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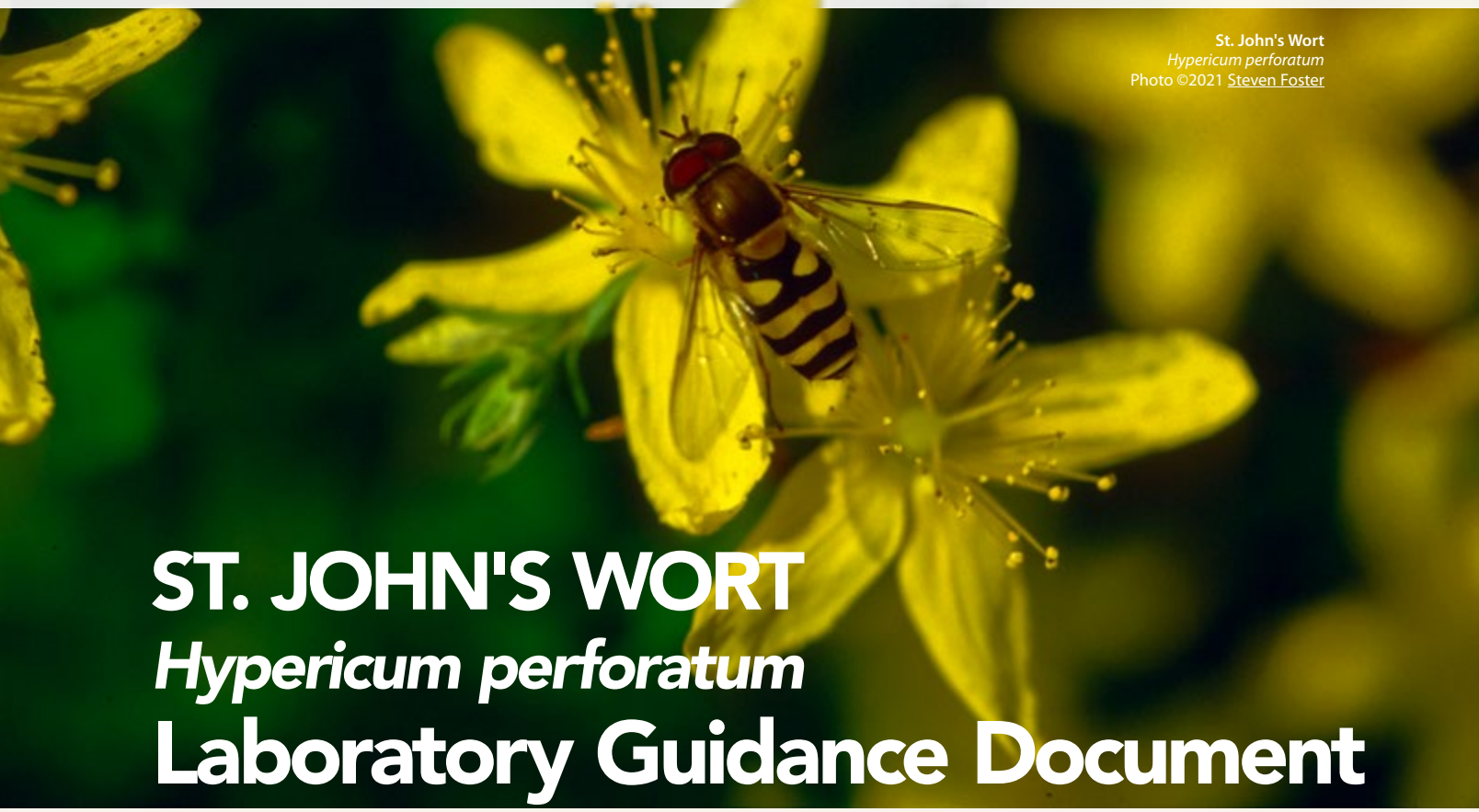
St. John's Wort
Hypericum perforatum
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ST. JOHN'S WORT
Hypericum perforatum
Laboratory Guidance Document

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1. Purpose

For centuries, St. John's wort (SJW; *Hypericum perforatum* L., Hypericaceae) has been used as an herbal remedy for various medical conditions both externally and internally in many countries. Although it is a well-known and widely used traditional medicinal plant, concerns about its safety and herb-drug interactions caused a significant decrease in market sales starting in the early 2000s. The adulteration history of St. John's wort (SJW) goes back to 1875 in the United States; the American Pharmaceutical Association mentioned *Ascyrum stans* and *A. crux-andreae* as the substitutes of SJW in its report on adulterations and sophistications.[1] More recently, many *Hypericum* species (*H. androsaemum, H. barbatum, H. crux-andreae, H. hirsutum, H. maculatum, H. montanum, H. patulum,* and *H. tetrapterum*) and synthetic dye mixtures (E123 Amaranth, E133 Brilliant Blue, E110 Sunset Yellow, and E102 Tartrazine) are reported as adulterants of SJW. [2,3]

This Laboratory Guidance Document presents a review of the various analytical methods used to differentiate between authentic SJW plant, powder/extracts and ingredients containing adulterating materials. This document can be used in conjunction with the *Hypericum perforatum* Botanical Adulterants Bulletin published by the ABC-AHP-NCNPR Botanical Adulterants Prevention Program in 2017.[2,3]

2. Scope

The analytical methods described in this paper are reviewed with the specific purpose of identifying the strengths and limitations of existing methods for differentiating SJW from its known potential adulterants. Analysts can use this review

to select the appropriate choice of techniques for their specific SJW products. The positive evaluation of a specific method for testing SJW material in the products' particular matrix in this Laboratory Guidance Document does not reduce or remove the responsibility of laboratory personnel to demonstrate adequate method performance in their own laboratory using accepted protocols. Such protocols are outlined in the US Food and Drug Administration's (FDA) Final Rule for Current Good Manufacturing Practices for Dietary Supplements (21 CFR Part 111) and by AOAC (Association Of Official Analytical Collaboration) International, International Organization for Standardization (ISO), World Health Organization (WHO), International Council for Harmonisation (ICH), and national pharmacopeial bodies, as may be applicable, depending on the regulatory requirements of the country, in which the SJW powder/extract is offered for sale, re-sale, and/or processing into finished consumer products.

3. Common and Scientific Names

3.1 Common name: St. John's wort

3.2 Other common names:

English: common St. John's wort, perforate St. John's wort, common goatweed, Klamath weed, racecourse weed, tipton weed[4,5]

Chinese: guàn yè lián qiáo (贯叶连翘), qiān céng lóu (千层楼)[4-6]

Dutch: sint janskruid, St. Jan's kraut[4,6]

French: herbe à mille trous, herbe de la Saint Jean, herbe de millepertuis, millepertuis commun, millepertuis perforé, toursaine[4,5]

German: echtes Johanniskraut, Eisenblut, Hexenkraut, Herrgottsblut, Jageteufel, Johannisblut, Johanniskraut, Tüpfel-Hartheu, Tüpfel-Johanniskraut[4,5]

Greek: spathohorto, valsamo[7]

Italian: erba di San Giovanni, erba della Madonna, brunnulidda, iperico, iperico perforato, perforate, pelatro, trascalan[4,6,8]

Japanese: seiyoutogiri[4]

Portuguese: flor de são João, hipericao, milfurada[4]

Russian: zveroboj obyknovenny, zwieroboij[4-6]

Spanish: corazoncillo, hierba de San Juan, hipericon[4]

South African: johanneskruid[4,5]

Swedish: äkta johannesört, johannesört[4,5]

Turkish: binbirdelik otu, sarı kantaron[9]

3.3 Latin binomial: *Hypericum perforatum* L.[10,11]

3.4 Synonyms: *Hypericum officinale* Gaterau, *Hypericum officinarum* Crantz, *Hypericum vulgare* Lam.[10]

3.5 Botanical family: Hypericaceae (formerly placed into the Clusiaceae [syn. Guttiferae])

4. Botanical Description and Geographical Range

The genus *Hypericum* includes over 500 species divided between two subgenera; one mainly Old World, in which dark hypericin-containing glands are present, and the other mainly New World, in which such glands are absent. It is further divided into 36 sections using differences in morphological characters. *Hypericum perforatum* is placed in series *Hypericum*, along with 10 other *Hypericum* species to which it is most closely related. It is morphologically and geographically an intermediate between *H. maculatum* and *H. attenuatum*.[12,13] Robson described four intergrading subspecies of SJW: subsp. *perforatum,* subsp. *chinense,* subsp. *songaricum,* and subsp. *veronense*[14,15] but they are rarely recorded in the published literature or databases.[12] The native range of *H. perforatum* subsp. *chinense* is China, subsp. *songaricum* has two distinct populations, one in Ukraine and neighboring southern Russia, the other from Kazachstan and Kyrgystan to China's Xinjiang province, and subsp. *veronense* is from Turkey west to Southern Europe as far as Macronesia, and south to Saudi Arabia and Sudan. Hence *H. perforatum* subsp. *perforatum* is native from Europe to Central Siberia and Northwest Turkey, its native range overlapping with subsp. *veronense,* in many countries.[10,12]

