Rhizoma et Radix Notopterygii



Figure 1 A photograph of Rhizoma et Radix Notopterygii

Official Name: Rhizoma et Radix Notopterygii

Chinese Name: 羌活

Chinese Phonetic Name: Qianghuo

2. SOURCE

Rhizoma et Radix Notopterygii is the dried rhizome and root of *Notopterygium incisum* Ting ex H. T. Chang (Apiaceae/Umbelliferae). The rhizome and root is collected in the spring and autumn. After removal of the clay and rootlets, the rhizomes and roots are dried under the sun or by heat to obtain Rhizoma et Radix Notopterygii.

3. DESCRIPTION

The rhizomes are cylindrical and somewhat curved, showing scars of aerial stem at the apices, 4-13 cm long, 6-25 mm in diameter. Internodes short, forming dense raised annulations like a silkworm (known as Canqiang); or elongated like the nodes of a bamboo (known as Zhujieqiang). Externally reddishbrown to brownish-black in colour, the point where the outer bark had fallen off appears brownish-yellow. Nodes show numerous dotted or tuberculate root scars and brown scales. Texture light and fragile, easily broken. Fracture uneven, with numerous radial clefts, bark brownish-yellow to dark brown, oily, with brown oil dots, wood yellowish-white with distinct rays, pith yellow to yellowish-brown. Odour aromatic; taste slightly bitter and acrid (Fig. 1).

4. **IDENTIFICATION**

4.1 Microscopic Identification (Appendix III)

Transverse section

The cork consists of over 10 rows of flat cells, sometimes a rhytidome is present. Cortex narrow. Phloem broad, ray cells mostly broken, forming many large clefts. Cambium in a ring. Xylem rays usually broken as clefts, with many vessels present. Phloem and pith with numerous secretory canals, which are sub-rounded or irregularly long-rounded, varying in size, up to 450 µm in diameter (Fig. 2).

Powder

Colour brownish-yellow. Fragments of secretory canals easily visible, usually containing yellowishbrown, linear secretion in the canals. Parenchyma cells mainly elongated, mostly containing pale yellowish secretion and oily droplets. Mainly reticulate vessels, with dense pits; spiral and bordered pitted vessels are also observed. Cork cells in lateral view multiseriate, flat, sometimes with a rhytidome outside, containing yellowish-brown matter; surface view polygonal or irregular in shape, thin-walled, crookedly sinuate. Secretory masses yellowish-brown, varying in size and up to 100 µm in diameter (Fig. 3).

4.2 Physicochemical Identification

Procedure

Weigh 0.5 g of the powdered sample and put into a test tube, then add 5 mL of diethyl ether. Sonicate (490 W) the mixture for 60 min. Allow the solid residues to settle. Transfer 2 mL of the supernatant to another test tube. Add 3 drops of hydroxylamines hydrochloride solution (7%, v/v) and mix. Adjust the pH to about 9 with sodium hydroxide solution (10%, w/v). Heat the mixture in a water bath for 1 min. Cool down to room temperature. Adjust the pH to about 3 with hydrochloric acid (6.2%, v/v), then add 1 drop of ferric chloride solution (1%, w/v). A red colouration is observed in the lower layer.

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

Standard solutions

Isoimperatorin standard solution Weigh 1.0 mg of isoimperatorin CRS (Fig. 4) and dissolve in 1 mL of methanol. Notopterol standard solution Weigh 1.0 mg of notopterol CRS (Fig. 4) and dissolve in 1 mL of methanol.

Developing solvent system

Prepare a mixture of toluene and ethyl acetate (4:1, v/v).

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Figure 2 Microscopic features of transverse section of Rhizoma et Radix NotopterygiiA. Sketch B. Section illustration C. Secretory canal [(i) smaller size and (ii) larger size]1. Cork 2. Clefts 3. Phloem 4. Cambium 5. Xylem 6. Pith 7. Secretory canals

川木理 摘活 Radix Saposhnikovise P3 風 茶冬 Radix Angelicae Pubescentis 中藤 Redix Adyranthis Bidentatee Radix Aucklandiae Rhizoma et Radix Notopterygii



Figure 3 Microscopic features of powder of Rhizoma et Radix Notopterygii (under the light microscope)

1. Secretory canal fragments 2. Secretory masses 3. Parenchyma cells 4. Reticulate vessel

5. Bordered-pitted vessels 6. Cork cells

Test solution

Weigh 1.0 g of the powdered sample and put into a 50-mL centrifugal tube, then add 10 mL of methanol. Sonicate (490 W) the mixture for 30 min. Centrifuge at about $1800 \times g$ for 10 min and then 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 isoimperatorin standard solution, notopterol standard solution and the test solution (1 µL each) to the plate. Develop over a path of about 8 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (366 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 $R_{\rm f}$ values, corresponding to those of isoimperatorin and notopterol.

(i)







Figure 4 Chemical structures of (i) isoimperatorin and (ii) notopterol

4.4 High-Performance Liquid Chromatographic Fingerprinting (Appendix XII)

Standard solution

Isoimperatorin standard solution for fingerprinting, *Std-FP (100 mg/L)* Weigh 1.0 mg of isoimperatorin CRS and dissolve in 10 mL of methanol.

