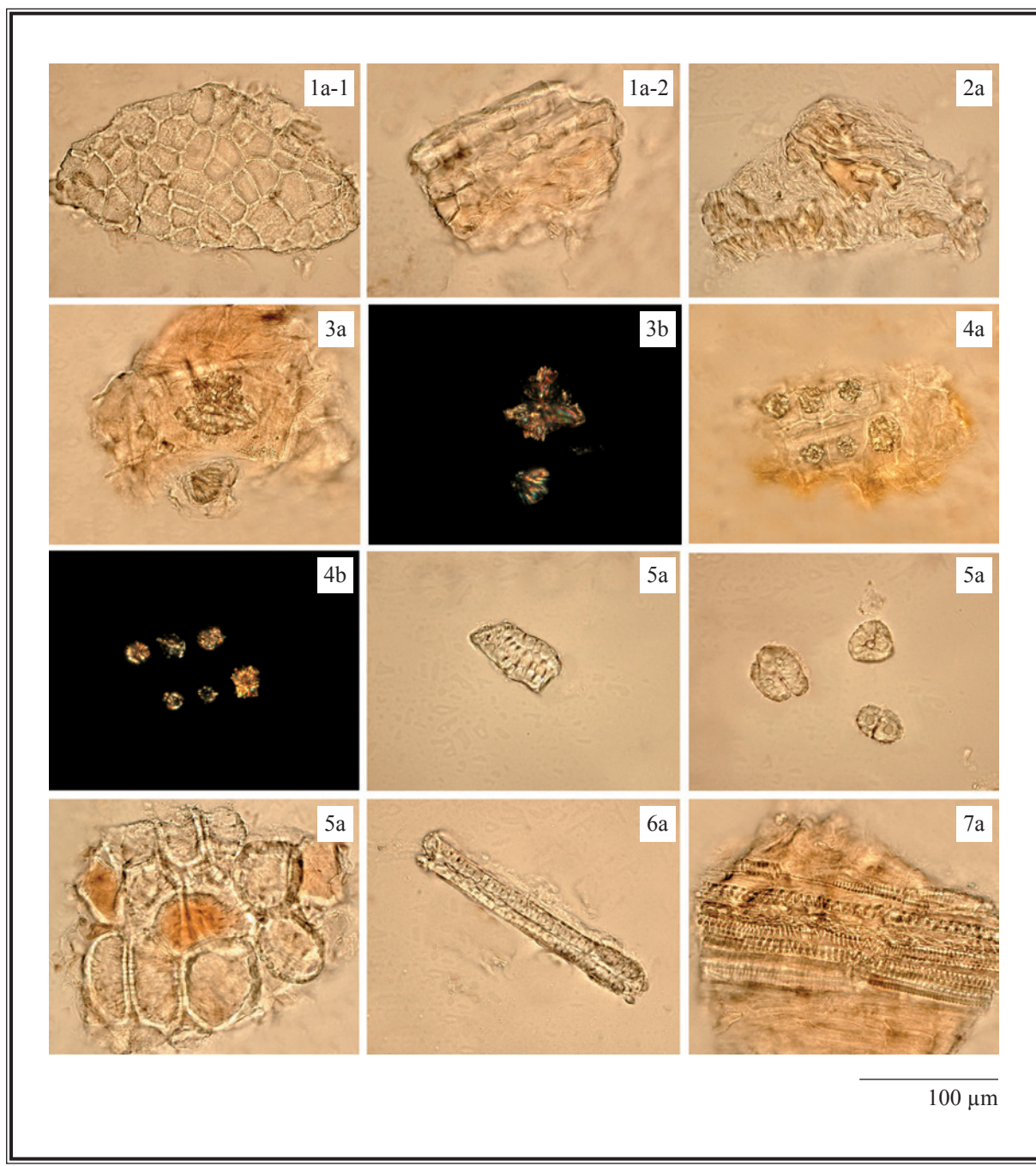


Figure 3 Microscopic features of powder of Corni Fructus

1. Exocarp cells (1-1 in surface view, 1-2 in lateral view)
 2. Mesocarp cells 3. Inulin masses 4. Clusters of calcium oxalate 5. Stone cells 6. Fibre 7. Spiral vessels
 a. Features under the light microscope b. Features under the polarized microscope