SJW is an upright, stoloniferous, yellow-flowered, and herbaceous perennial plant that typically grows from a woody, branched rootstock to 1-3' tall, and features a showy display of star-shaped, yellow flowers (0.75-1.5" diameter) that bloom in pyramidal compound cymes in the summer. Its flowers have five yellow petals peppered with black dots (hypericin glands), a pistil with three styles, and a center boss of bushy yellow stamens; stem-clasping, elliptic to oblong leaves (1.25" long) that have translucent dots and black marginal punctations. The foliage has an unpleasant aroma when bruised or rubbed.[4,16]

The genus *Hypericum* is distributed globally, although generally absent from environments that are subject to extreme heat, cold, dryness, or moisture. SJW is native to Europe, Asia, Northern and Southern Africa, South America, Australia, and New Zealand. It was first brought to North America by settlers in 1696 and has naturalized over time throughout much of the continent.[4,16]

Hyperici herba is defined as the whole, fragmented, and dried flowering tops of the plant, harvested during flowering time.[17] It is sold cut, powdered, and as oil macerate, dry and liquid extract.[6]

5. Adulterants and Confounding Materials

Many *Hypericum* species such as *H. androsaemum, H. barbatum, H. crux-andreae, H. hirsutum, H. humifusum, H. maculatum, H. montanum, H. patulum,* and *H. tetrapterum* have been reported as adulterants of SJW.[1,18-22] Additionally, *H. undulatum* has been mentioned as potential adulterant (See Table 1), but there is no published evidence for such adulteration.[23,24] Also, admixtures of undeclared synthetic dyes have been identified as a means to bolster absorbance readings when measuring hypericin by spectrophotometry and to impart a visually acceptable color; such dyes include E123 Amaranth (FD&C Red #2), E133 Brilliant Blue (FD&C Blue #1), E110 Sunset Yellow (FD&C Yellow #6), and E102 Tartrazine (FD&C Yellow #5).[24,25]

Table 1. Scientific and common names of known SJW (*H. perforatum*) adulterants

Species [a]	Synonym(s) [a]	Common name [b,c,d]
H. androsaemum L.	*Androsaemum androsaemum* (L.) Huth *A. officinale* All. *A. vulgare* Gaertn. *Hypericum bacciferum* Lam. *H. bacciforme* Bubani *H. floridum* Salisb.	Tutsan, Sweet-amber
H. barbatum Jacq.	*Hypericum barbatum* subsp. *calabricum* (Spreng.) Peruzzi & N.G.Passal. *H. calabricum* Spreng. *H. heufleri* R. Keller *H. ilicianum* Formánek *H. macedonicum* Boiss. & Orph. *H. richeri* Rochel *H. trichanthum* Boiss. & Spruner	
H. crux-andreae (L.) Crantz	*Ascyrum cruciatum* St.-Lag. *A. crux-andreae* L. *A. cuneifolium* Chapm. *A. grandiflorum* Raf. *A. simplex* Zeyh. ex Turcz. *A. stans* var. *obovatum* Chapm. ex Torr. & A.Gray *Hypericoides crux-andreae* (L.) Poir. *Hypericum parviflorum* Salisb. *H. stans* (Michx. ex Willd.) P.B.Adams & N.Robson	St. Peter's wort
H. hirsutum L.	*Adenosepalum hirsutum* (L.) Fourr. *Hypericopsis hirsuta* (L.) Opiz *Hypericum ferrugineum* Banks ex Pursh *H. villosum* Crantz	Hairy St. John's wort
H. humifusum L.	*Holosepalum humifusum* (L.) Fourr. *Hypericum decumbens* Peterm. *H. exiguum* Bubani *H. liottardii* Vill. *H. losae* Sennen ex Losa *H. repens* Georgi *H. rubrum* Wight ex Dyer	Trailing St. John's wort
H. maculatum Crantz	*Hypericum quadrangulum* var. *maculatum* (Crantz) Choisy	Spotted St. John's wort
H. montanum L.	*Adenosepalum montanum* (L.) Fourr. *Hypericopsis montana* (L.) Opiz *Hypericum confertum* Moench *H. elegantissimum* Crantz *H. glandulosum* Gilib. *H. tauricum* Ledeb.	Pale St. John's wort
H. patulum Thunb.	*Eremanthe patula* (Thunb.) K.Koch *Hypericum argyi* H.Lév. & Vaniot *H. hookerianum* var. *dentatum* S.N.Biswas *Komana patula* (Thunb.) Y. Kimura *Norysca patula* (Thunb.) Voigt	Golden cup St. John's wort
H. tetrapterum Fr.	*Hypericum quadrialatum* Wahlb.	Four-petal St. John's wort
H. undulatum Schousb. ex Willd.	*Hypericum acutum* subsp. *undulatum* (Schousb. ex Willd.) Rouy *H. acutum* var. *undulatum* (Schousb. ex Willd.) Pau *H. maculatum* subsp. *undulatum* (Schousb. ex Willd.) P.Fourn. *H. quadrangulum* var. *undulatum* (Schousb. ex Willd.) Choisy *H. quadrangulum* f. *undulatum* (Schousb. ex Willd.) Borg *H. tetrapterum* var. *undulatum* (Schousb. ex Willd.) S.Coult. *H. tetrapterum* subsp. *undulatum* (Schousb. ex Willd.) P.Silva	Wavy St. John's wort

a According to Plants of the World Online, Kew Science[26]
b According to Tropicos database[27]
c According to Plants for A Future database[28]
d According to USDA Plants Database[29]

6. Identification and Distinction using Macro-anatomical Characteristics

Macro-anatomical characteristics of *H. perforatum* have been published in several pharmacopeial monographs, books, and manuscripts.[4,6,17,18,20,30,31] Descriptions in the American Herbal Pharmacopoeia (AHP) are detailed, and the monograph includes the adulterant *Hypericum* species in a table format. The characteristics that can be used to distinguish SJW from other species are as follows: The stems: opposing ribs, two-winged, and glabrous; the leaves: egg-shaped to elongated, obtuse, with a short petiole, translucent, and marked with black dots (hypericin glands); the sepals: lanceolate, acute, twice as long as the ovary; and the petals: oblique, egg-shaped, one-side serrated, also marked with hypericin glands and lines.[6] Additionally, the most common adulterants, *H. maculatum* and *H. montanum* have quadrangular stems and round stems, respectively. The leaves of *H. barbatum* contain no or very few punctiform hypericin glands and these glands are also absent in the leaves of *H. androsaemum*.[20,21] The American Herbal Products Association (AHPA) Botanical Identity References Compendium contains colored photos of distinctive botanical characteristics of SJW.[30]

7. Identification and Distinction using Micro-anatomical Characteristics

Detailed descriptions of the micro-anatomical characteristics of SJW, including line drawings[4,13] and color microscopic images[20,31] have been published. Although specific diagnostic elements such as hypericin glands, secretory cells, and tricolpate pollen grains showing a smooth and faintly warted exine, and clusters of calcium oxalate crystals are described in detail, there is no information about the microscopic distinction criteria of aerial parts of SJW and the potential adulterant *Hypericum* species so far (except that trichomes are absent in SJW, which helps to distinguish SJW from *H. hirsutum* having many trichomes).

For this reason, the microscopic examination should be combined with other testing methods (genetic or chemical) for authentication of SJW or detection of adulteration.

