Nardostachyos Radix et Rhizoma

Figure 1 A photograph of Nardostachyos Radix et Rhizoma

A. Nardostachyos Radix et Rhizoma
B. Magnified transverse section of root

1. NAMES

Official Name: Nardostachyos Radix et Rhizoma

Chinese Name: 甘松

Chinese Phonetic Name: Gansong

2. SOURCE

Nardostachyos Radix et Rhizoma is the dried root and rhizome of *Nardostachys jatamansi* DC. (Valerianaceae). The root and rhizome is collected in spring and autumn, soil and foreign matter removed, then dried under the sun or in a shaded area to obtain Nardostachyos Radix et Rhizoma.

3. DESCRIPTION

Subconical, mostly twisted or curved, 5-18 cm long. Rhizome short and small, top with the remnants of stem and leaves, in the form of long and narrow membranaceous-flaky or fibrous structure. Externally blackish-brown, inner layer brown or yellow. Root simple or multiple fascicled, branched or juxtaposed, 3-10 mm in diameter. Externally brown, shrunken, with rootlets. Texture lax and fragile, easily broken. Fracture rough, bark dark brown, usually splintered, wood yellowish-white. Odour characteristic; taste bitter and pungent, with a cooling sensation (Fig. 1).

4. IDENTIFICATION

4.1 Microscopic Identification *(Appendix III)*

Transverse section

Root: The outside surrounded by several concentric rings of cork tissue (named as cork ring). Cork rings separate phloem and xylem into 2-5 bundles in the inner part, every bundle surrounded by several concentric cork rings, phloem cells often broken and formed numerous big clefts, xylem at the centre part. Remanent of xylem usually observed between cork rings (Fig. 2).

Powder

Colour dark brown. Fragments of remained basal leaves numerous, cells pale yellow to brown, rectangular or elongated polygonal, 20-31 μm in diameter, 50-90 μm long, walls beaded-thickened; another fragments of cells subrectangular, up to 200 μm long, sometimes walls beaded-thickened. Cork cells mostly irregularly polygonal, walls relatively thin, lumens contain yellow to brownish-yellow oily contents. Vessels reticulate or scalariform, 7-40 μm in diameter, small vessels often in bundles. Stone cells scattered singly or in groups, subrectangular, subsquare or subrounded, 33-64 μm in diameter, up to 200 μm long. Inulin relatively abundant, often occur in parenchymatous cells, fan-shaped or irregular, with radial striations; bright yellow with radial striations under the polarized microscope (Fig. 3).