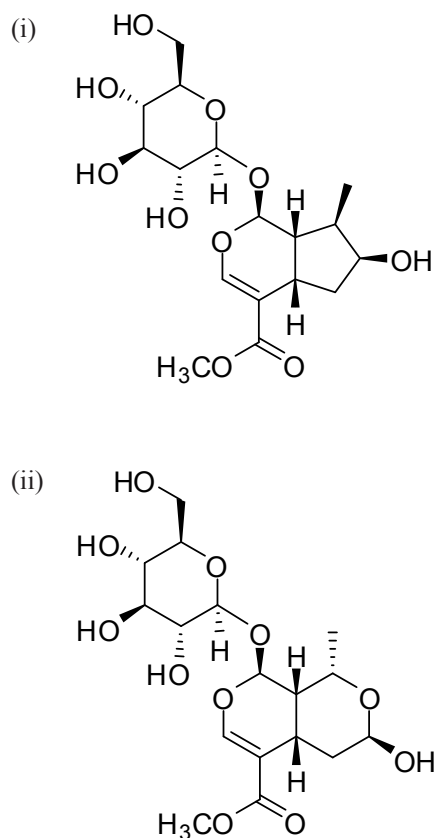


Figure 4 Chemical structures of (i) loganin and (ii) morroniside

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

Standard solution

Loganin standard solution for fingerprinting, Std-FP (80 mg/L)

Weigh 4.0 mg of loganin CRS and dissolve in 50 mL of methanol (50%).

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about $1800 \times g$ for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (50%). Centrifuge at about $1800 \times g$ for 10 min. Combine the supernatants 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 a DAD (240 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30°C during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	0.2% Phosphoric acid (% v/v)	Acetonitrile (% v/v)	Elution
0 – 15	98 → 92	2 → 8	linear gradient
15 – 30	92 → 90	8 → 10	linear gradient
30 – 60	90 → 88	10 → 12	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of loganin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of loganin should not be more than 5.0%; the RSD of the retention time of loganin peak should not be more than 2.0%; the column efficiency determined from loganin peak should not be less than 20000 theoretical plates.

The *R* value between peak 4 and the closest peak in the chromatogram of the test solution should not be less than 1.0 (Fig. 5).

Procedure

Separately inject loganin Std-FP and the test solution (10 μL each) into the HPLC system and record the chromatograms. Measure the retention time of loganin peak in the chromatogram of loganin Std-FP and the retention times of the four characteristic peaks (Fig. 5) in the chromatogram of the test solution. Identify loganin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of loganin Std-FP. The retention times of loganin 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 four characteristic peaks of Corni Fructus extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Corni Fructus extract

Peak No.	RRT	Acceptable Range
1	0.27	± 0.03
2	0.57	± 0.03
3 (morroniside)	0.63	± 0.03
4 (marker, loganin)	1.00	-