8. Organoleptic Identification

St. John's wort powder is greenish to yellowish-brown, and the yellow becomes more pronounced as the percentage of flowers and unopened buds increases. Its odor is distinct, slightly sweet and aromatic, somewhat balsamic and the taste is slightly sweet, mildly bitter, somewhat resinous, and astringent.[6] The appearance of SJW dry extract is described as a brownish-grey powder in the monograph of the European Pharmacopoeia (Ph Eur).[17] Solutions of SJW extracts in methanol are normally red to brownish in color due to their hypericin content. However, SJW extracts adulterated with the admixture of synthetic dyes (tartrazine, amaranth, sunset yellow, and brilliant blue) are reported to produce green methanolic solutions.[24] Therefore, organoleptic evaluation of the extract can give hints about the authenticity of SJW powders and extracts, but is not appropriate for authentication as a stand-alone assay.

9. Genetic Identification and Distinction

Besides determining phylogenetic relationships, DNA-based identification techniques have been used in many studies to authenticate SJW and to detect adulterations in powders, extracts, or finished herbal products.[3,7,13,22,32-38] While using universal primers in general DNA barcoding techniques with Sanger sequencing has been shown to provide mixed results, additional methods such as DNA mini-barcoding, qualitative polymerase chain reaction (qPCR), and next-generation sequencing (NGS) are used as additional tools for identifying processed ingredients used in finished herbal products.[39] Crocket et al. published one of the first studies on genetic profiling of SJW and related species by analyzing the sequence of the nuclear ribosomal Internal Transcribed Spacer region (ITS).[32] In eukaryotes, the ITS region consists of the ITS1 spacer (between 18S and 5.8S rRNA genes) and ITS2 spacer (between 5.8S and 28S rRNA genes) (Figure 1 below).

Howard et al. analyzed nine *Hypericum* species using universal primers to amplify the approximate 750 bp ITS region. Primers were designed based on the ITS region to amplify two species-specific "microcode" sequences of approximately 80 bp and 160 bp for molecular identifi-

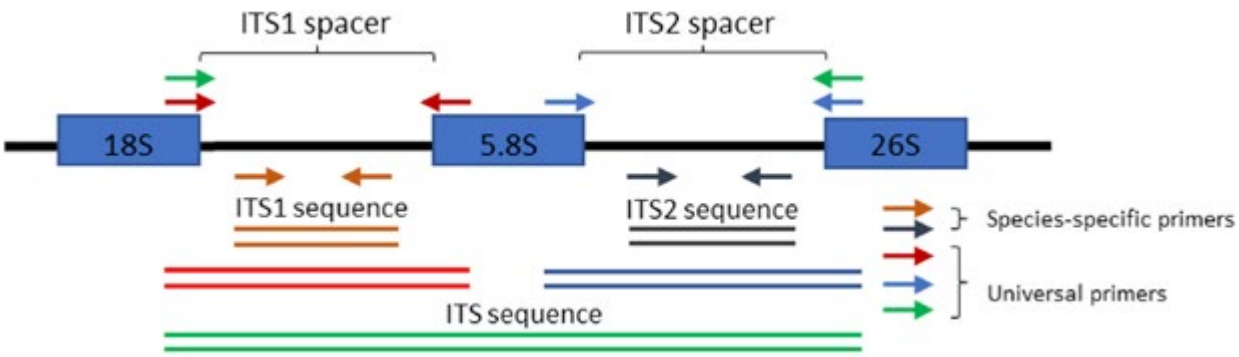


Figure 1. The nuclear ribosomal Internal Transcribed Spacer (ITS) region. In eukaryotes, the ITS region consists of the ITS1 spacer (between 18S and 5.8S rRNA genes) and ITS2 spacer (between 5.8S and 28S rRNA genes). Forward and reverse primers (arrows) can be used to amplify the whole region or parts of it. Species-specific primers are often based on the spacer sequences. Image provided by Natascha Techen (University of Mississippi).

cation of SJW. Three commercial SJW products, all capsules filled with dried ground plant material were analyzed. Two of these products, gave a positive result for SJW. The PCR amplification of the whole ITS region was successful for the third sample, labeled as a mixture of SJW with other botanical ingredients, but did not result in a product with SJW specific primers. It was concluded that the lack of SJW specific PCR products was due to the absence of target *H. perforatum* DNA. The assay was found to be sensitive enough to detect 0.75 ng *H. perforatum,* equal to just 0.1% of the total DNA in the test sample.[33]

Another DNA-based identification method was developed to distinguish between seven different *Hypericum* species to detect SJW and other *Hypericum* spp. in single-ingredient dietary supplements and complex mixtures. After the selection of most divergent areas of the ITS region, species-specific PCR primers were designed to anneal specifically to areas where the four target species differ from each other in their sequence. The resulting characteristic PCR product was 222 bp (*H. perforatum*), 67 bp (*H. androsaemum*), 131 bp (*H. athoum*), and 231 bp (*H. ascyron*). The three commercial SJW products used in the previous study were also analyzed by using this technique, and two of them were determined to contain SJW, while the third product, again, did not provide the expected SJW signal, concluding that no target DNA was present.[34]

Thirteen commercial SJW products, only one containing powdered herb, representing six capsules, five tablets, and two tinctures were analyzed using the same primer combinations as Howard et al.[33] *Hypericum perforatum* species-specific PCR products were amplified in each of the products, indicating the presence of SJW material. However, only four products (all capsules) yielded the full length ITS region, indicating that the DNA in the other nine products was degraded or fragmented.[35]

Costa et al. assessed DNA mini-barcodes (ITS1 and *matK*) of 13 commercial herbal infusions labeled to contain either *H. perforatum* or *H. androsaemum* by real-time PCR assays coupled to high resolution melting (HRM) analysis. DNA mini-barcoding uses smaller DNA segments for PCR amplification and thus often permits species identification even if DNA is fragmented during the manufacturing process. One product labeled to contain only *H. androsaemum* also tested positive for *H. perforatum* DNA and another product labeled as containing *H. perforatum* tested positive for *H. androsaemum* DNA. All other products tested positive only for the DNA of the *Hypericum* species indicated on the label of the product. The confidence level for species identification using HRM was between 98.5-99.9%.[3]

Amplicon metabarcoding (AMB) of the ITS1 and ITS2 spacers was used to authenticate 38 single ingredient and 40 complex herbal products labeled to contain SJW. The

St. John's Wort
Hypericum perforatum
Photo ©2021 Steven Foster

ability of the method to detect adulterations was evaluated in comparison to thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC)-mass spectrometry (MS). The presence of *H. perforatum* was confirmed in 68% (26 out of the 38 sequenced samples) by using AMB. Also, other *Hypericum* species were detected: *H. humifusum* in 21% (eight), *H. tetrapterum* in 13% (five), and *H. hirsutum* in 3% (one) of the samples. These other *Hypericum* species were always found in combination with *H. perforatum*, suggesting that adulteration by admixture is more widespread than an attempted complete substitution. However, it may also indicate hybridization or incidental harvesting based on overlapping harvest regions.[22]

Scotti et al. analyzed 20 commercial SJW products, combining chemical and genetic techniques: nuclear magnetic resonance (NMR)-based metabolomics combined with principal component analysis (PCA), high-performance thin-layer chromatography (HPTLC) analysis, and DNA-barcoding. DNA-barcoding confirmed the identity of three SJW samples. Specific PCR tests gave a positive signal with a further three SJW samples but also with three "Chinese SJW" samples. While the chemical methods agreed with each other, DNA-barcoding identification was limited by its ability to extract viable DNA from all samples.[36]

In another study by Howard et al., two assays were designed to analyze DNA, comparing data to those from a curated database of selected *Hypericum* ITS sequences: a quantitative PCR (qPCR) assay based on a species-specific primer pair spanning the ITS1 and ITS2 regions (Figure 1), and an NGS assay separately targeting the ITS1 and ITS2 regions. The ability of the assays to detect *H. perforatum* DNA sequences in 20 processed SJW medicines, including capsules (n = 8), tablets (n = 10), and teabags (n = 2), was investigated. Both assays detected *H. perforatum* DNA in five samples. High-quality sequence data were obtained from three of them, two teabags containing dried SJW, and one capsule containing SJW powder. Additionally, the qPCR assay was also able to detect lower levels of DNA in two further samples for amplicons less than 200 bp. Five samples contained highly fragmented DNA and the remaining samples were devoid of amplifiable DNA, making genetic authentication challenging if not impossible. The NGS assay confirmed that *H. perforatum* was the major species in all five samples for which the ITS sequence was obtained, though trace contaminants were also detected.[37]

