

St. John's Wort

St. John's Wort consists of the dried flowering tops or aerial parts of *Hypericum perforatum* Linn é (Fam. Hypericaceae), gathered shortly before or during flowering. It contains not less than 0.04 percent of the combined total of hypericin ($C_{30}H_{16}O_8$) and pseudohypericin ($C_{30}H_{16}O_9$) and not less than 0.6 percent of hyperforin ($C_{35}H_{52}O_4$).

Packaging and storage— Store in tight containers, protected from light and moisture.

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

Botanic characteristics—

Macroscopic— The two-edged stem is greenish yellow, rounded, and has two ribs running longitudinally on opposite sides. The plant is adversifoliate, its leaves are sessile, ovoid or elongated, up to 3.5 cm in length, smooth-edged, and hairless with translucent perforations. The very numerous yellow, short-stemmed, pentamerous flowers form false umbels shaped like grape clusters. The five lanceolate and black-dotted sepals are one-half the length of the dark yellow petals, which are shaped like slanted ovals and whose edges are set with dark red glands. The numerous stamens are joined in three to six bundles (usually three). The ovary is surmounted by three styles. Some ovaries are already developed into greenish, elongated, oval triovular capsules with various degrees of maturity. When chopped, the crude plant material is distinguished by numerous yellow to yellowish-brown flower buds and individual petals with dark red glands at the edges. The light green to brown-green leaf fragments, characterized by plicate marcescence, appear stippled when held up to the light. The greenish yellow or reddish-brown hollow stem fragments are distinguished by two longitudinal edges.

Histology— The stems have elongated epidermal cells with straight beaded, anticlinal walls; cuticle smooth; frequent paracytic stomata with two small adjacent epidermal cells; cortex of five to six rows of collenchyma; stele with secondary growth consisting of a compacted ring of phloem, with a wide area of lignified xylem and small areas of intraxylary phloem; parenchymatous pith, lignified and pitted in older stems; oil glands may occur in the cortex and phloem.

The upper surface of the leaf has polygonal cells with sinuous, slightly beaded anticlinal walls; cells of lower surface smaller, with anticlinal walls more wavy with frequent paracytic, sometimes anomocytic, stomata; smooth cuticle, thicker on upper surface, straight-walled, elongated

epidermal cells of veins occasionally beaded. Dorsiventral, single palisade lamina; large oil glands equal to depth of spongy mesophyll. Midrib containing single, collateral bundle with small area of lignified xylem. Trichomes and calcium oxalate are absent.

The sepal of the flower has characteristics resembling those of the leaf. Petal, narrow, elongated, thin-walled, epidermal cells with straight anticlinal walls on the outer surface and wavy on the inner surface. Stamen, lignified fibrous layer of anther wall; elongated, thin-walled cells of filament with striated cuticle; subprolate pollen grains, about 20 μm in diameter with three pores and a smooth exine. Ovary, small polygonal cells with underlying oil glands; seed testa, brown, thick-walled hexagonal cells.

Identification— Weigh and finely powder about 50 g of St. John's Wort, and transfer 10 g of the powder to a suitable flask. Add 100 mL of methanol, and mix, with frequent shaking, for 15 minutes. Filter, and use the filtrate as the test solution. Apply separately, as bands, 20 μL of the test solution and 20 μL of a Standard solution of USP Hyperoside RS in methanol containing 0.5 mg per mL to a suitable thin-layer chromatographic plate coated with a 0.50-mm layer of chromatographic silica gel mixture, and allow the bands to dry. Develop the chromatograms in a solvent system consisting of the upper layer of a mixture of ethyl acetate, water, glacial acetic acid, and formic acid (10:2.6:1.1:1.1) until the solvent front has moved about three-fourths of the length of the plate. Remove the plate from the chromatographic chamber, mark the solvent front, and allow the plate to air-dry until the solvents evaporate. Spray the plate with a 1% solution of 2-aminoethyl diphenylborinate in methanol and allow the plate to air-dry. Immediately after, spray the plate with a 5% solution of polyethylene glycol 400 in alcohol, and allow the plate to air-dry. Examine the plate under UV light at 365 nm. The chromatogram of the test solution exhibits several zones having a yellowish-orange fluorescence, one of which, appearing at an RF value of about 0.5, corresponds in RF value and intensity to a similar zone in the chromatogram of the Standard solution. The chromatogram of the test solution exhibits also two zones of red fluorescence, one at an RF value of about 0.85 (presence of hypericin) and the other at an RF value of about 0.80 (presence of pseudohypericin), and two zones of higher blue fluorescence (presence of chlorogenic and neochlorogenic acids) located below yellow to yellowish-orange hyperoside zone.

