

0039 - Devil's Claw for Iridoids by HPLC

Botanical Name: *Harpagophytum procumbens*

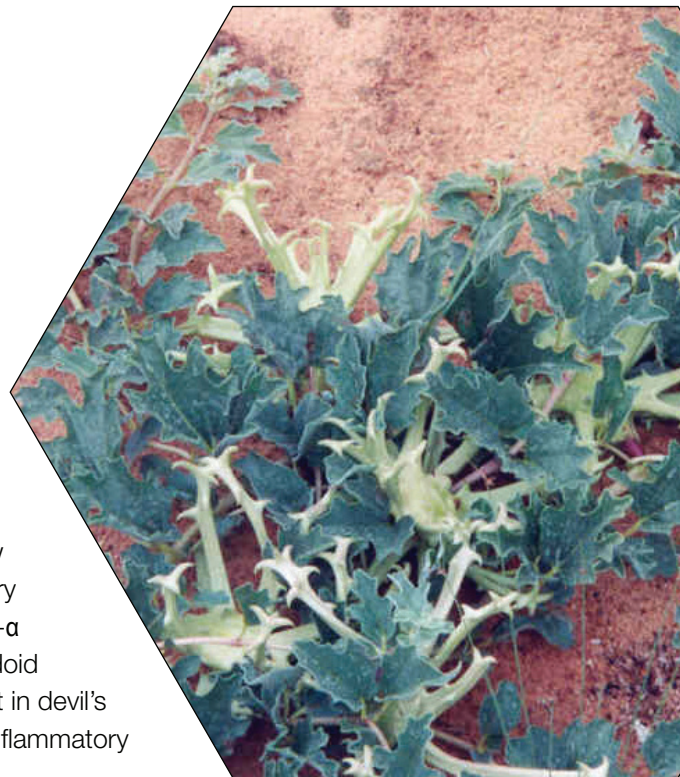
Common Names: Devil's Claw

Parts of Plant Used: Tubers and roots

Uses: As an anti-inflammatory agent to treat arthritis and rheumatism and to alleviate back pain.

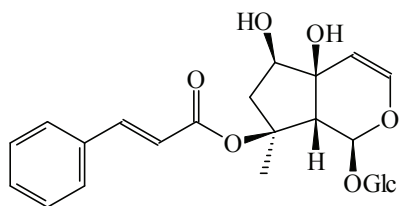
Modes of Action:

Several clinical trials have proven that devil's claw root extracts are effective in fighting osteoarthritis, alleviating back pain, and treating low back pain.¹⁻⁵ Although the mechanism of devil's claw's anti-inflammatory activity is still unclear, extracts of devil's claw were found to inhibit TNF- α synthesis in LPS-stimulated primary human monocytes.⁶ The major iridoid harpagoside has not been proven clinically to be the active component in devil's claw, but generally iridoids are believed to contribute to the total anti-inflammatory effect.

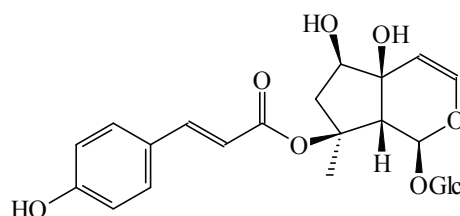


Chemistry and Chemical Markers for Quality Control:

Iridoids are believed to be the major chemical components in devil's claw with harpagoside as the dominant iridoid. Other iridoids purified from devil's claw include procumbide and its 6'-O-p-coumaroyl ester, procumboside, 8-O-(p-coumaroyl)-harpagide.⁷⁻⁹ Several phenylpropanoic acid glycosides (aceteoside, isoaceteoside, verbascoside, 6-acetylacteoside, 2,6-diacetylacteoside) were also identified from the root extract.^{7,8,9,10} Other compounds include sterols, triterpenes, phenolic acid, flavonoids, and a phenylethanoic derivative.^{8,11,12} In the U.S. market, harpagoside is used as marker compound for quality control of devil's claw.



Harpagoside



8-O-(p-Coumaroyl)-harpagide

Methods of Analysis

Various methods have been used to analyze the iridoids in the root of devil's claw including HPLC with UV detection, HPTLC methods with UV detection and visualization with sulfuric acid, a colorimetric method to determine the total glucosides, and GC to analyze the silyl derivatives.¹³⁻¹⁹ HPLC is the most accepted method. Various solvents have been used to extract iridoids from the root of devil's claw including hot water, methanol, and aqueous alcohol solutions.

Method 1:

The method of Baghdikian et al.¹⁵ was used. This method analyzes two iridoids, harpagoside and 8-O-(p-coumaroyl)-harpagide in two species *Harpagophytum procumbens* and *Harpagophytum zeyheri*.

Sample Preparation:

Infuse powdered roots in boiling water (500 mL) and then macerate for 12 hours at 40°C.

