

Cortex Phellodendri Amurensis



Figure 5 A photograph of Cortex Phellodendri Amurensis

1. NAMES

Official Name: Cortex Phellodendri Amurensis

Chinese Name: 關黃柏

Chinese Phonetic Name: Guanhuangbo

2. SOURCE

Cortex Phellodendri Amurensis is the dried bark of *Phellodendron amurense* Rupr. (Fam. Rutaceae). The bark is collected in autumn, the coarse part removed, then dried in the sun to obtain Cortex Phellodendri Amurensis.

3. DESCRIPTION

Tabular or shallowly channeled, varying in length and width, 2–8 mm in thickness. Outer surface relatively even and brownish-yellow or pale brownish-yellow, sometimes showing remnants of greyish-brown coarse bark; inner surface yellow or yellowish-brown, with longitudinal ridges. Texture light and hard, fracture fibrous, yellow, showing lobe-like layers. Odour, slight; taste, bitter, viscous on chewing. (Fig. 5)

4. IDENTIFICATION

4.1 Microscopic Identification (Appendix III)

Transverse section

The transverse section shows residual cork consisting of several rows of cork cells. Cortex parenchymatous cells varying in rows and up to 14 rows. Stone cells sparsely scattered in cortex. Phloem rays 1–5 cells wide, slightly curved. Phloem fibres usually in bundles, arranged in several rows discontinuously. Mucus cells spheroidal or ellipsoid, scattered. Prisms of calcium oxalate up to 39 µm in diameter, mostly present in parenchymatous cells of the cortex and phloem. (Fig. 6)

Powder

Yellow or yellowish-brown. Stone cells suborbicular, fusiform or irregular shape, scattered or arranged in groups, some branched, sharp at the top, walls thickened, with distinct striations, about 11–94 μm in diameter, some up to about 260 μm in length; showing a polychrome when examined under a polarizing microscope. Fibres yellow or light yellow, often in bundles, walls thickened, with linear cell cavity, surrounded by parenchymatous cells containing prisms of calcium oxalate, forming crystal fibres; showing a polychrome when examined under a polarizing microscope. Prisms of calcium oxalate are up to 39 μm in diameter. End walls of sieve element fastigiated, with compound sieve plates observed. (Fig. 7)

4.2 Physicochemical Identification

Reagent

Potassium iodobismuthate solution

Dissolve 0.85 g of bismuth subnitrate in 10 mL of acetic acid and 40 mL of water, then mix with 20 mL of aqueous potassium iodide solution (40%, w/v).

Procedure

Weigh 1.0 g of the powdered sample and put into a 100-mL conical flask, then add 10 mL of ethanol and 2 drops of hydrochloric acid. Sonicate the mixture for 30 min. Filter and adjust the pH to about 7 with aqueous sodium carbonate solution (10%, w/v). Re-filter and transfer 1 mL of the neutralized filtrate to a test tube. Evaporate to dryness on a water bath, then cool to room temperature. Dissolve the residue in 2 mL of dilute hydrochloric acid (3%, v/v). Transfer 0.5 mL of the solution to a test tube, add 3–5 drops of potassium iodobismuthate solution and allow the precipitate to settle. Reddish-orange precipitate is observed.

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

Standard solutions

Berberine chloride standard solution

Weigh 1.0 mg of berberine chloride and dissolve in 2 mL of methanol.

Palmatine chloride standard solution

Weigh 1.0 mg of palmatine chloride and dissolve in 2 mL of methanol.

Developing solvent system

Prepare a mixture of 1-butanol, water and glacial acetate acid (4:1:1.5, v/v).