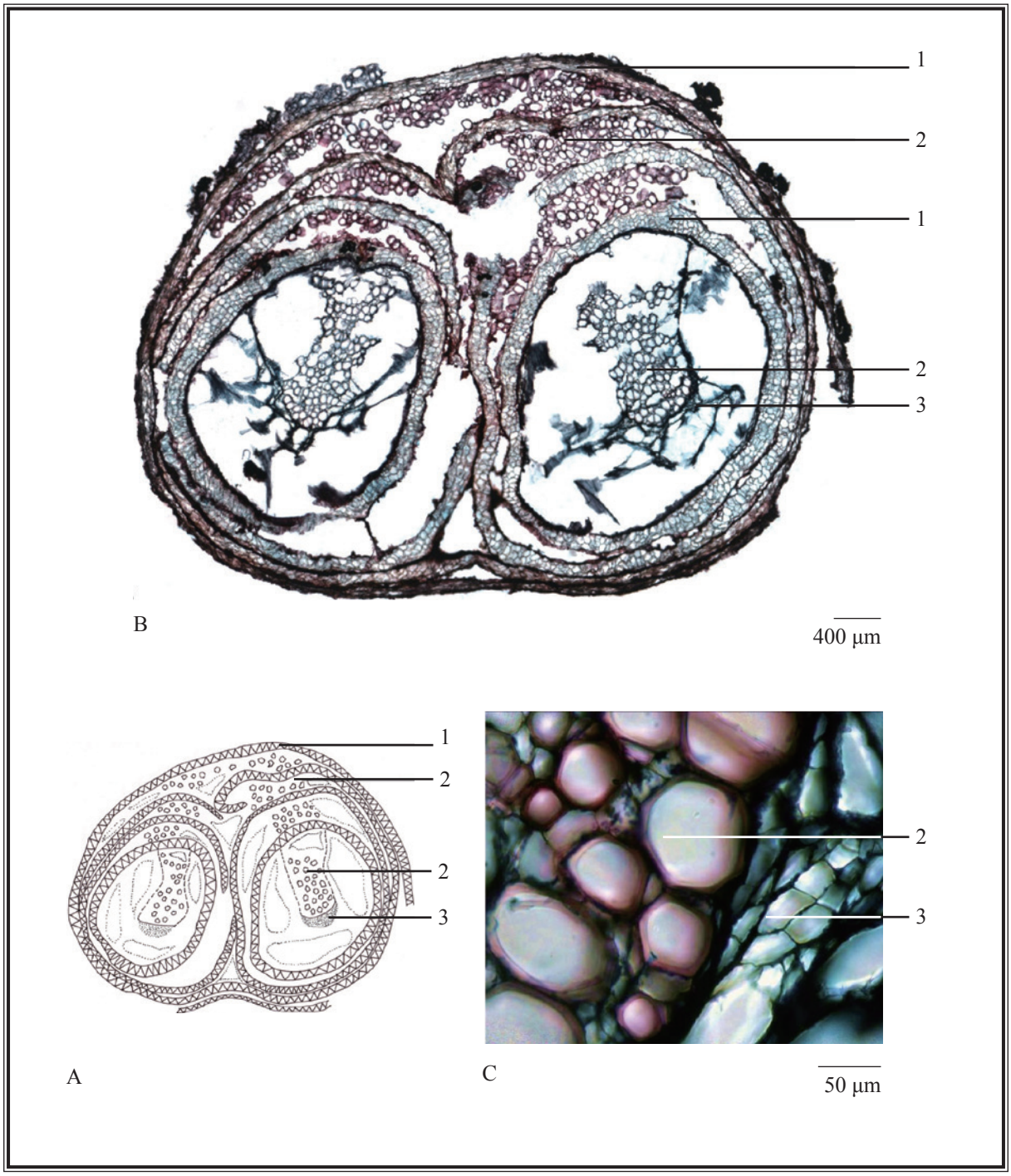

Figure 2 Microscopic features of transverse section of root of Nardostachyos Radix et Rhizoma