In a study from 2020, twenty *Hypericum* species were selected for DNA barcoding, representing closely related species, samples used in commerce, and potential adulterants. Their DNAs were extracted and subjected to amplification of the genomic nuclear ITS region and the chloroplast regions *trnH-psbA*, *rbcL*, and *matK*. The chloroplast regions showed suboptimal characteristics that already have been noted for many other plant groups; in contrast to that, the ITS region was useful as a barcode. According to the results, three subtypes of *H. perforatum* and two subtypes of *H. maculatum* were differentiated.[38] Molecular phylogenetic studies have characterized two distinct gene pools in European *H. perforatum* populations, although their relationship to the subspecies is not clear. Thus, many sequences and reference samples are needed to be collected to ensure that any intraspecific variation that might be found in commercial samples are captured and incorporated into the genetic sequence databanks.[38]

Comments: *Hypericum humifusum*, *H. tetrapterum* and *H. hirsutum* were detected as the adulterants of SJW by amplicon metabarcoding studies. Additionally, adulteration with *H. androsaemum* was proven by real-time PCR assays coupled to high resolution melting (HRM) analysis. The DNA sequencing technologies are highly reliable and useful methods for the identification of medicinal plants in herbal products under specific conditions, such as the presence of DNA of sufficient length and quality, sufficient primer affinity for successful PCR amplification and, in certain cases, absence of contaminating DNA. Plant DNA is a relatively stable molecule that can be easily extracted from fresh or dried plant material by simple methods. However, manufacturing processes of herbal products that involve extensive heat treatment, irradiation, distillation, filtration, UV light exposure, and/or supercritical fluid extraction lead to either complete removal of DNA or degradation of DNA into smaller fragments. Hence, DNA barcoding does not provide reliable results for certain processed herbal products such as extracts and tinctures in which the DNA is not present at all or is highly degraded.[39] It is also well known that adulteration by chemical compounds, e.g., undeclared dyes, usage of the wrong part of the relevant plant, some extracts, or presence of poor-quality ingredients such as spent (i.e., previously extracted) plant material cannot be detected by these methods. Such limitations of the DNA-based methods make them unsuitable as a stand-alone tool for identifying and authenticating plant species.

10. Chemical Identification and Distinction

There are many analytical methods available for authenticating *H. perforatum* and differentiating it from other *Hypericum* species as well as potential adulterants, such as undeclared synthetic dyes. These methods are cited in the Laboratory Methods section below (Section 10.2). Distinction based on the phytochemical profile requires detailed knowledge of the constituents of *H. perforatum* and its adulterants. Below is a summary of the phytochemical composition of SJW and its known adulterants, including the chemical structures of their principal compounds (Figure 2). Additionally, the main secondary metabolites in *H. perforatum* and other *Hypericum* species are summarized for comparison in Table 2 (references are given in section 10.1). When the distinction is based on chromatographic or spectral patterns, identification of specific constituents may not be necessary.

10.1 Chemistry of *Hypericum perforatum* and potential adulterants

Hypericum perforatum

SJW contains numerous compounds such as naphthodianthrones, phloroglucinols, flavonoids, phenylpropanoids, xanthones, and terpenes. The characteristic constituents (Figure 2) are naphthodianthrones (0.06–0.4%) and phlo-

roglucinols (0.2–4%).[40] Naphthodianthrones include hypericin, pseudohypericin, protohypericin, protopseudohypericin, cyclopseudohypericin, and emodin-anthrone. Protohypericin and protopseudohypericin are the biosynthetic precursors which are transformed into hypericin and pseudohypericin by exposure to light. Pseudohypericin content in SJW is two to three times greater than hypericin.[6,18,41-45]

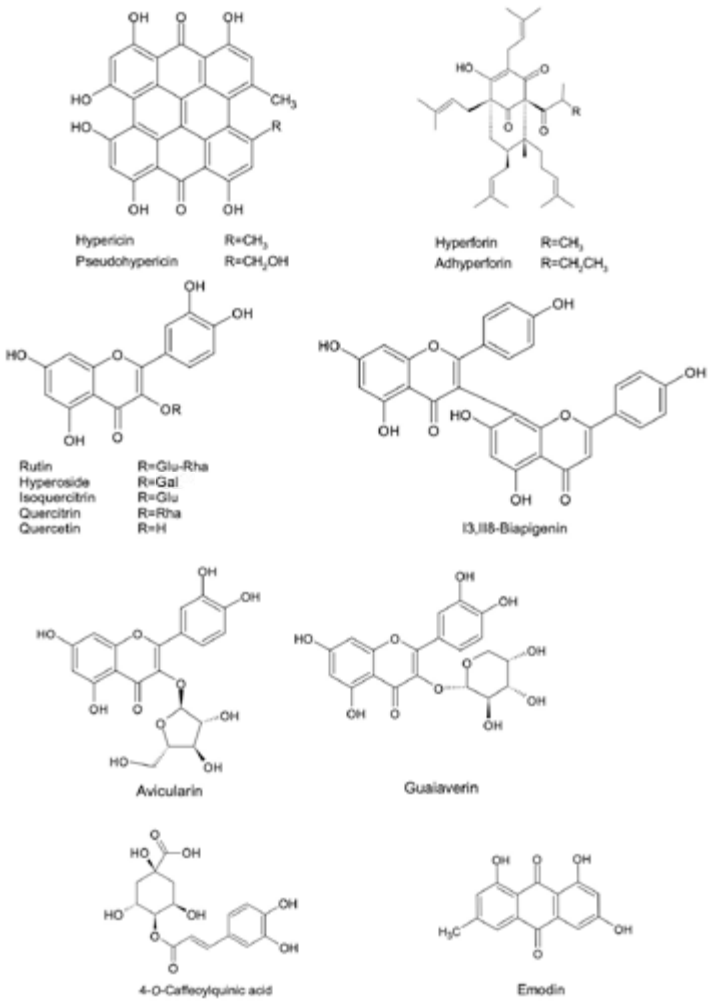
The main phloroglucinols are hyperforin and adhyperforin. They have limited stability and their oxidated derivatives such as furohyperforin are also present. Additionally, hyperfirin, adhyperfirin, and hyperpolyphyllirin are determined as other phloroglucinols.[8,45-50] Average amounts of hypericin (0.008-0.282%), pseudohypericin (0.009-0.211%), and hyperforin (up to 0.482%) in the flowering tops of *H. perforatum* samples from various provenances are given in a review paper by Lazzara et al.[51]

Quercetin glycosides (2-4%), including avicularin, guaiaverin (syn. guaijaverin), hyperoside, isoorientin, isoquercitrin (quercetin-3-*O*-glucoside), miquelianin (quercetin-3-*O*-glucuronide), quercitrin (quercetin-3-*O*-rhamnoside), rutin, and other flavonoids, such as apigenin-7-*O*-glucoside, astilbin (syn. dihydroquercitrin), kaempferol, kaempferol-3-*O*-glucuronide, kaempferol-3-*O*-glucoside, luteolin, luteolin-7-*O*-glucoside, myricetin, myricetin 3-*O*-glucoside, naringenin, naringenin-7-*O*-glucoside, quercetin, quercetin-3-*O*-sulfate, taxifolin (syn. dihydroquercetin), and biflavonoids (amentoflavone, I3,II8-biapigenin) were determined in the aerial parts of SJW.[18,41,42,45,46,48,52-59]

Other constituents include benzoates and cinnamates (3,5-dihydroxybenzoic acid, chlorogenic acid, *p*-coumaric acid, cryptochlorogenic acid, ellagic acid, ferulic acid, gallic acid, genistic acid, isoferulic acid, neochlorogenic acid, quinic acid, rosmarinic acid, shikimic acid, and *p*-coumaroylquinic acid); polymeric and oligomeric proanthocyanidins (procyanidins A2, B1, B2, B3, B5, B7, and C1), catechin and epicatechin monomers, epigallocatechin, anthocyanins (cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside), 7-epiclusianone, esculin, *cis*-piceid, phlorizin, skyrin-2-*O*-glucopyranoside, trace amounts of xanthones (dimethylmagniferin, magniferin, 1,3,6,7-tetrahydroxyxanthone), and essential oil (0.1-0.25%) containing mainly monoterpenes, sesquiterpenes, and higher *n*-alkanes.[41,45,53-55,58-61]