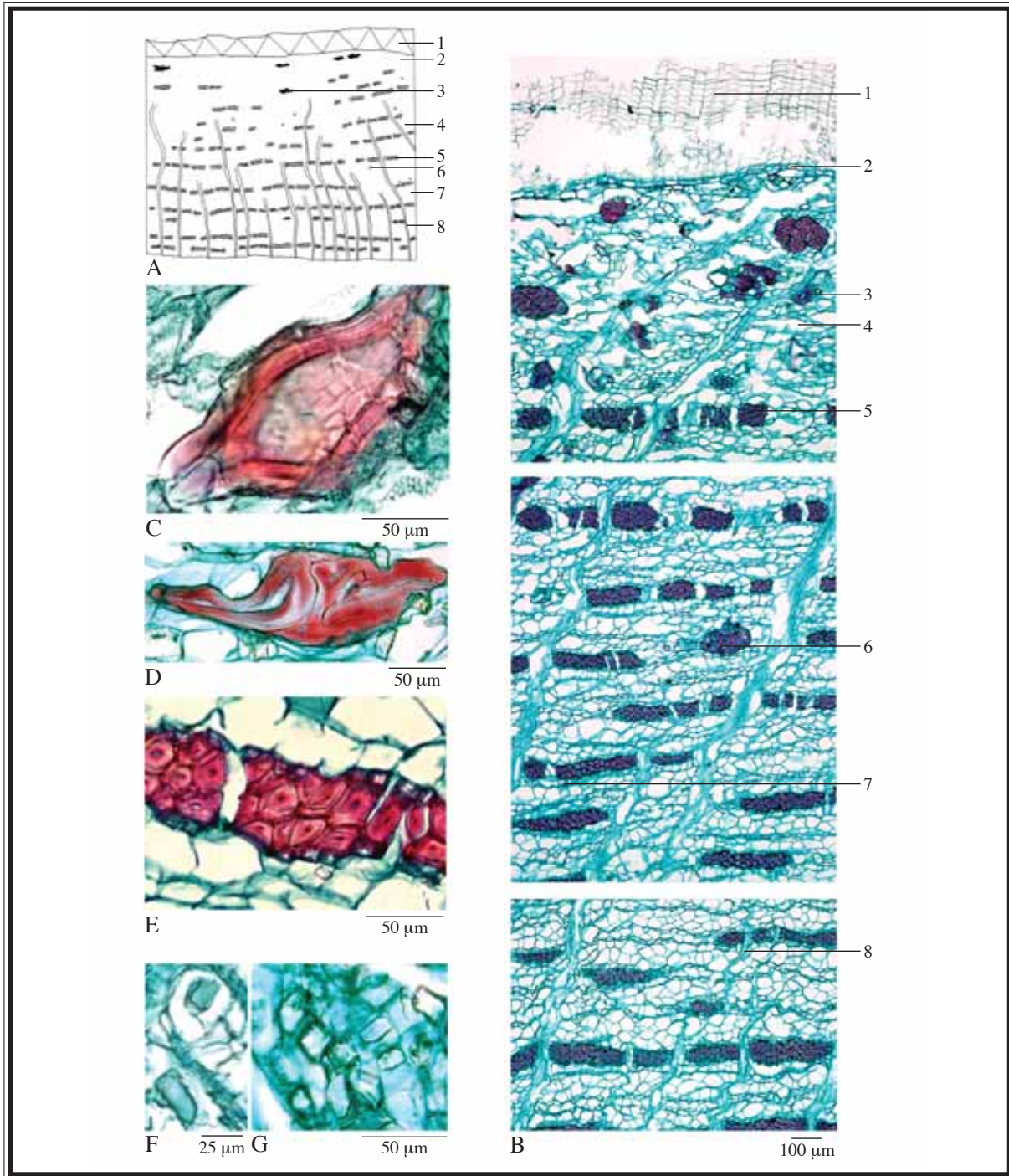


Figure 6 Microscopic features of transverse section of Cortex Phellodendri Amurensis

A. Sketch B. Section illustration C. Single stone cell D. Stone cells in a group
E. Phloem fibres F. Mucus cells G. Prisms of calcium oxalate

