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Application Note

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

0059 - Passion Flower for Flavonoids by HPLC

Botanical Name: *Passiflora incarnata L.*

Common Names: Apricot vine, maypop, wild passion flower

Parts of Plant Used: Dried aerial parts

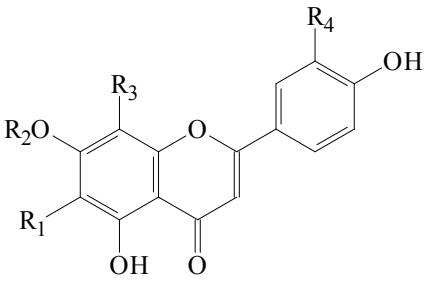
Uses: Treatment of anxiety, nervousness, and minor sleeplessness. Usually used as an infusion or in the form of extracts, mainly combined with other sedative herbs such as hops, kava, and valerian.

Modes of Action:

Passiflora incarnata L. is widely used in herbal sedatives. Passion flower is used in combination with hops, valerian, and kava frequently; therefore, clinical studies using this individual herb are not available. Many pharmacological studies confirm the sedative effects of passion flower and some studies also found anxiolytic effects. Many compounds including maltol, ethyl maltol, indole alkaloids, and flavonoids were found to be responsible, in part, for its activity. But the mechanism of action is unknown.[1–8]

Chemical Markers:

The main chemical components in passion flower are flavone-di-C-glycosides of apigenin and luteolin types including shaftoside, isoshaftoside, isoorientin, orientin, isovitexin, vitexin, saponarin, vicenin-2, and lucenin-2. Passion flower also contains coumarins, maltol, phenolic acids, phytosterols, small amounts of indole alkaloids (harmane, harmine, harmol), and small amounts of essential oil.[9,10] The volatile constituents of *P. incarnata* were investigated by GC and the main chemicals were identified as hexanal, benzyl alcohol, linalool, 2-phenylethyl alcohol, 2-hydroxybenzoic acid methyl ester, carvone, *trans*-anethole, eugenol, isoeugenol, ß-ionone, α-bergamotol, phytol, and two fatty acids (palmitic and oleic).[11] Although the active compounds in passion flower have not been identified, the flavonoids are used as marker compounds for quality control of crude herb and its related preparations.



Name	R_1	R_2	R_3	R_4
Vicenin 2	Glc	H	Glc	H
Schafloside	Glc	H	Ara	H
Isoschafioside	Ara	H	Glc	H
Isoorientin	Glc	H	H	OH
Orientin	H	H	Glc	OH
Isovitexin	Glc	H	H	H
Vitexin	H	H	Glc	H

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Methods of Analysis

Various methods have been developed to analyze the chemicals in passion flower including GC, GC–MS, and GC–FTIR for volatile analysis, HPLC for alkaloids and flavonoid analysis, LC–MS for identifying the flavonoids, capillary electrophoresis for flavonoid analysis, and TLC and colorimetry for determination the total amounts of flavonoids and alkaloids.[12–20]

The flavonoids in passion flower can be extracted easily with methanol, ethanol, or aqueous methanol and ethanol.

Method 1:

The method of Bokstaller and Schmidt19 can be used to identify and quantify the flavonoids vicenin-2, schaftoside, isoorientin, orientin, isovitexin-2"-glucoside, vitexin, and isovitexin.

Sample Preparation:

Prepare samples in 50% ethanol.

Chromatography:

Column: Merck LiChrospher 100RP, 5 µm, 250 × 4 mm with a guard column LiChrospher 100RP-18, 5 µm, 4 × 4 mm.
Mobile phase: Solvent A = acetonitrile–water (110:690, wt/wt), solvent B = acetonitrile–water (120:180, m/m). Both solutions were adjusted to pH 2.8 with phosphoric acid (85%).
Gradient:

Time (minutes)	%A	%B
0	100	0
13	100	0
13.5	0	100
16.5	0	100
17	100	0
25	100	0

Flow rate: 1.2 mL/minute
Injection volume: 10 µL
Detection wavelength: 340 nm
Column temperature: Not controlled

Validation Data:

Not available

Method 2:

The method of Raffaelli et al.18 can be combined with MS detection to identify 10 flavonoids including vicenin-2, schaftoside, isoschaftoside, isoorientin-2"-O- ß-glucopyranoside, isoorientin, isovitexin-2"-O-ß-glucopyranoside, swertisin, orientin, isovitexin, and vitexin.

Sample Preparation:

Prepare in 50% ethanol.

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Chromatography:
Column: LiChrospher C18, 250 × 4.6 mm.
Mobile phase: Isocratic condition, water–tetrahydrofuran–isopropanol–acetonitrile (88:8:1.6:2.4); the mobile phase also contained 0.07% formic acid.
Flow rate: 1.0 mL/minute
Injection volume: 10 µL
Detection wavelength: 340 nm
Column temperature: Not controlled

Validation Data:
Not specified

Method 3:

The method of Abourashed et al.[14] was used to analyze six flavonoids, schaftoside, isoschaftoside, isoorientin, orientin, isovitexin and vitexin, in different species of Passiflora.

Sample Preparation:
Load 2 g of powdered sample onto a stainless steel cartridge and extract in a Dionex accelerated speed extractor with the following program: solvent = 80% methanol; temperature = 40°C; pressure = 1000 psi; cycle duration = 10 minutes with solvent flush cycle; repeat three times.

Chromatography:
Column: Phenomenex Hypersil ODS, 5 µm, 150 × 4.6 mm with a SecurityGuard cartridge, C18, 4 × 3 mm.
Mobile phase: Solvent A = 0.01% phosphoric acid at pH 3.1, solvent B = acetonitrile–tetrahydrofuran–isopropanol (80:80:20 vol/vol/vol).
Gradient:

Time (minutes)	%A	%B
0	100	0
5	100	0
60	0	100

Between each injection, wash with methanol for 5 minutes, and equilibrate with solvent A for 20 minutes.
Flow rate: 1.0 mL/minute
Injection volume: 10 µL
Detection wavelength: 280 nm
Column temperature: Not controlled

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