As mentioned before (section 4), the distinction of four *H. perforatum* subspecies was based on minor morphological traits and with a well-defined geographical distribution. Although the morphology of these subspecies was examined in detail, there are few studies about their chemical profile. The chemical composition of *H. perforatum* subsp. *perforatum* was examined and hyperforin, hypericin, pseudohypericin, hyperoside, isoquercitrin, naringenin, rutin, quercetin, quercitrin, amentoflavone, ferulic acid, chlorogenic acid, caffeic acid, and *p*-hydroxybenzoic acid were determined as constituents.[62-65]

Figure 2. Representatives of the main classes of secondary metabolites in *H. perforatum* and other *Hypericum* species



Hyperoside, isoquercitrin, quercetin, quercitrin, a small amount of rutin, chlorogenic acid, hyperforin, hypericin, and traces of pseudohypericin were detected in *H. perforatum* subsp. *veronense* samples.[63,65] On the other hand, *H. perforatum* subsp. *angustifolium* samples collected from Italy were found to contain chlorogenic acid, rutin, hyperoside, isoquercitrin, quercetin, quercitrin, hypericin and hyperforin.[64] Filippini et al. analyzed *H. perforatum* subsp. *perforatum* and subsp. *veronense,* collected from Italy, by HPLC-DAD to compare their chemical constituents. Chlorogenic acid, hyperoside, isoquercitrin, quercitrin, I3,II8-biapigenin, hyperforin, and hypericin were common in both subspecies, and rutin was only found in subsp. *veronense*.[66] The fourth subspecies, *H. perforatum* subsp. *chinense*, was thought to represent a specific chemotype differing from the other three subspecies. While avicularin was initially considered to be a unique marker for subsp. *chinense*, both Spanish (*H. perforatum* subsp. *perforatum* or *veronense*) and Chinese samples (*H. perforatum* subsp. *chinense*) were found to contain this flavonol-glycoside.[12,14,67]

Table 2. Summary of secondary metabolites in *H. perforatum* and other *Hypericum* species[a]

	Name of the Species and Presence of the Secondary Metabolites[b,c]										
Constituents	***AND***	***BAR***	***CRU***	***HIR***	***HUM***	***MAC***	***MON***	***PAT***	***PER***	***TET***	***UND***
Naphtodianthrones											
Hypericin	+	+		+	+	+	+	+	+	+	+
Protohypericin									+		
Pseudohypericin	+	+		+	+	+	+	+	+	+	+
Phloroglucinols											
Adhyperforin	+			+				+	+	+	+
Hyperfirin									+		+
Hyperforin	+	+		+	+	+	+	+	+	+	+
Flavonoids											
Apigenin				+		+			+ (Glu)		+ (Glu)
Avicularin/Guaiaverin	+			+		+	+		+		
Hyperoside	+	+		+	+	+	+	+	+	+	+
Isoorientin									+		
Isoquercitrin	+	+		+	+	+	+	+	+	+	+
Kaempferol	+			+		+	+		+	+	
Luteolin	+		+ (Gly)	+		+	+		+		
Myricetin				+		+	+		+	+ (Glu)	+ (Glu)
Naringenin	+			+		+ (Glu)	+		+		
Orientin	+	-		+		-			-		
Quercetin	+	+	+ (Gly)	+	+	+	+	+	+	+	+
Quercitrin	+	+		+	+	+	+	+	+	+	+
Rutin	+	+		+	+	+	+	+	+	+	
Proanthocyanidins											
Catechin	+			+		+	+	+	+		+
Epicatechin	+			+	+	+	+		+		+
Epigallocatechin	+			+		+	+		+		
Anthraquinones											
Emodin						+		+	-	+	
Emodin-anthrone									+		
Biflavonoids											
Amentoflavone	+			+	+	+	+	+	+	+	+
I3,II8-Biapigenin	+	+		+	+	+	+	+	+	+	
Benzoates & Cinnamates											
Caffeic acid	+	+		+	+	+			+	+	
Chlorogenic acid	+	+		+	+	+	+	+	+	+	+
p-Coumaric acid				+		+	+		+	+	
Cryptochlorogenic acid		+		+		+	+		+		
Ellagic acid				+		+	+		+		
Ferulic acid				+		+			+	+	
Gallic acid				+		+	+		+		
Neochlorogenic acid	+			+	+	+	+		+		
Shikimic acid	+								+	+	

+ reported from the species
- absent in the species

[a] The lack of reporting of a specific constituent in a given *Hypericum* species may not necessarily mean that it is not present as not all these species have been thoroughly investigated
[b] *AND: Hypericum androsaemum, BAR: H. barbatum, CRU: H. crux-andreae, HIR: H. hirsutum, HUM: H. humifusum, MAC: H. maculatum, MON: H. montanum, PAT: H. patulum, PER: H. perforatum, TET: H. tetrapterum, UND: H. undulatum,*
[c] Glu: Glucoside, Gly: Glycoside

Hypericum androsaemum

Hypericin, pseudohypericin, small amounts of hyperforin and adhyperforin, hypersampsones D and E, avicularin, hyperoside, isoquercitrin, kaempferol-3-*O*-glucoside, quercetin, quercitrin, rutin, amentoflavone, I3,II8-biapigenin, catechin, epicatechin, 2,4-dihydroxybenzoic acid, caffeic acid, caffeoylquinic acid derivatives (chlorogenic acid, neochlorogenic acid), and shikimic acid were determined in *H. androsaemum* samples.[45,57,68-71] In another study, absence of naphthodianthrones was reported, as well as the presence of acylphloroglucinols (1.069%), cinnamic acids (0.203%), flavonoids (0.079%), and biflavones (0.167%) in Italian samples.[70] Çırak et al. confirmed the presence of small amounts (0.008-0.035% in the dry extract [DE]) of avicularin in this species.[69] On the other hand, caffeic acid, fumaric acid, rosmarinic acid, astragalin, eupatilin, herniarin, hyperoside, kaempferol, luteolin, 3-*O*-methyl-quercetin, myricitrin, naringenin, orientin, penduletin, quercetin, quercitrin, rutin, scutellarein, taxifolin, catechin, epicatechin, epigallocatechin, and epigallocatechin gallate were determined in the methanol extract of *H. androsaemum* leaves.[72]

Hypericum barbatum

Hypericin, pseudohypericin, a small amount of hyperforin, hyperoside, isoquercitrin, quercetin, quercitrin, rutin, I3,II8-biapigenin, caffeic acid, chlorogenic acid, and cryptochlorogenic acid were described as the components of *H. barbatum* samples.[52,57,68,73,74] The amounts of hypericin (0.030%), pseudohypericin (0.043%), hyperforin (0.007%), hyperoside (0.155%), and quercitrin (0.019%) were measured in an *H. barbatum* sample from Serbia by Smelcerovic et al.[73]

Hypericum crux-andreae

Despite a comprehensive literature search, no information was found on the chemical profile of flowering tops or aerial parts of this species. The only study from 1983 was aimed to isolate and characterize the leaf flavonoids of *H. crux-andreae.* Flavonoids were identified by UV spectral data. After acid hydrolysis, aglycones and glycosides were co-chromatographed with authentic standards on TLC. Results revealed the presence of two luteolin-6-*C*-glycosides, and a quercetin-3-*O*-glycoside.[75]

Hypericum hirsutum

In the extract of *H. hirsutum*, the main flavonoid is hyperoside (0.471% dry extract [DE]), followed by rutin (0.383% DE), isoquercitrin (0.329% DE), smaller amounts of quercitrin (0.194% DE), and quercetin (0.005% DE).[76] Additionally, hypericin, pseudohypericin, a small amount of hyperforin, adhyperforin, apigenin, avicularin, quercetin-3-*O*-sulfate, kaempferol, kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside, luteolin, luteolin-7-*O*-glucoside, myricetin, naringenin, naringenin-7-*O*-glucoside, amentoflavone, I3,II8-biapigenin, esculin, phlorizin, catechin, epicatechin, epigallocatechin, procyanidin B1, procyanidin B2, 3,5-dihydroxybenzoic acid, caffeic acid, caffeoylquinic acid derivatives (chlorogenic acid, cryptochlorogenic acid, neochlorogenic acid), *p*-coumaric acid, ellagic acid, ferulic acid, gallic acid, gentisic acid, and *cis*-piceid were determined as the constituents of *H. hirsutum*.[52,57,58,64,65,68,73,76-79] Napoli et al. determined the content of naphthodianthrones (hypericin: 0.015%), acylphloroglucinols (hyperforin: 0.225%), cinnamic acids (0.047%), flavonoids (0.130%) and biflavones (0.093%) in Italian samples.[70] Kladar et al. reported that *H. hirsutum* samples collected from Serbia had lower amounts of hypericin and hyperforin but higher levels of rutin compared to *H. perforatum*.[78] It was remarkable that flavone-*C*-glycosides such as orientin and 2''-*O*-acetyl-orientin were detected only in a *H. hirsutum* sample, but not in *H. perforatum, H. barbatum,* and *H. maculatum*.[18,57] Although Huck-Pezzei et al. reported that kushenol G and H were the marker compounds of this species, the data could not be corroborated and these prenylated flavonols have not been reported in any *Hypericum* species. Additionally, the mass spectral data given in the Huck-Pezzei et al. study are confusing, that as the *m/z* values determined are not in agreement with those of kushenol G and H.*[23]