1. Cork 2. Cortex 3. Stone cells 4. Phloem 5. Phloem fibres 6. Mucus cells
7. Prisms of calcium oxalate 8. Phloem rays

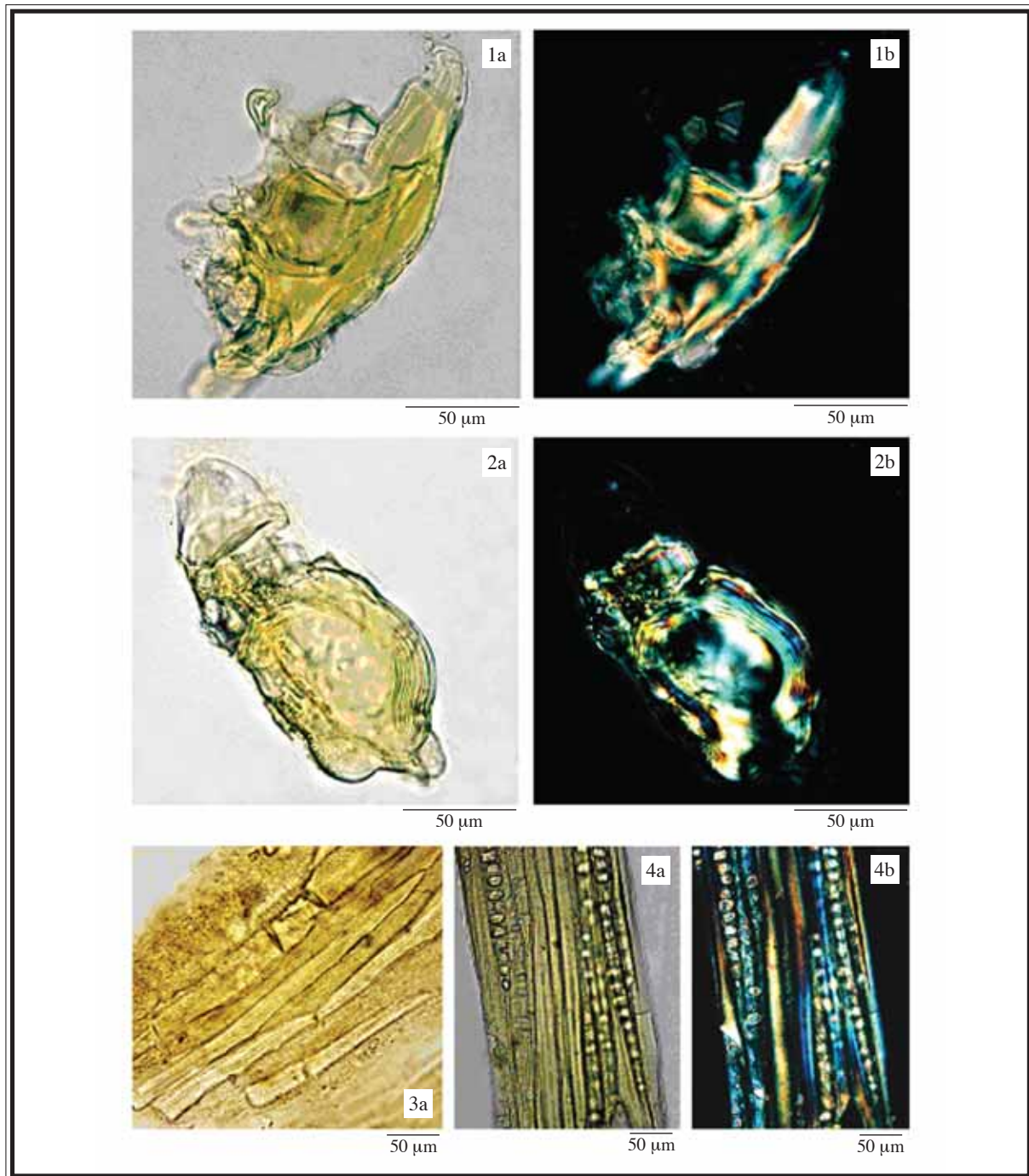


Figure 7 Microscopic features of powder of *Cortex Phellodendri Amurensis*

1. Irregular stone cell 2. Suborbicular stone cell 3. Sieve tubes 4. Crystal fibres

a. Features under a light microscope b. Features under a polarizing microscope

Test solution

Weigh 1.0 g of the powdered sample and put into a 100-mL conical flask, then add 20 mL of methanol. Sonicate the mixture for 30 min, filter and then evaporate to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 2 mL of methanol.

