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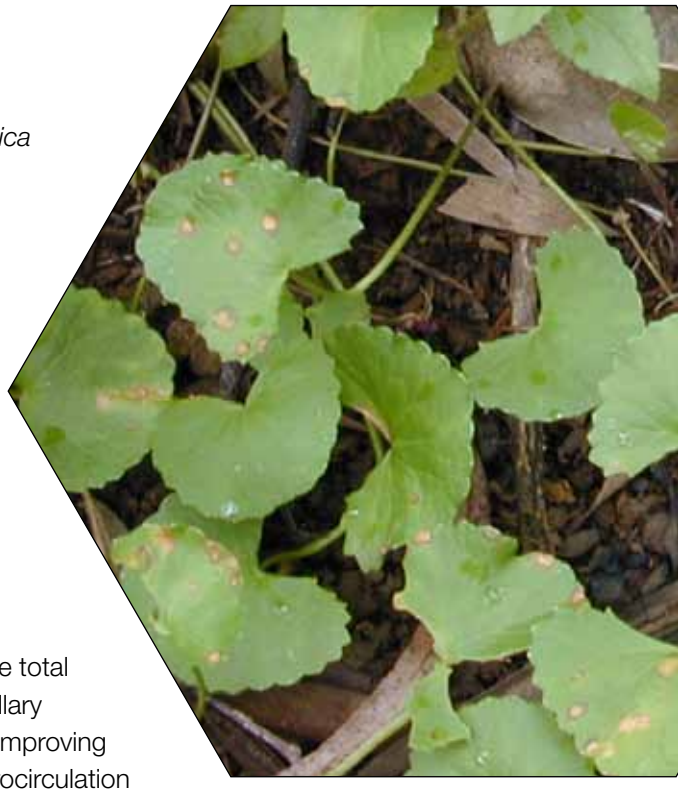
Application Note

As published in "The Handbook of Analytical Methods for Dietary Supplements"

0051 - Gotu Kola for Triterpenes by HPLC



Botanical Name: *Centella asiatica* (L.) Urban; *Hydrocotyle asiatica*

Common Names: Hydrocotyle, India pennywort

Parts of Plant Used: Leaves, dried aboveground parts

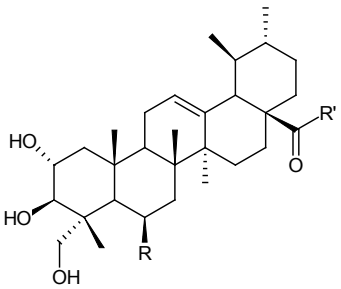
Uses: To improve memory, to improve blood flow throughout the body by strengthening the veins and capillaries, as a wound-healing agent, as a topical application for skin conditions such as ulcers, wounds, and eczema.

Modes of Action:

Several clinical trials have evaluted the health benefits of gotu kola.[1] The total triterpenes fraction has been used to treat edema and to increase capillary filtration in venous hypertension,[2] to treat diabetic microangiopathy by improving microcirculation and decreasing capillary permeability,[3] to improve microcirculation in venous hypertension,[4] and to stimulate maturation of the scar by the production of type 1 collagen.[5]

Chemical Markers:

The main chemical components in gotu kola are triterpenes including ursane- and oleanane-type triterpene glycosides. The triterpenes include asiaticoside, madecassoside (asiaticoside A), asiaticoside B, scheffoleoside A, madecassic acid, asiatic acid, terminolic acid, and centellasaponins B, C, and D.[6,7] Other identified components include flavonoids (quercetin, kaempferol, 3-glucosylquercetin, 3-glucosylkaempferol, ß-sitosterol, daucosterol, vanillic acid, 11-oxohenicosanyl-cyclohexane, dotriacont-8-1-oic acid, carotenoids, and chlorophyll.[8–11] The essential oils of gotu kola aerial parts were analyzed by GC and GC–MS and the major chemical category found was the sesquiterpenoids, with ß-caryophyllene, α-humulene, and germacrene-D as the most abundant.[12] As the triterpenes are the major and also the bioactive components in gotu kola, they are used as chemical markers for quality control of this herb. The marker compounds include asiaticoside, madecassoside, asiatic acid, madecassic acid, terminolic acid, and asiaticoside B.



Asiatic acid: R = H, R' = OH
Madecassic acid: R = OH, R' = OH
Asiaticoside: R = H, R' = O-Glu(6-1)-Glu(4-1)-Rham
Madecassoside: R = OH, R' = O-Glu(6-1)-Glu(4-1)-Rham

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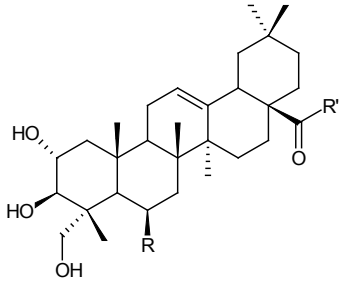


Terminolic acid: R = OH, R' = OH
Asiaticoside B: R = OH, R' = O-Glu(6-1)-Glu(4-1)-Rham

Methods of Analysis

Most published analytical methods for total triterpenes in gotu kola use reversed-phase HPLC with gradient elution and low-wavelength detection.[13–17] Most of these methods are not accurate for analysis of triterpenes in gotu kola because they cannot separate madecassic acid from terminolic acid and/or madecassoside from asiaticoside.[13]

Extraction is of key importance for the accurate analysis of total triterpenes. Usually pure methanol, 90% methanol aqueous solution, and 80% methanol aqueous solution are suitable solvents for extraction; organic solvents (acetone, ethyl acetate, chloroform) are not suitable for triterpene glycosides. Triterpenes are not very soluble in water, so the increased level of water in the extraction solvent will decrease the extraction efficacy of triterpenes such as asiatic acid and madecassic acid.