[Total ash](#) [561](#) : not more than 5.0%.

[Water content](#) [561](#) : not more than 10.0%.

[Foreign organic matter](#) [561](#) : not more than 2.0%.

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

[Microbial enumeration tests](#) [61](#) and [Tests for specified microorganisms](#) [62](#) — 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 and *Escherichia coli* and for absence of *Staphylococcus aureus*.

Content of hypericin and pseudohypericin— [note—Conduct all sample preparations with minimal exposure to subdued light, and use low-actinic glassware to protect solutions from light.]

Solvent: a mixture of methanol and acetone (1:1).

Solution A— Dilute 3 mL of phosphoric acid with water to 1000 mL, and mix.

Solution B— Use acetonitrile.

Solution C— Use methanol.

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

Standard solution 1— Quantitatively dissolve an accurately weighed quantity of USP Oxybenzone RS in Solvent to obtain a solution having a known concentration of about 0.0025 mg per mL.

Standard solution 2— Quantitatively dissolve an accurately weighed quantity of USP Powdered St. John's Wort Extract RS in Solvent to obtain a solution having a known concentration of about 1 mg per mL.

Test solution— Weigh and pulverize about 10 g of St. John's Wort. Transfer about 1 g, accurately weighed, to a round-bottom flask equipped with a condenser and protected from light, add about 50 mL of Solvent and a magnetic stirring bar, and heat at 60 °C for two hours while stirring. Cool to room temperature, and pass through filter paper into a 50-mL volumetric flask. Wash the flask and the residue on the filter with Solvent, dilute to volume with the washings, and mix. Pass the solution through a polytetrafluoroethylene membrane filter having a 0.45- μ m or finer porosity, and use the filtrate.

Chromatographic system— The liquid chromatograph is equipped with a 270-nm and a 588-nm detector, a 4.6-mm × 25-cm column that contains packing L1, and a guard-column that contains packing L1. The flow rate is about 1 mL per minute. The column temperature is maintained at 30 . After equilibration with 100% Solution A, the chromatograph is programmed to provide a linear gradient elution as follows.

Time (minutes)	Solution A (%)	Solution B (%)	Solution C (%)
0–10	100→85	0→15	0
10–30	85→70	15→20	0→10
30–40	70→10	20→75	10→15
40–55	10→5	75→80	15
55–56	5→100	80→0	15→0
56–66	100	0	0

Chromatograph Standard solution 1, and record the peak responses at 270 nm as directed for Procedure: the column efficiency, N, determined from oxybenzone is not less than 100,000 theoretical plates; the tailing factor for oxybenzone is not more than 1.5; and the relative standard deviation for replicate injections is not more than 2.0%. Chromatograph Standard solution 2, and record the peak responses at 270 nm and 588 nm as directed for Procedure: the chromatograms obtained are similar to the respective Reference Chromatograms provided with USP Powdered St. John's Wort Extract RS.

Procedure— Separately inject equal volumes (about 20 µL) of Standard solution 1 and the Test solution into the chromatograph, record the chromatograms, and measure the areas at 270 nm of the relevant peaks in the chromatogram of the Test solution. Calculate the percentage of hypericin (C₃₀H₁₆O₈) and pseudohypericin (C₃₀H₁₆O₉) in the portion of St. John's Wort taken by the formula:

$$5(C/FW)(r_U / r_S)$$

in which C is the concentration, in mg per mL, of USP Oxybenzone RS in Standard solution 1; F is the response factor of the relevant analyte relative to that of oxybenzone and is 1.30 for hypericin and 1.24 for pseudohypericin; W is the quantity, in g, of St. John's Wort taken to prepare the Test solution; r_U is the peak area of the relevant analyte obtained from the Test solution; and r_S is the peak area of oxybenzone obtained from Standard solution 1.

Content of hyperforin— Using the chromatograms obtained in the test for Content of hypericin and pseudohypericin, calculate the percentage of hyperforin ($C_{35}H_{52}O_4$) in the portion of St. John's Wort taken by the formula:

$$5(C/0.46W)(r_U/r_S)$$

in which 0.46 is the response factor of hyperforin, relative to that of oxybenzone; r_U is the area of the hyperforin peak in the chromatogram of the Test solution; and the other terms are as defined therein.