Procedure

Carry out the method by using a HPTLC silica gel F₂₅₄ plate and a freshly prepared developing solvent system as described above. Develop over a path of about 5 cm. Apply separately berberine chloride standard solution, palmatine chloride standard solution and the test solution (1 µL each) to the plate. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate in UV light (365 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 berberine chloride and palmatine chloride.

4.4 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)**Standard solution**

Berberine chloride standard stock solution, Std-Stock (1000 mg/L)

Weigh 4.0 mg of berberine chloride and dissolve in 4 mL of methanol.

Berberine chloride standard solution for fingerprinting, Std-FP (100 mg/L)

Pipette 0.5 mL of berberine chloride Std-Stock to a 5-mL volumetric flask and make up to the mark with methanol.

Test solution

Weigh 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of methanol and weigh. Sonicate for 30 min and weigh again. Add an appropriate amount of methanol to compensate the weight loss, if any. Mix and centrifuge at about 3000 x g for 5 min. Filter through a 0.45-µm PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (346 nm) and a column (3.9 x 300 mm) packed with ODS bonded silica gel (4 µm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows –

Time (min)	0.1% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0–48	100 → 50	0 → 50	linear gradient
48–55	50 → 0	50 → 100	linear gradient
55–60	0	100	isocratic

System suitability requirements

Perform at least five replicate injections each with 5 μ L of berberine chloride Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of berberine chloride should be not more than 3.0%; the RSD of the retention time of berberine chloride peak should be not more than 2.0%; the column efficiency determined from berberine chloride peak should be not less than 50,000 theoretical plates.

The *R* value between peaks 1 and 2 (Fig. 8) in the test solution should be not less than 1.0.

Procedure

Separately inject berberine chloride Std-FP and the test solution (5 μ L each) into the HPLC system and record the chromatograms. Measure the retention time of berberine chloride peak in the chromatogram of berberine chloride Std-FP and the retention times of the six characteristic peaks (Fig. 8) in the chromatogram of the test solution. Under the same HPLC conditions, identify berberine chloride peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of berberine chloride Std-FP. The retention times of berberine chloride 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 six characteristic peaks of Cortex Phellodendri Amurensis extract are listed in Table 4.

Table 4 The RRTs and acceptable ranges of the six characteristic peaks of Cortex Phellodendri Amurensis extract

Peak No.	RRT	Acceptable Range
1	0.46	±0.03
2	0.47	±0.03
3	0.57	±0.03
4 (jatrorrhizine chloride)	0.88	±0.03
5 (palmatine chloride)	0.97	±0.03
6 (marker, berberine chloride)	1.00	-