Test solution

Weigh 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add 20 mL of methanol. Sonicate (490 W) the mixture for 60 min. Centrifuge at about $1800 \times g$ for 5 min. Filter through a 0.45-µm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (255 nm) and a column (4.6×250 mm) packed with ODS bonded silica gel (5 μ m particle size). The flow rate is about 0.8 mL/min. Programme the chromatographic system as follows –

Time	Water	Acetonitrile	Flution
(min)	(%, v/v)	(%, v/v)	Elution
0 – 5	60	40	isocratic
5 - 45	60 → 30	40 → 70	linear gradient
45 - 50	30 → 0	70 → 100	linear gradient

System suitability requirements

Perform at least five replicate injections each with $10 \,\mu\text{L}$ of isoimperatorin Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of isoimperatorin should not be more than 3.0%; the RSD of the retention time of isoimperatorin peak should not be more than 2.0%; the column efficiency determined from isoimperatorin peak should not be less than 50000 theoretical plates.

The R value between peak 3 and peak 4 in the chromatogram of the test solution should not be less than 1.5 (Fig. 5).

Procedure

Separately inject isoimperatorin Std-FP and the test solution (10 μ L each) into the HPLC system and record the chromatograms. Measure the retention time of isoimperatorin peak in the chromatogram of the isoimperatorin Std-FP and the retention times of the five characteristic peaks (Fig. 5) in the chromatogram of the test solution. Under the same HPLC conditions, identify isoimperatorin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of isoimperatorin Std-FP. The retention times of isoimperatorin 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 five characteristic peaks of Rhizoma et Radix Notopterygii extract are listed in Table 1.

 Table 1
 The RRTs and acceptable ranges of the five characteristic peaks of Rhizoma et Radix

 Notopterygii extract

Peak No.	RRT	Acceptable Range
1 (bergapten)	0.50	±0.03
2	0.57	±0.03
3 (notopterol)	0.81	±0.03
4	0.84	±0.03
5 (marker, isoimperatorin)	1.00	-



Figure 5 A reference fingerprint chromatogram of Rhizoma et Radix Notopterygii 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. 5).

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 1.0%.
- **5.6** Ash (Appendix IX)

Total ash: not more than 6.5%. Acid-insoluble ash: not more than 3.0%.

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

6. EXTRACTIVES (Appendix XI)

Water-soluble extractives (cold extraction method): not less than 19.0%. Ethanol-soluble extractives (cold extraction method): not less than 22.0%.

7. ASSAY

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

Standard solution

Isoimperatorin standard stock solution, Std-Stock (1000 mg/L) Weigh accurately 10.0 mg of isoimperatorin CRS and dissolve in 10 mL of methanol. Isoimperatorin standard solution for assay, Std-AS Measure accurately the volume of the isoimperatorin Std-Stock, dilute with methanol to produce a series of solutions of 5, 20, 60, 100, 200 mg/L for isoimperatorin.

Test solution

Weigh accurately 0.2 g of the powdered sample and put into a 50-mL centrifugal tube, then add accurately 20 mL of methanol and weigh. Sonicate (490 W) the mixture for 60 min and weigh again. Add

an appropriate amount of methanol to compensate the weight loss, if any. Mix and centrifuge at about $1800 \times g$ for 5 min. Filter through a 0.45-µm RC filter.

Chromatographic system

The liquid chromatograph is equipped with a detector (255 nm) and a column (4.6×250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows –

Time	Water	Acetonitrile	Elution	
(min)	(%, v/v)	(%, v/v)	Elution	
0 - 30	50 → 30	50 → 70	linear gradient	

System suitability requirements

Perform at least five replicate injections each with 10 μ L of isoimperatorin Std-AS (60 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of isoimperatorin should not be more than 3.0%; the RSD of the retention time of isoimperatorin peak should not be more than 2.0%; the column efficiency determined from isoimperatorin peak should not be less than 30000 theoretical plates.

The R value between isoimperatorin 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 isoimperatorin Std-AS (10 μ L each) into the HPLC system and record the chromatograms. Plot the peak areas of isoimperatorin against the corresponding concentrations of isoimperatorin Std-AS. Obtain the slope, y-intercept and the r^2 value from the 5-point calibration curve.

Procedure

Inject 10 μ L of the test solution into the HPLC system and record the chromatogram. Identify isoimperatorin peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of isoimperatorin Std-AS. The retention times of isoimperatorin peaks from the two chromatograms should not differ by more than 2.0%. Measure the peak area and calculate the concentration (in milligram per litre) of isoimperatorin in the test solution, and calculate the percentage content of isoimperatorin in the sample by using the equations indicated in Appendix IV(B).

Limits

The sample contains not less than 0.21% of isoimperatorin $(C_{16}H_{14}O_4)$, calculated with reference to the dried substance.



1 2 3 4 5 6

Lane	Sample	Results	
1	Blank (Methanol)	Negative	
2	Sample	Notopterol and isoimperatorin	
	(Rhizoma et Radix Notopterygii)	positive	
2	Sample duplicate	Notopterol and isoimperatorin	
3	(Rhizoma et Radix Notopterygii)	positive	
4	Standard (Issimnaratorin)	Isoimperatorin	
	Standard (IsoImperatorm)	positive	
5	Standard (Notantaral)	Notopterol	
	Standard (Notopteror)	positive	
6	Spiked sample	Notontorol and isoimportorin	
	(Sample plus notopterol and		
	isoimperatorin)	positive	

Figure 1 TLC results of Rhizoma et Radix Notopterygii extract observed under UV light (366 nm)