Hypericum humifusum

Two naphthodianthrones (hypericin, pseudohypericin), a phloroglucinol (hyperforin), four cinnamic acid derivatives (caffeic acid, chlorogenic acid, gentisic acid, neochlorogenic acid), four flavonol glycosides (hyperoside, isoquercitrin, rutin, quercitrin), quercetin, epicatechin, and two biflavonoids (amentoflavone, I3,II8-biapigenin) were identified in the aerial parts of *H. humifusum*.[79-82] Total hypericins in *H. humifusum* methanol and ethanol extracts were determined as 0.121% and 0.124% according to the spectrophotometric method in Ph Eur 8.0.[80] On the other hand hyperforin, hypericin and pseudohypericin contents were measured as 0.147%, 0.033% and 0.091%, respectively, by HPLC.[79]

Hypericum maculatum

A high amount of hyperoside (0.977% DE) and a low concentration of rutin (0.007% DE)[76] were observed among hypericin, pseudohypericin, hyperforin, apigenin, isoquercitrin, isorhamnetin, kaempferol, kaempferol-3-*O*-glucoside, luteolin, luteolin-7-*O*-glucoside, myricetin, naringenin-7-*O*-glucoside, quercetin, quercetin-3-*O*-arabinoside (avicularin or guaiaverin), isoquercitrin, quercetin-3-*O*-sulfate, quercitrin, amentoflavone, I3,II8-biapigenin, esculin, phlorizin, catechin, epicatechin, epigallocatechin, procyanidin B1, procyanidin B2, 3,5-dihydroxybenzoic acid, caffeic acid, caffeoylquinic acid derivatives (chlorogenic acid, cryptochlorogenic acid, neochlo-

* It is not possible to assign a structure to the constituents reported as kushenols G and H by Huck-Pezzei et al. based on the available information. However, the HPTLC data and mass spectrometric fragments provided of the marker compound characteristic for SJW of Chinese origin agree with the $[M + Na]^+$ and $[M + K]^+$ ions of the flavonol glycosides avicularin and guaiaverin, rather than kushenols G and H. Avicularin/guaiaverin have been reported as markers to differentiate SJW of Chinese and European origin in 2019 (see section 10.2.2).

rogenic acid), *p*-coumaric acid, ellagic acid, ferulic acid, gallic acid, gentisic acid, and *cis*-piceid in *H. maculatum* samples.[22,57,58,71,73,83-85] *Hypericum maculatum* subsp. *maculatum* was found to contain significant amounts of amentoflavone and quercitrin, which were higher than those in the same type of *H. perforatum* extracts. Additionally, rutin contents were determined as 0.29-1.58% DE in the same study.[85] *Hypericum perforatum* reportedly has a similar hypericin content as its adulterant *H. maculatum*, but *H. maculatum* has a lower content of hyperforin, 0.004–0.018%.[22] Hypericin, pseudohypericin, and hyperforin contents of a Serbian *H. maculatum* sample were determined as 0.003%, 0.004%, and 0.005%, respectively.[73] On the other hand, it was reported that *H. maculatum*, *H. patulum,* and *H. tetrapterum* contain emodin, which can be used as a marker compound to distinguish it from *H. perforatum*.[18,71]

Hypericum montanum

Hypericin, pseudohypericin, trace amounts of hyperforin, hyperoside, isoquercitrin, kaempferol, kaempferol-3-glucoside, luteolin, luteolin-7-*O*-glucoside, myricetin, naringenin, naringenin-7-*O*-glucoside, quercetin, quercetin-3-*O*-arabinoside (avicularin or guaiaverin), quercetin-3-*O*-sulfate, quercitrin, a very small amount of rutin, amentoflavone, I3,II8-biapigenin, esculin, catechin, epicatechin, epigallocatechin, procyanidin B2, 3,5-dihydroxybenzoic acid, caffeoylquinic acid derivatives (chlorogenic acid, cryptochlorogenic acid, neochlorogenic acid), *p*-coumaric acid, ellagic acid, gallic acid, and *cis*-piceid were determined in *H. montanum* collected from different countries.[52,58,64,65,68,80] In another study, the absence of acylphloroglucinols was revealed and the content of naphthodianthrones (0.672%), cinnamic acids (0.128%), flavonoids (0.507%), and biflavones (0.588%) was measured in Italian samples.[70]

Hypericum patulum

Raclariu et al. determined the hypericin (0.853%) and hyperforin (0.015%) contents of *H. patulum*.[22] Additionally, the presence of pseudohypericin, adhyperforin, hyperoside, isoquercitrin, kaempferol-3-*O*-glucoside, naringin, quercetin, quercitrin, rutin, catechin, chlorogenic acid, amentoflavone and I3,II8-biapigenin was confirmed.[22,63,70,71] On the other hand, *H. maculatum*, *H. patulum,* and *H. tetrapterum* contain emodin that can be used as a marker compound to distinguish these species from *H. perforatum*.[18,71]

Hypericum tetrapterum

According to Gitea et al., the main flavonoid in *H. tetrapterum* is hyperoside (0.545% DE) followed by isoquercitrin (0.319%), quercitrin (0.161% DE), and—in a much smaller amount—rutin (0.0011% DE).[76] Caffeic acid, chlorogenic acid, ferulic acid, gentisic acid, *p*-coumaric acid, and shikimic acid were found to be the main phenolic acids. Additionally, the presence of a small amount of hypericin, protopseudohypericin, pseudohypericin, hyperpolyphyllirin, hyperforin, adhyperforin miquelianin, myricetin 3-*O*-glucoside, quercetin, kaempferol, amentoflavone, I3,II8-biapigenin, skyrin-2-*O*-glucoside, and geranyl phlorisobutyrophenone was reported[45,64,65,70,73,76] In Italian samples, the contents of naphthodianthrones were found to be less than those in *H. perforatum* (0.858% and 1.336%, respectively); acylphloroglucinols (0.480% DW), cinnamic acids (0.135%), flavonoids (0.992%), and biflavones (0.938%) were also identified.[70] In Serbian samples, hypericin, pseudohypericin, and hyperforin contents were determined as 0.009%, 0.010%, and 0.011% respectively.[73] *Hypericum tetrapterum* also contains emodin, which previously was mentioned as a marker compound for distinction of a number of *Hypericum* species with *H. perforatum*.[18]

Hypericum undulatum

The secondary metabolite composition of *H. undulatum* was determined by different research groups. Rainha et al. compared the profiles of phenolic compounds of samples grown in the field with those from micropropagation in cell culture. The major phenolic compound in *H. undulatum* culture-grown samples was found to be chlorogenic acid, followed by epicatechin, quercitrin, and isoquercitrin. The wild-grown plants presented hyperoside as the main phenolic compound, followed by isoquercitrin, chlorogenic acid, and quercetin. Rutin and apigenin-7-*O*-glucoside, which were among the flavonoids detected in the extracts from cultured wavy SJW, were absent in wild-grown samples.[86] Presence of chlorogenic acid and several additional caffeoylquinic acid derivatives, flavonoids (amentoflavone, apigenin-7-*O*-glucoside, astilbin, catechin, epicatechin, hyperoside, isoquercitrin, miquelianin, myricetin 3-*O*-glucoside, quercetin, quercitrin, quercetin-7-*O*-arabinoside), naphthodianthrones (hypericin, protopseudohypericin, pseudohypericin), phloroglucinols (adhyperforin, 7-epiclusianone, furohyperforin, hyperforin, hyperfirin, hyperpolyphyllirin, hypersampsones D and E), and geranyl phlorisobutyrophenone have been confirmed by using different techniques.[45,86]