mAu

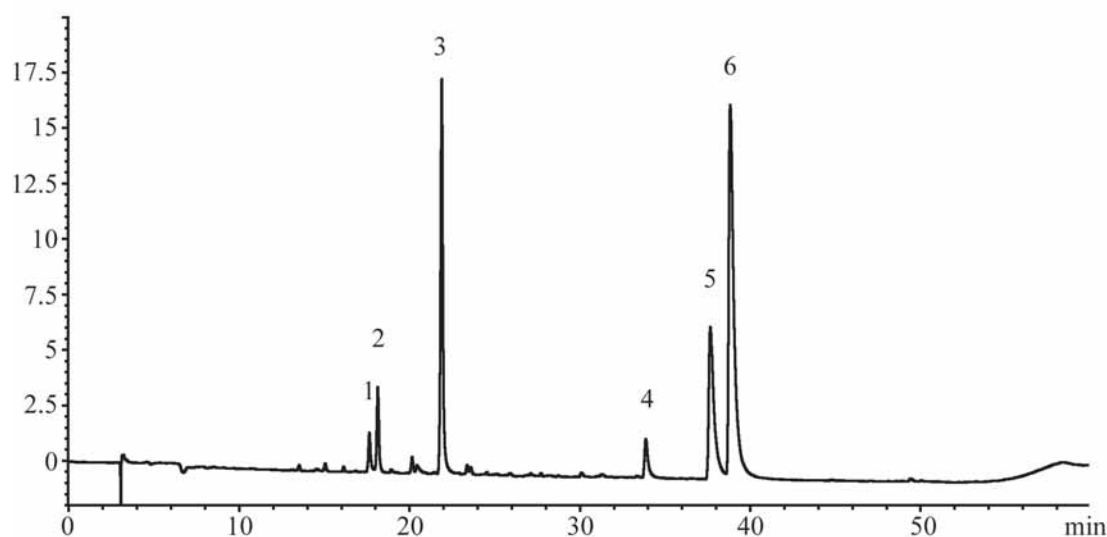


Figure 8 A reference fingerprint chromatogram of Cortex Phellodendri Amurensis extract

For positive identification, the sample must give the above six characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 8).

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 XIV*): meet the requirements.
- 5.5 **Foreign Matter** (*Appendix VIII*): not more than 1.0%.

5.6 Ash (*Appendix IX*)

Total ash: not more than 8.5%.

Acid-insoluble ash: not more than 1.0%.

5.7 Water Content (*Appendix X*): not more than 11.0%.

6. EXTRACTIVES (*Appendix XI*)

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

Ethanol-soluble extractives (cold extraction method): not less than 12.0%.

7. ASSAY

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

Standard solution

Mixed berberine chloride and palmatine chloride standard stock solution, Std-Stock (1000 mg/L each)

Weigh accurately 10.0 mg of berberine chloride and 10.0 mg of palmatine chloride, and dissolve in 10 mL of methanol.

Mixed berberine chloride and palmatine chloride standard solution for assay, Std-AS

Measure accurately the volume of the mixed berberine chloride and palmatine chloride Std-Stock, dilute with methanol to produce a series of solutions of 1, 10, 100, 200 and 400 mg/L for both berberine chloride and palmatine chloride.

Test solution

Weigh accurately 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add accurately 10 mL of methanol and weigh. Sonicate the mixture for 30 min and weigh again. Add an appropriate amount of methanol to compensate the weight loss, if any. Mix and centrifuge at about 3000 x g for 5 min. Filter through a 0.45- μ m PTFE filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (346 nm) and a column (3.9 x 300 mm) packed with ODS bonded silica gel (4 μ m particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows –

Time (min)	0.1% Trifluoroacetic acid (%, v/v)	Acetonitrile (%, v/v)	Elution
0–20	90 → 10	10 → 90	linear gradient

System suitability requirements

Perform at least five replicate injections each with 5 µL of the mixed berberine chloride and palmatine chloride Std-AS (200 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak areas of berberine chloride and palmatine chloride should be not more than 5.0%; the RSD of the retention times of berberine chloride peak and palmatine chloride peak should be not more than 2.0%; the column efficiency determined from berberine chloride peak and palmatine chloride peak should be not less than 30,000 theoretical plates.

The *R* value between berberine chloride peak and the closest peak in the test solution should be not less than 1.5.

Calibration curves

Inject a series of the mixed berberine chloride and palmatine chloride Std-AS (5 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of berberine chloride and palmatine chloride against the corresponding concentrations of the mixed berberine chloride and palmatine chloride Std-AS. Obtain the slopes, y-intercepts and the r^2 values from the corresponding 5-point calibration curves.

Procedure

Inject 5 µL of the test solution into the HPLC system and record the chromatogram. Identify berberine chloride peak and palmatine chloride peak in the chromatogram of the test solution by comparing their retention times with those in the chromatogram of the mixed berberine chloride and palmatine chloride Std-AS. The retention times of berberine chloride peaks and palmatine chloride peaks in both chromatograms should not differ from their counterparts by more than 5.0%. Measure the peak areas and calculate the concentrations (in milligram per litre) of berberine chloride and palmatine chloride in the test solution, and calculate the percentage contents of berberine chloride and palmatine chloride in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.33% of berberine [calculated as berberine chloride ($C_{20}H_{18}NO_4Cl$)], and not less than 0.18% of palmatine [calculated as palmatine chloride ($C_{21}H_{22}NO_4Cl$)], calculated with reference to the dried substance.