

Hawthorn Leaf with Flower

Hawthorn Leaf with Flower consists of the dried tips of the flower-bearing branches up to 7 cm in length of *Crataegus monogyna* Jacq. emend Lindman. or *Crataegus laevigata* (Poir.) DC., also known as *Crataegus oxycantha* Linn é (Fam. Rosaceae). It contains not less than 0.6 percent of C-glycosylated flavones, expressed as vitexin (C₂₁H₂₀O₁₀), and not less than 0.45% of O-glycosylated flavones, expressed as hyperoside (C₂₁H₂₀O₁₂), calculated on the dried basis.

Packaging and storage— Store in a well-closed container, protected from light.

Labeling— The label states the Latin binomial and, following the official name, the parts of the plant contained in the article. The label also states the following cautionary statement:

“Cardiotonic Herb. Not recommended for use without the advice of a health care practitioner.”

Botanic characteristics—

Macroscopic— It shows fragments of dark brown, lignified branches, usually from 1 mm to not more than 2.5 mm in diameter, bearing alternate petiolate leaves, with small, often deciduous styles, and bearing numerous white flowers in a corymbose arrangement. The leaves are more or less strongly lobate, and their margins are slightly or very slightly serrate. *C. laevigata* has pinnatilobate to pinnatifid leaves, divided into three, five, or seven obtuse lobes; the leaves of *C. monogyna* are almost pinnatisect with three to five acute lobes. The adaxial surface of the leaf is dark green to brownish green; the abaxial surface is lighter, greyish green, and shows a dense network of clearly visible veinlets and slightly prominent principal veins. The leaves of *C. laevigata* and *C. monogyna* are glabrous or bear isolated trichomes. The flowers consist of a brownish-green tubulous calyx, ending in its upper part in five triangular segments, and of five yellowish white to brownish free petals, rounded to widely oval, shortly unguiculate, and with numerous stamens. The ovary, fused to the tubulous calyx, bears one to three long styles and consists of the same number of carpels, each containing one fertile ovule. *C. monogyna* has one style and one carpel, and *C. laevigata* has two or three styles and carpels.

Microscopic— When reduced to a fine powder and examined under a microscope, the yellowish-green powder shows the following characteristics: unicellular covering trichomes, usually with thick walls and wide lumens, almost straight to somewhat curved, pitted at the base; fragments of leaf epidermis with cells that have sinuous to polygonal walls and large anomocytic stomata surrounded by four to seven subsidiary cells; clusters of parenchymatous cells containing

calcium oxalate crystals, usually from 10 to 20 μm in length; fragments of petals showing rounded polygonal epidermal cells, strongly papillose, with thick walls, the cuticle of which clearly shows wavy striations; fragments of anthers whose endothecium has an arched and regularly thickened margin; fragments of stems containing collenchymatous cells, vessels and fibers of lignified sclerenchyma, with narrow lumens; numerous rounded to elliptical triangular pollen grains up to 45 μm in diameter, with free exines and three germinal pores.

Identification—

A: [Thin-Layer Chromatographic Identification Test 201](#) —

Adsorbent: 0.50-mm layer of chromatographic silica gel mixture.

Test solution: 0.1 g per mL, prepared as follows. Weigh and finely powder about 10 g of Hawthorn Leaf with Flower. Transfer 1 g of the powder to a suitable flask, and add 10 mL of methanol. Heat the flask on a water bath maintained at 65 °C for 5 minutes, cool, filter, and use the filtrate.

Standard solution: 0.1 mg each of chlorogenic acid, rutin, USP Hyperoside RS, and USP Vitexin RS per mL, in methanol. [Note—Reserve a portion of this solution for use in Identification test B.]

Developing solvent system: a mixture of ethyl acetate, water, glacial acetic acid, and formic acid (10:2.6:1.1:1.1).