Comments: Çırak et al. conducted a study to determine the chemical and morphological variability of *H. perforatum* collected from different locations of Northern Turkey in the same week. Significant chemical and morphological variations were detected among the samples, such as the hypericin content among populations, which ranged between 0.044 – 0.282%, rutin between not detected and 0.877%, and hyperoside between 0.541 – 2.228%.[56] In another study, secondary metabolite contents of aerial parts of plants from Turkish populations of *H. androsaemum* and *H. polyphyllum* growing at different altitudes were compared. All the assayed compounds were detected in both species at varying levels depending upon the growing altitude, except for hypericins and rutin which did not accumulate in *H. androsaemum*. It was observed that overall, the compounds were more abundant in plants from higher altitudes.[69] According to the study by Kladar et al. different combinations of ecological factors available at each collection site affected the distribution of secondary metabolites in *H. maculatum* subsp. *maculatum* samples during all three assessed ontogenetic

St. John's Wort
Hypericum perforatum
Photo ©2021 Steven Foster

phases (before flowering, flowering, after flowering).[85] Additionally, Filippini et al. have profiled three different subspecies of *H. perforatum* in different maturation phases, evaluating the variations in the context of their major secondary metabolites. HPLC analysis indicated that the three subspecies show a different profile during the developmental stages and plant material at different developmental stages show a different metabolite profile specifically with respect to hyperforins and hypericins.[66] There are many environmental factors such as geographic location, seasonal variations, and different subspecies that affect the amount and spectrum of secondary metabolites of SJW extracts. Thus, detection of adulterations by other *Hypericum* species can be challenging by if only checking the chemical profile of the herbal extract or commercial product.

Synthetic Dyes

St. John's wort preparations may be quantified for their content of hypericin derivatives by spectrophotometric measurements. Hypericin and pseudohypericin have an intense red color and a characteristic UV/Vis spectrum with a maximum at 590 nm. Although the European Pharmacopoeia still includes this method as a quantitative assay for Hyperici herba, it has been shown that certain food dyes, e.g., Amaranth and Brilliant Blue FCF, are used as adulterants since they absorb light in the same range as the hypericins and can therefore enable substandard material to pass the analytical test.[17,87] The researchers examined SJW extracts or commercial products and proved that Tartrazine, Amaranth, Brilliant Blue FCF, and Sunset Yellow FCF were used as adulterants.[24,25] Sunset Yellow FCF and Tartrazine are presumably added to offset the visual aspects of the blue color imparted when SJW extract is adulterated with Amaranth and Brilliant Blue FCF.

10.2 Laboratory methods

Many studies have been conducted on the analytical methods to identify SJW, assess its quality, and/or determine evidence of adulteration. Not all the reported methods are necessarily suitable for all these purposes or all forms of SJW in the marketplace. Unless otherwise noted, all methods summarized here refer to aerial parts of SJW. Table 4 provides a summary of different methods of analysis of SJW.

10.2.1 UV Spectrometry

The naphthodianthrone content in St. John's wort herb and herb extracts can be quantified by measuring the naturally occurring pigments, mainly hypericin, and pseudohypericin. They both absorb visible light with a maximum absorption at 590 nm and are highly fluorescent in methanol when exposed to UV light. Thus, official pharmacopeial monographs recommend modified versions of a spectrophotometric assay for total naphthodianthrones expressed as hypericin.[6,17,88,89] Gitea et al. used this method to evaluate total hypericin contents of *H. perforatum, H. hirsutum, H. maculatum*, and *H. tetrapterum*. All the studied *Hypericum* species complied with the limits imposed by the Ph Eur (minimum 0.08 % total hypericins, expressed as hypericin).[90]

Comments: Many researchers reported adulteration of SJW extracts with synthetic food dyes. It is possible to mimic the UV spectrum and produce substandard material that passes the spectrophotometric assay with these dyes.[24,25,87] Additionally, as described above, total hypericin contents of the adulterant species can be within the limits of Ph Eur. Thus, the lack of specificity makes this assay unsuitable to detect SJW adulteration.

10.2.2 Thin-layer chromatography (TLC) / High-performance thin-layer chromatography (HPTLC)

Methods described in the following publications were covered in this review: USP 2021,[91] Ph Eur 8.1,[17] AHP Monograph,[6] AHPA Botanical Identity References Compendium,[30] HPTLC Association,[92,93] Camag Application Note,[94] Abdel-Tawab et al.,[95] Berghöfer and Hölzl,[18] Booker et al.,[25] Frommenwiler et al.,[24] Huck-Pezzei et al.,[23] Jesionek et al.,[96] Kırmızıbekmez et al.,[97] Kitanov,[98] Maleš et al.,[99] Marrelli et al.,[100] Mulinacci et al.,[101] Raclariu et al.,[22] and Scotti et al.[67]

Comments: Various TLC/HPTLC methods are used to separate, identify, and quantify constituents of SJW and its potential adulterants. Ethanol (70%, 80%) or methanol is most commonly used to prepare the sample extracts; also, a defatting step may be added.[98] Silica gel is the most frequently used stationary phase. Most mobile phases use ethyl acetate combined with acetic acid or formic acid, and possibly other organic solvents (acetone, chloroform, hexane, methylene chloride, toluene) or water. Different techniques, such as TLC-densitometry, examination under UV light, or visualization after spraying with various reagents (KOH in ethanol, natural products-polyethylene glycol reagent, ceric ammonium molybdate solution) were applied to evaluate the chromatograms.

USP specifies the acceptance criterion as the presence of a blue zone due to hyperforin that must correspond in color and position to that in the TLC chromatogram of standard SJW solution.[91] According to Ph Eur, the TLC chromatogram of SJW extract needs to have the yellow band of rutin, the blue zone of chlorogenic acid, and the yellow band of hyperoside in the lower third of the chromatogram. In the top third of the chromatogram, two red bands due to hypericin and pseudohypericin and one yellow band due to quercetin must be visible. Also, additional yellow and fluorescent bands can be seen in the chromatogram of SJW extract.[17] The AHP monograph mentions the Rf values of hypericin and pseudohypericin.[6] Berghöfer and Hölzl reported that SJW can be differentiated from adulterants by the presence of hyperforin and rutin, which are absent or negligible in other species. However, rutin can easily be added from other sources, such as unrelated *Styphnolobium japonicum* (Fabaceae), and some *Hypericum* species also contain hyperforin at relevant concentrations, making the determination of these two constituents alone insufficient as criteria to detect adulteration. *Hypericum barbatum* shows characteristic red-orange fluorescing flavonoid glycosides; *H. hirsutum* and *H. androsaemum* can be distinguished by the presence of the compound orientin; *H. montanum*

contains a turquoise fluorescing phenolic acid in addition to chlorogenic acid; and *H. maculatum, H. patulum,* and *H. tetrapterum* contain emodin.[18]

USP[91] mentions an HPTLC method for the analysis of SJW flowering tops, their powder, and extract. The HPTLC Association[92] uses similar conditions and provides the HPTLC fingerprints of USP powdered SJW extract reference standard (RS), SJW flowering top samples from China, and SJW flowering top extracts. In the AHPA Botanical Identity References Compendium[30], the USP method was used to compare SJW samples with three other *Hypericum* species. A very faint zone at the Rf corresponding to rutin can be used to distinguish *H. montanum*, *H. hirsutum*, and *H. undulatum* from SJW, which exhibits a much stronger band (Figure 3). Additional zones can be used to distinguish among the four species. Kırmızıbekmez et al. also used the USP method to separate and quantify rutin, miquelianin, hyperoside, and rutin in SJW from Turkey.[97]

Huck-Pezzei et al. used a more polar mobile phase to identify adulteration of 32 SJW samples by HPTLC. Under 365 nm and, after applying derivatization reagent, they found that SJW of Chinese origin contained a yellow-orange band under hypericin in the chromatogram. They suggested that it might be kushenol G and H due to *H. hirsutum* adulteration, but as mentioned before, the data provided do not support the presence of these molecules in any *Hypericum* species.[23]

A number of authors used the method proposed by the HPTLC Association (Figure 3).[24,25,67,100] Marrelli et al. employed the method to examine the chemical variability of four samples of *H. perforatum* subsp. *veronense* from Italy and mentioned that the constituents can be well separated and easily visualized by this method.[100]