Method 1:

Schaneberg et al.[13] were the first to separate totally the six triterpenes of interest: asiaticoside, madecassoside (asiaticoside A), asiaticoside B, madecassic acid, asiatic acid, and terminolic acid.

Sample Preparation:

Place gotu kola powders (about 500 mg) into a 15-mL screw-cap polypropylene centrifuge tube and extract three times by sonication for 10 minutes. Centrifuge and transfer the supernates to a 10-mL volumetric flask and fill to volume with methanol.

Chromatography:

Column: Phenomenex Aqua, C18, 200 Å, 5 µm, 150 × 4.6 mm guard column (Security Guard C18 cartridge system from Phenomenex).
Mobile phase: Solvent A = water (0.01% trifluoroacetic acid), solvent B = acetonitrile (6.0% methyl *tert*-butyl ether and 0.01% trifluoroacetic acid), solvent C = acetonitrile (12.0% methyl *tert*-butyl ether and 0.01% trifluoroacetic acid), solvent D = acetonitrile.
Gradient:

Time (minutes)	%A	%B	%C	%D	Curve
0	81	19	-	-	0
15	67	33	-	-	10
20	67	0	33	-	6
40	59	-	41	-	10
50	55	-	45	-	-
60	-	-	-	100	-
70	81	19	-	-	-

Flow rate: 1.0 mL/minute
Injection volume: 10 µL
Detection wavelength: 206 nm

Validation Data:

Linearity: 5.0 to 850 mcg/mL with a correlation coefficient over 0.995 for all six compounds.
Accuracy: The percent recoveries were from 98.39 to 100.02 for different triterpenes.
Precision: Not specified
Selectivity: Peak identification was determined against standards.
Ruggedness: Not specified
Robustness: Not specified
LOD/LOQ: LOD = 0.010 ng.

Method 2:

The unpublished method of Mingfu Wang was used.

Chromatography:

Column: Phenomenex Prodigy ODS3, 5 µm, 3.2 × 150 mm.
Mobile phase: Solvent A = water (0.2% phosphoric acid), solvent B = acetonitrile.
Gradient:

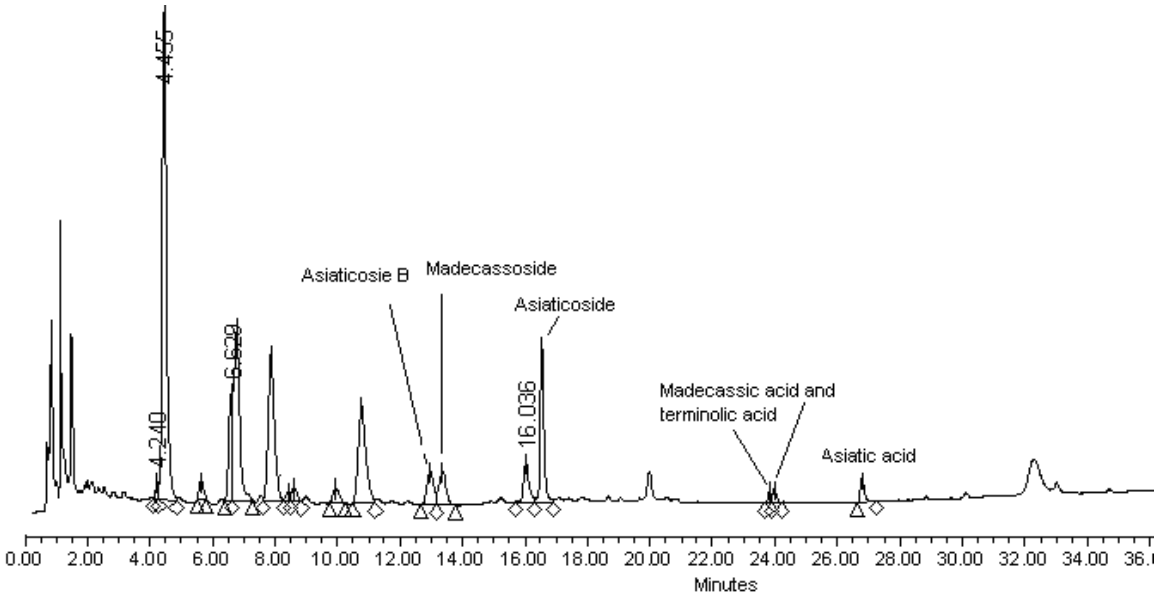
Time (minutes)	%A	%B
0	82	18
12	78	22
25	55	45
35	20	80
36	82	18
45	82	18

Flow rate: 1.2 mL/minute
Injection volume: 10 µL
Detection wavelength: 203 nm

Validation Data:

Linearity: 10 to 200 mcg/mL with a correlation coefficient over 0.999 for berberine and hydrastine.
Accuracy: The average percent recoveries were 98.25 and 104.2 for berberine and hydrastine, respectively.
Precision: Not specified
Selectivity: Peak identification was determined against standards.
Ruggedness: Not specified
Robustness: Not specified
LOD/LOQ: LOQ = 0.4 mcg/mL for both compounds, LOD = 0.1 mcg/mL for both compounds.

Representative HPLC Chromatogram of Gotu Kola Run by Method 2



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