A. Sketch B. Section illustration C. Xylem and phloem

1. Cork 2. Xylem 3. Phloem

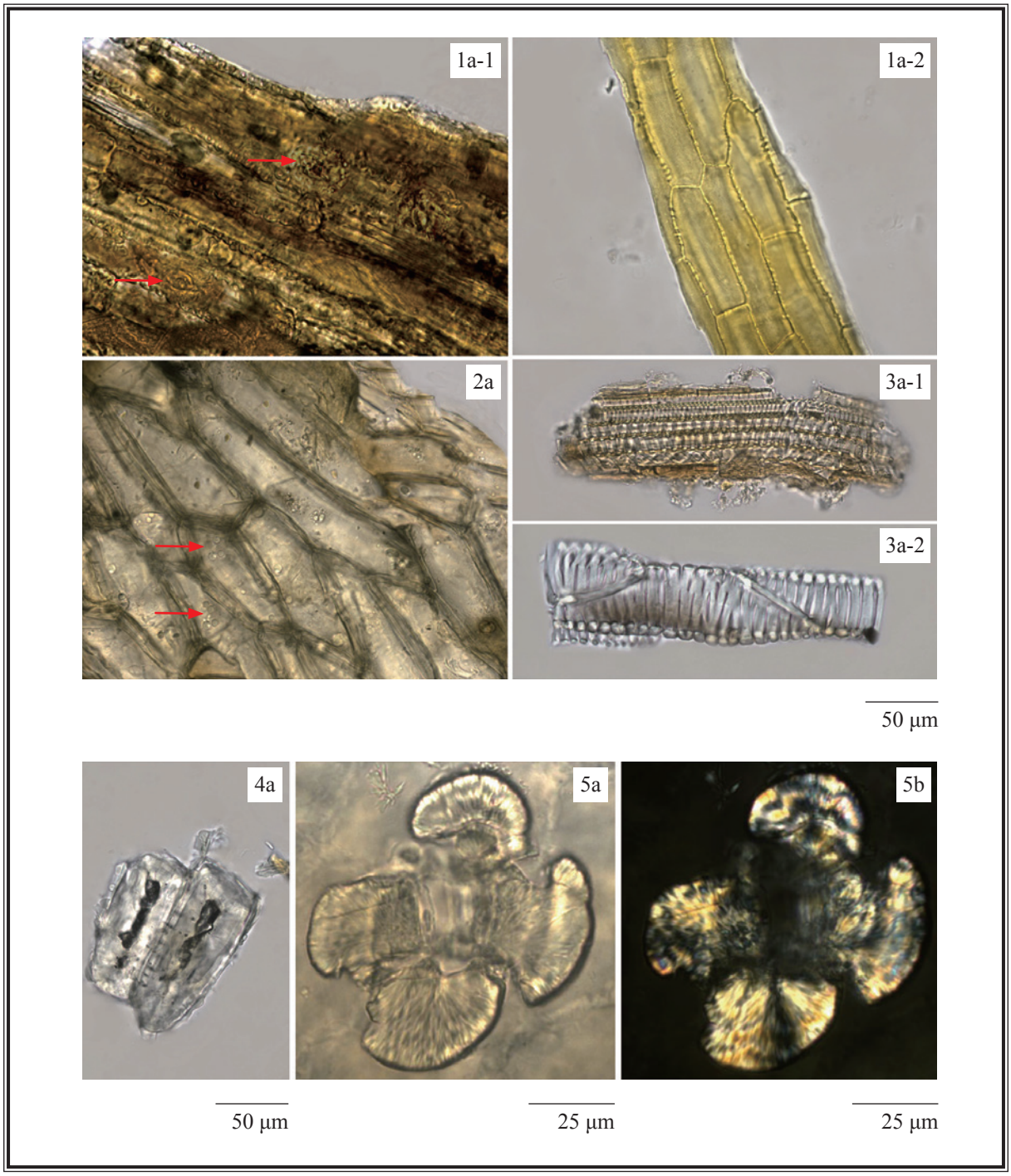

Figure 3 Microscopic features of powder of Nardostachyos Radix et Rhizoma

1. Fragments of remained basal leaf (1-1 elongated polygonal cells and stomata, 1-2 subrectangular cells)
2. Cork cells with oil droplets 3. Vessels (3-1 scalariform vessels, 3-2 reticulate vessel)
4. Stone cells 5. Inulin

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

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

Standard solution

Nardosinone standard solution

Weigh 1.0 mg of nardosinone CRS (Fig. 4) and dissolve in 0.5 mL of petroleum ether (60-80ºC).

Developing solvent system

Prepare a mixture of petroleum ether (60-80ºC) and ethyl acetate (4:1, v/v).

Spray reagent

Weigh 0.5 g of vanillin and dissolve in 100 mL of sulphuric acid.

Test solution

Weigh 0.5 g of the powdered sample and place it in a 50-mL conical flask, then add 20 mL of petroleum ether (60-80ºC). Sonicate (100 W) the mixture for 20 min. Filter the mixture.

Procedure

Carry out the method by using a HPTLC silica gel F_{254} plate, a twin trough chamber and a freshly prepared developing solvent system as described above. Apply separately nardosinone standard solution and the test solution (10 µL each) 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 7 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 5-10 min). Examine the plate under visible light. Calculate the R_f value by using the equation as indicated in Appendix IV (A).

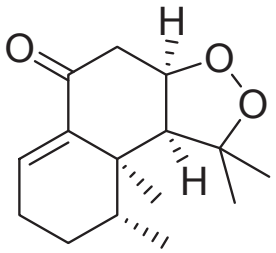

Figure 4 Chemical structure of nardosinone

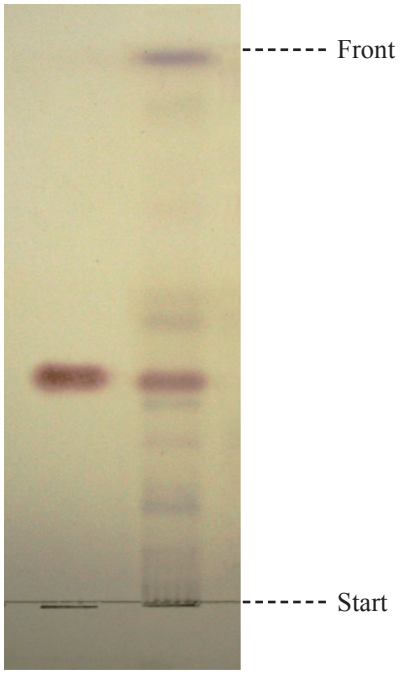

Figure 5 A reference HPTLC chromatogram of Nardostachyos Radix et Rhizoma extract observed under visible light after staining

1. Nardosinone 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 nardosinone (Fig. 5).

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

Standard solution

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

Weigh 1.0 mg of nardosinone 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 15 mL of methanol. Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3500 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Combine the supernatants 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 (250 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows (Table 1) –

Table 1 Chromatographic system conditions

Time (min)	Water (%, v/v)	Acetonitrile (%, v/v)	Elution
0 – 20	60 → 40	40 → 60	linear gradient
20 – 60	40 → 10	60 → 90	linear gradient

System suitability requirements

Perform at least five replicate injections, each using 20 μL of nardosinone Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of nardosinone should not be more than 5.0%; the RSD of the retention time of nardosinone peak should not be more than 2.0%; the column efficiency determined from nardosinone peak should not be less than 25000 theoretical plates.

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

Procedure

Separately inject nardosinone Std-FP and the test solution (20 μL each) into the HPLC system and record the chromatograms. Measure the retention time of nardosinone peak in the chromatogram of nardosinone Std-FP and the retention times of the four characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify nardosinone peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of nardosinone Std-FP. The retention times of nardosinone 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 Nardostachyos Radix et Rhizoma extract are listed in Table 2.