Procedure— Proceed as directed in the chapter, except to dry the plate at 105 °C, and spray the plate while still hot with 10 mL of a solution of 2-aminoethyl diphenylborinate in methanol (1 in 100) and then with 10 mL of a solution of polyethylene glycol 4000 in methanol (5 in 100). Allow the plate to air-dry for about 30 minutes, and examine the plate under long-wavelength UV light: the chromatogram of the Standard solution exhibits an intense orange zone (at R_F value of about 0.3) due to rutin; a light blue fluorescent zone (at R_F value of about 0.4) due to chlorogenic acid; a yellowish-orange zone (at R_F value of about 0.55) due to hyperoside; and a yellowish green zone (at R_F value of about 0.65) due to vitexin. The chromatogram of the Test solution, in addition to the zones due to rutin, chlorogenic acid, hyperoside, and vitexin, exhibits a yellowish-green zone (at R_F value of about 0.35) due to vitexin-2-rhamnoside; a light blue fluorescent zone (at R_F value of about 0.6) due to spiraeoside; and a light blue fluorescent zone near the solvent front (at R_F

value of about 0.9) due to caffeic acid. The chromatogram of the Test solution also exhibits other zones with weaker fluorescence.

B: Solution A—Prepare a filtered and degassed mixture of tetrahydrofuran, methanol, and acetonitrile, (92.4:4.2:3.4).

Solution B— Prepare a filtered and degassed solution of 0.5% phosphoric acid in water.

Mobile phase— Use variable mixtures of Solution A and Solution B as directed for Chromatographic system. Make adjustments if necessary.

Standard solution— Use the Standard solution reserved from Identification test A.

Test solution— Transfer about 3 g of finely powdered Hawthorn Leaf with Flower to a 100-mL round-bottom flask, add 60 mL of a mixture of methanol and water (80:20), and heat on a hot water bath under reflux for 1 hour. Cool, filter, and collect the filtrate in a separate flask. Transfer the residue from the filter back to the flask, add 40 mL of a mixture of methanol and water (80:20), and heat on a hot water bath under reflux for 10 minutes. Cool, filter, and combine the filtrate with the filtrate obtained from the first extraction. Evaporate the solvent from the combined filtrates under vacuum to a volume of about 20 mL. Dilute the resulting solution with a mixture of methanol and water (80:20) to 25.0 mL. Filter 5.0 mL of this solution through a freshly conditioned solid-phase extraction column containing 360 mg of packing L1, collect the filtrate in a 10-mL volumetric flask, dilute with a mixture of methanol and water (80:20) to volume, and mix.

Chromatographic system— The liquid chromatograph is equipped with a 336-nm detector and a 4.0-mm × 10-cm column that contains 5-µm packing L1. The flow rate is about 1.0 mL per minute.

The column temperature is maintained at 25 . The chromatograph is programmed as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Elution
0–12	12	88	isocratic
12–25	12→18	88→82	linear gradient
25–30	18	82	isocratic

Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the relative retention times are about 0.26 for chlorogenic acid, 1.0 for vitexin, 1.16 for rutin, and 1.4 for hyperoside; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— Separately inject equal volumes (about 5 μL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the retention times for the major peaks: the relative retention times in the chromatogram of the Test solution are about 1.53 for acetyl vitexin-2 β -D-glucopyranoside, 1.0 for vitexin, 0.73 for isovitexin, and 0.67 for vitexin-2 β -D-glucopyranoside; and the retention times of the peaks for chlorogenic acid, vitexin, rutin, and hyperoside in the chromatogram of the Test solution correspond to those in the chromatogram of the Standard solution.

[Microbial enumeration](#) [2021](#) — The total bacterial count does not exceed 10⁴ cfu per g, the total combined molds and yeasts count does not exceed 100 cfu per g, and it meets the requirements of the tests for absence of *Salmonella* species, *Escherichia coli*, and *Staphylococcus aureus*.

[Loss on drying](#) [731](#) — Dry 1.0 g of finely powdered Hawthorn Leaf with Flower at 105 °C for 2 hours: it loses not more than 10.0% of its weight.

[Foreign organic matter](#) [561](#) : not more than 8.0% of lignified matter.