Chromatography:

Column: Waters μ Bondapak C18 10 μ m, 300 \times 3.9 mm with a precolumn Guard Pak μ Bondapak C18.

Mobile phase: Water–methanol (43:57) isocratic.

Flow rate: 1.0 mL/minute

Injection volume: 20 μ L

Detection wavelength: 278 nm

Validation Data:

Linearity: Not specified

Accuracy: Not specified

Precision: RSD 2.92% for harpagoside and RSD 1.46% for 8-O-(p-coumaroyl)-harpagide.

Selectivity: Peak identification was determined against standards.

Ruggedness: Not specified

Robustness: Not specified

LOD/LOQ. Not specified

Method 2:

The method of Guillerault et al.¹⁷ was used. This method analyzes three iridoids, harpagoside, harpagide, and 8-O-(p-coumaroyl)-harpagide with two detection methods: UV and light scattering.

Sample Preparation:

For drugs, add 5 g of drug to 70 mL of methanol, warm for 15 minutes, filter, and then evaporate in vacuo. Dissolve the residue in 50 mL of water, and concentrate 2 mL of this preparation on SEP PACK. Elute with 10 mL of water and methanol separately. Evaporate the methanol part under vacuum and dissolve in 0.5 mL of water for HPLC.

For the extract, disperse 5 g in 100 mL of water, and concentrate 2 mL of the extraction solution and prepare as for drugs.

Chromatography:

Column: Phenomenex RP-18 Bondclone, 10 μ m, 300 \times 3.9 mm with a precolumn Guard Pak μ Bondapak C18.

Mobile phase: Water–methanol

Gradient: 0 to 10 minutes, 15% methanol; 10 to 45 minutes, 50% methanol; and 45 to 60 minutes, 15% methanol.

Flow rate: 1.0 mL/minute

Injection volume: 20 μ L

Detection wavelength: 270, 305, and 312 nm for harpagoside and 8-O-(p-coumaroyl)-harpagide and light scattering for harpagide.

Validation Data:

Linearity: 0.041 to 0.5 mg/mL for 8-O-(p-coumaroyl)-harpagide and 0.1 to 0.8 mg/mL for harpagide and 0.13 to 1 mg/mL for harpagoside by light-scattering detection.

Accuracy: Not specified

Precision: RSD all less than 5% for the different detection methods.

Selectivity: Peak identification was determined against standards.

Ruggedness: Not specified

Robustness: Not specified

LOD/LOQ. Limit of detection for 8-O-(p-coumaroyl)-harpagide was 2 mcg/mL by UV at 312 and 50 mcg/mL by light scattering; for harpagoside it was 4 mcg/mL by UV at 278 nm and 50 mcg/mL by light scattering; and for harpagide it was 60 mcg/mL by light-scattering detection.

Method 3:

The unpublished method of Mingfu Wang was used.

Sample Preparation:

Weigh 300-mg samples, add 35 mL of methanol, sonicate for 25 minutes, and shake for 15 minutes. Allow the flask to cool to room temperature, and fill to volume with methanol.

Chromatography:

Column: Phenomenex Luna C18 (2), 5 µm, 250 × 4.6 mm.

Mobile phase: Water (adjusted to pH 2.5 with phosphoric acid)–methanol isocratic (34:66).

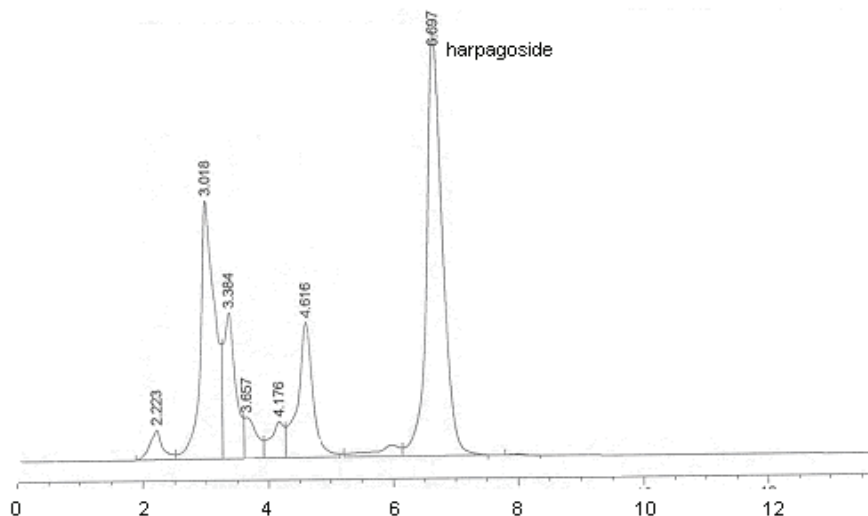
Flow rate: 0.8 mL/minute

Injection volume: 10 µL

Detection wavelength: 278 nm

Validation Data:

Not available

Representative HPLC chromatogram run by method 3.

References:

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