Table 2 The RRTs and acceptable ranges of the four characteristic peaks of Nardostachyos Radix et Rhizoma extract

Peak No.	RRT	Acceptable Range
1 (marker, nardosinone)	1.00	-
2	1.08	± 0.03
3	1.26	± 0.03
4	1.51	± 0.03

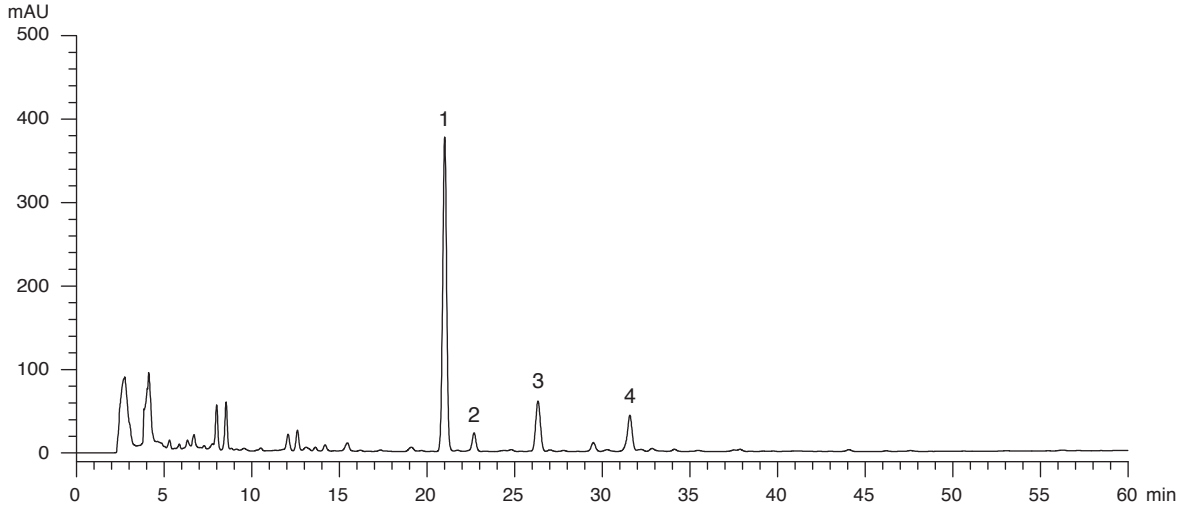

Figure 6 A reference fingerprint chromatogram of Nardostachyos Radix et Rhizoma extract

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. 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 22.0%.
Acid-insoluble ash: not more than 1.0%.

5.7 Water Content *(Appendix X)*

Toluene distillation method: not more than 10.0%.

6. EXTRACTIVES *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 8.0%.
Ethanol-soluble extractives (cold extraction method): not less than 9.0%.

7. ASSAY

7.1 Assay of Nardosinone

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

Standard solution

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

Weigh accurately 5.0 mg of nardosinone CRS and dissolve in 5 mL of methanol.

Nardosinone standard solution for assay, Std-AS

Measure accurately the volume of the nardosinone Std-Stock, dilute with methanol to produce a series of solutions of 2.5, 5, 12.5, 25, 50 mg/L for nardosinone.

Test solution

Weigh accurately 0.2 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 15 mL of methanol. Sonicate (100 W) the mixture for 30 min. Centrifuge at about 3500 × *g* for 10 min. Transfer the supernatant to a 50-mL volumetric flask. Repeat the extraction for two more times. Combine the supernatants 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 (250 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 0.8 mL/min. The mobile phase is a mixture of acetonitrile and water (60:40, v/v). The elution time is about 30 min.

System suitability requirements

Perform at least five replicate injections, each using 20 µL of nardosinone Std-AS (12.5 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of nardosinone should not be more than 5.0%; the RSD of the retention time of nardosinone peak should not be more than 2.0%; the column efficiency determined from nardosinone peak should not be less than 8000 theoretical plates.

The *R* value between nardosinone 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 nardosinone Std-AS (20 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of nardosinone against the corresponding concentrations of nardosinone Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 20 µL of the test solution into the HPLC system and record the chromatogram. Identify nardosinone peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of nardosinone Std-AS. The retention times of nardosinone 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 nardosinone in the test solution, and calculate the percentage content of nardosinone in the sample by using the equations as indicated in Appendix IV (B).

Limits

The sample contains not less than 0.29% of nardosinone ($C_{15}H_{22}O_3$), calculated with reference to the dried substance.

7.2 Assay of Volatile Oil

Weigh accurately 25 g of the powdered sample and place it in a 1000-mL round-bottomed flask. Add 350 mL of water and a few glass beads, shake and mix well. Carry out the method as directed in Appendix XIII (Method A).

Limits

The sample contains not less than 2.0% (v/w) of volatile oil.