[Total ash](#) [561](#) : not more than 9.0%.

[Pesticide residues](#) [561](#) : meets the requirements.

[Heavy metals, Method III](#) [231](#) : 0.002%.

Content of C-glycosylated flavones—

Solution A— Prepare a 0.5% solution of phosphoric acid in water.

Solution B— Prepare a mixture of tetrahydrofuran, isopropyl alcohol, and acetonitrile (10:8:3).

Mobile phase— Prepare a filtered and degassed mixture of Solution A and Solution B (88:12).

Make adjustments if necessary.

Standard solution— Dissolve an accurately weighed quantity of USP Vitexin RS in Solution B, with heating if necessary, to obtain a solution having a known concentration of about 0.3 mg per mL.

Test solution— Weigh and finely powder about 100 g of Hawthorn Leaf with Flower. Transfer about 4 g of the powder, accurately weighed, to a continuous-extraction apparatus fitted with a flask containing about 80 mL of methanol, and extract the test specimen for 5 hours. Cool, remove

the flask, and evaporate the solvent from the extract under vacuum to about 40 mL. Transfer this solution to a 50-mL volumetric flask, dilute with methanol to volume, and mix. Transfer 10.0 mL of the solution to a suitable flask fitted with a reflux condenser, add 4 mL of 25% hydrochloric acid, and heat the flask under reflux on a water bath at 65 °C for 90 minutes. Cool, transfer the contents of the flask to a 50-mL volumetric flask, dilute with methanol to volume, and mix.

Chromatographic system— The liquid chromatograph is equipped with a 336-nm detector and a 4-mm × 10-cm column that contains packing L1. The flow rate is about 1.0 mL per minute.

Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the column efficiency is not less than 3000 theoretical plates; the tailing factor is between 0.8 and 2; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— Separately inject equal volumes (about 5 µL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the areas of the major peaks. Calculate the percentage of C-glycosylated flavones, expressed as vitexin (C₂₁H₂₀O₁₀), in the portion of Hawthorn Leaf with Flower taken by the formula:

$$25(C/W)(\sum r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Vitexin RS in the Standard solution; W is the weight, in g, of Hawthorn Leaf with Flower taken to prepare the Test solution; SrU is the sum of the peak areas of vitexin and isovitexin, with a relative retention time of about 1.0 and 0.85, respectively, in the chromatogram of the Test solution; and rS is the vitexin peak area obtained from the Standard solution.

Content of O-glycosylated flavones—

Mobile phase— Prepare a mixture of methanol, water, and phosphoric acid (100:100:1). Make adjustments if necessary.

Standard solution— Dissolve an accurately weighed quantity of USP Quercetin RS in methanol, and dilute quantitatively, and stepwise if necessary, to obtain a solution having a known concentration of about 0.05 mg per mL.

Test solution— Proceed as directed for Test solution under Content of C-glycosylated flavones, except to use 1 mL of 25% hydrochloric acid for 60 minutes instead of 4 mL of 25% hydrochloric acid for 90 minutes.

Chromatographic system— The liquid chromatograph is equipped with a 370-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute.

Chromatograph the Standard solution, and record the peak responses as directed for Procedure: the column efficiency is not less than 3000 theoretical plates; the tailing factor is between 0.8 and 2; and the relative standard deviation for replicate injections is not more than 2.0%.

Procedure— Separately inject equal volumes (about 10 µL) of the Standard solution and the Test solution into the chromatograph, record the chromatograms, and measure the areas of the major peaks. Calculate the percentage of O-glycosylated flavones, expressed as hyperoside (C₂₁H₂₀O₁₂), in the portion of Hawthorn Leaf with Flower taken by the formula:

$$(464.38/302.24)25(C/W)(r_U / r_S)$$

in which 464.38 and 302.24 are the molecular weights of hyperoside and quercetin, respectively; C is the concentration, in mg per mL, of USP Quercetin RS in the Standard solution; and r_U and r_S are the quercetin peak areas in the chromatograms obtained from the Test solution and Standard solution, respectively.