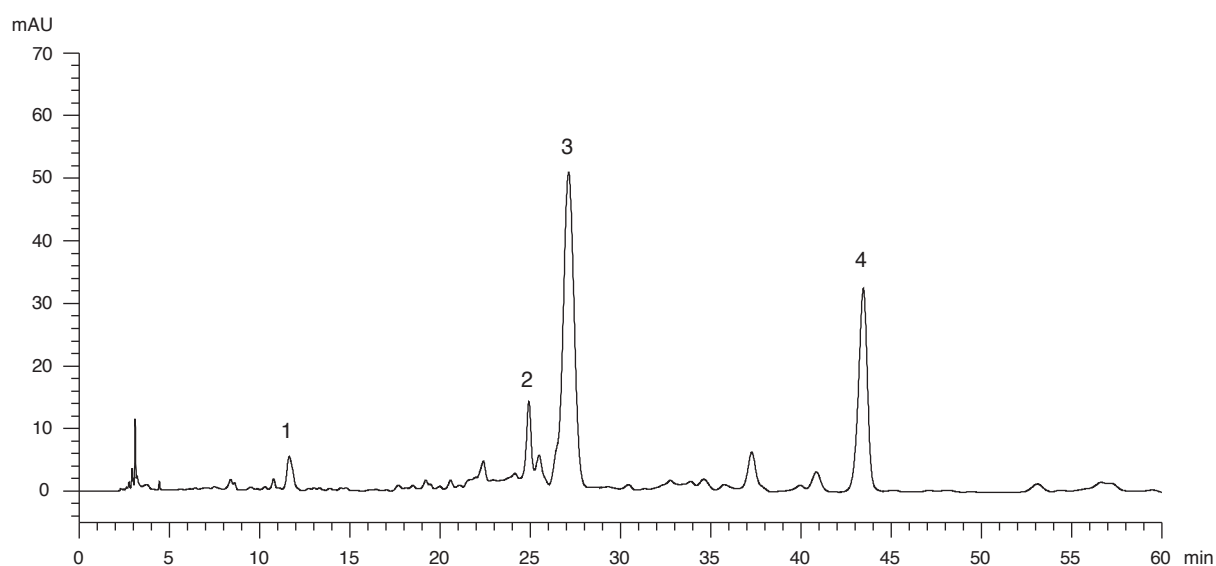


Figure 5 A reference fingerprint chromatogram of Corni Fructus extract

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. 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 5.0%.

Acid-insoluble ash: not more than 1.0%.

5.7 Water Content (Appendix X)

Oven dried method: not more than 25.0%.

6. EXTRACTIVES (Appendix XI)

Water-soluble extractives (cold extraction method): not less than 58.0%.

Ethanol-soluble extractives (cold extraction method): not less than 52.0%.

7. ASSAY

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

Standard solution

Mixed loganin and morroniside standard stock solution, Std-Stock (400 mg/L for loganin and 1000 mg/L for morroniside)

Weigh accurately 4.0 mg of loganin CRS and 10.0 mg of morroniside CRS, and dissolve in 10 mL of methanol (50%).

Mixed loganin and morroniside standard solution for assay, Std-AS

Measure accurately the volume of the mixed loganin and morroniside Std-Stock, dilute with methanol (50%) to produce a series of solutions of 20, 40, 80, 120, 200 mg/L for loganin and 50, 100, 200, 300, 500 mg/L for morroniside.

Test solution

Weigh accurately 0.5 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 20 mL of methanol (50%) and mix. Sonicate (270 W) the mixture for 30 min. Centrifuge at about $1800 \times g$ for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for one more time. Wash the residue with methanol (50%). Centrifuge at about $1800 \times g$ for 10 min. Combine the supernatants 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 a DAD (240 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The column temperature is maintained at 30°C during the separation. The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

Table 3 Chromatographic system conditions

Time (min)	0.2% Phosphoric acid (% v/v)	Acetonitrile (% v/v)	Elution
0 – 15	98 → 92	2 → 8	linear gradient
15 – 30	92 → 90	8 → 10	linear gradient
30 – 60	90 → 88	10 → 12	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 10 μL of the mixed loganin and morroniside Std-AS (80 mg/L for loganin and 200 mg/L for morroniside). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of loganin and morroniside should not be more than 5.0%; the RSD of the retention times of loganin and morroniside peaks should not be more than 2.0%; the column efficiencies determined from loganin and morroniside peaks should not be less than 5000 theoretical plates.

The *R* value between loganin peak and the closest peak; and the *R* value between morroniside peak and the closest peak in the chromatogram of the test solution should not be less than 1.5.

Calibration curves

Inject a series of the mixed loganin and morroniside Std-AS (10 μL each) into the HPLC system and record the chromatograms. Plot the peak areas of loganin and morroniside against the corresponding concentrations of the mixed loganin and morroniside 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 loganin and morroniside peaks in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed loganin and morroniside Std-AS. The retention times of loganin and morroniside peaks in the chromatograms of the test solution and the Std-AS should not differ by more than 2.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of loganin and morroniside in the test solution, and calculate the percentage contents of loganin and morroniside in the sample by using the equations indicated in Appendix IV(B).

Aurantii Fructus
枳殼

Orpiment
雌黃

Cistanches Herba
仙茅 肉苻蓉

雄黃
Realgar

Houttuyniae Herba
魚腥草

墨旱蓮
Ecliptae Herba

Smilacis Glabrae Rhizoma
土茯苓
五味子

Calomelas

Curculiginis Rhizoma

前胡

Peucedani Radix

蛇床子

Cnidii Fructus

Scutellariae Barbatae Herba

半枝蓮

Sophorae Flavescentis Radix

苦參

Hydrargyri Oxydum

Schisandrae Chinensis Fructus

紅粉

Isatidis Radix

Corni Fructus

板藍根

Limits

The sample contains not less than 0.65% of loganin ($C_{17}H_{26}O_{10}$) and not less than 1.3% of morroneiside ($C_{17}H_{26}O_{11}$), calculated with reference to the dried substance.