Frommenwiler et al. investigated the adulteration of SJW crude herbs, commercial products, and extracts. They detected an additional yellow band in SJW of Chinese origin, but additionally, they observed the absence of a yellow band at Rf = 0.18 in these samples. Also, a reversed-phase HPTLC method was used to study the adulteration of the SJW samples with food dyes. A mobile phase consisting of methanol-5% aqueous sodium sulfate (3:4) was used to investigate the presence of Tartrazine, Amaranth, Sunset Yellow FCF, and Brilliant Blue FCF.[24] This method is also explained in detail in the monograph of HPTLC Association[93] and Camag's application note (Figure 4).[94]

Using the method of the HPTLC Association, both Booker et al. and Scotti et al. aimed to evaluate the chemical profile of Chinese SJW and compare the variability of SJW samples collected from different geographical locations.[25, 67] The fingerprint of Chinese SJW was found to be very distinct from the other eight Chinese *Hypericum*

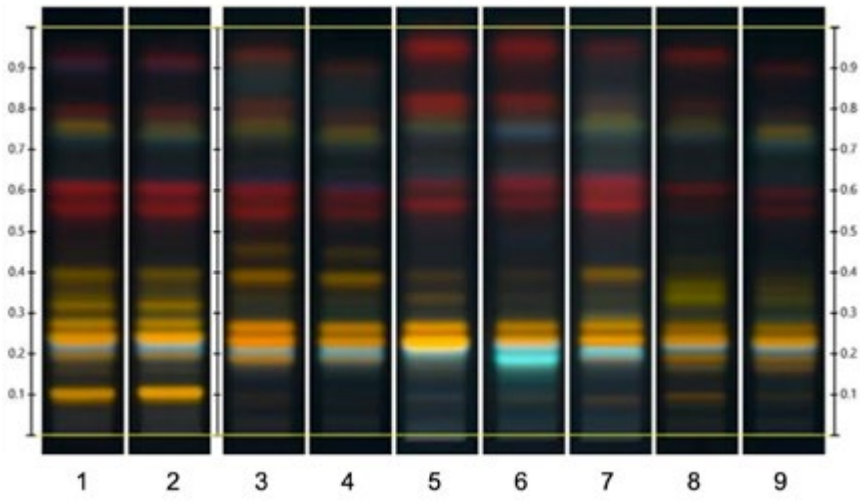


Figure 3. HPTLC analysis of Hypericum species. Lanes 1,2: *H. perforatum*; lanes 3,4: *H. undulatum*; lanes 5, 6: *H. montanum*; lane 7: *H. tetrapterum*; lanes 8, 9: *H. hirsutum*. Rutin is a yellow band at Rf = 0.1. Conditions as described by the HPTLC Association.

Image provided by Camag (Muttenz, Switzerland)

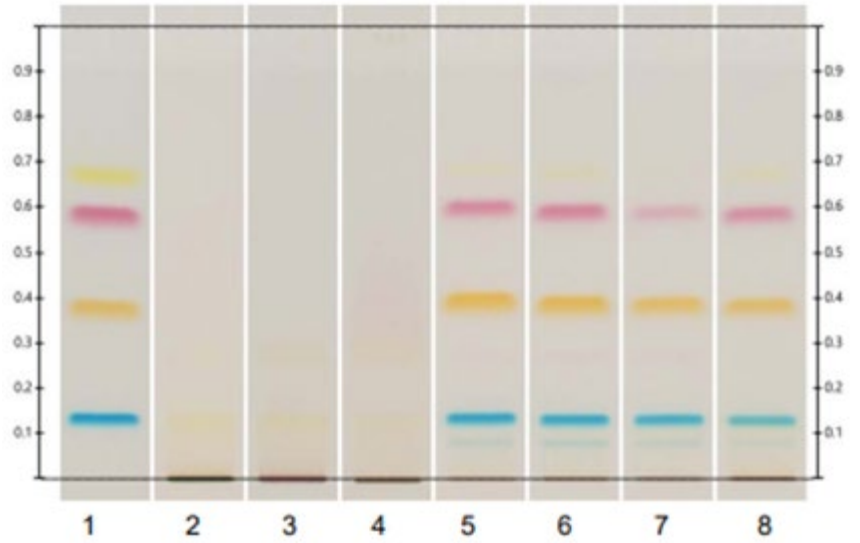


Figure 4. HPTLC system for the detection of food dyes. Lane 1: Brilliant Blue FCF, Sunset Yellow FCF, Amaranth, and Tartrazine (with increasing Rf); lane 2: St. John's wort herb; lanes 3-4: St. John's wort herb extract; lanes 5-7: St. John's wort herb bulk extract containing food dyes; lane 8: Commercial St. John's wort dietary supplement containing food dyes. Conditions as described by the HPTLC Association.

Image provided by HPTLC Association (Rheinfelden, Switzerland)

species tested by Scotti et al. A typical fingerprint of Chinese SJW was observed in all samples collected from China, with an extra compound, represented by the yellow band at Rf = 0.49 and the missing yellow band at Rf = 0.18. This seems to define a specific chemotype for specimens belonging to ssp. *chinense* that is geographically restricted to China. On the contrary, some samples from Spain also have the yellow band at Rf = 0.49 but they do not contain rutin or the yellow band at R_f = 0.18. According to NMR data, the compound at Rf = 0.49 is avicularin, but guaiaverin (Figure 2) is also found albeit at a much lower concentration in both Chinese and Spanish samples. Guaiaverin was also detected in a German sample. Based on this, the view of Chinese SJW containing some unique marker substances cannot be substantiated. The variability of the market products is strongly affected not only by the geographical origin of the plant material, but also harvest time, plant part harvested, and processing techniques.[67]

Raclariu et al. reported that several *Hypericum* species have indistinguishable TLC chromatograms from SJW, and that it is impossible to differentiate a mixture of *H. perforatum* and *H. maculatum* from an unadulterated *H. perforatum* sample.[22] While these authors state that TLC assays do not provide useful information about the concentrations of the main bioactive compounds hyperforin and hypericin, Mulinacci et al. proved that the quantitative data obtained from TLC-densitometry and HPLC-DAD analysis were in good agreement and statistical analysis of the findings revealed the equivalence of these two techniques.[101]

HPTLC is a robust, reliable, and suitable routine method for the detection of adulteration (dyes, incorrect species, or potential chemotypes of SJW) and can detect compositional differences between crude materials, extracts, and products. It should be kept in mind that representative sample number has to be used due to the chemical variability of the plant material. In view of the considerable number of *Hypericum* species that can be differentiated, the methods proposed by USP[91] and the HPTLC Association[92] are good choices for the detection of SJW with adulterating species. Additionally, the HPTLC Association's method for analyzing food dyes[93] is useful to detect the undeclared presence of synthetic colorants.

10.2.3 Infrared spectroscopy

Infrared (IR) Spectroscopy,[102] Near Infrared Spectroscopy (NIR),[103-105] and Fourier Transmission Infrared (FTIR) imaging[105] techniques conducted on SJW samples are discussed in this document. Methods include those published by Huck et al.,[103] Kokalj et al.,[102] Nichita et al.,[105] Rager et al.,[104] and Strzemski et al.[79]

Comments: IR spectroscopy was used for the evaluation of differences between *H. perforatum*, *H. hirsutum*, *H. montanum*, *H. dubium*, *H. maculatum*, and *H. tetrapterum*. Leaf samples (n = 10) were analyzed by four IR spectroscopy modes to identify the best one, and the KBr transmission mode in the spectral range of 450-4000 cm^{-1} provided the optimum results (97% correct species identification).[102]

Rager et al. developed a quantitative NIR method for the analysis of hyperforin and I3,II8-biapigenin. SJW dry extracts were studied in triplicate in the spectral range of 1100-2498 nm, and the data obtained were pre-treated and subjected to regression statistics. The Root Mean Square Error of Prediction was used for calibration and validation.[104] Huck et al. established a similar method for the quantification of naphthodianthrones and phloroglucinols in SJW dry extracts. RP-HPLC was used as a reference method in both studies. Spectra were acquired from SJW dry extracts over the spectral range of 4500-10000 cm^{-1} in transflection mode. Hypericin and hyperforin were analyzed via NIR with a standard error of estimation (SEE) of 0.52 and 0.50 µg/mL and standard error of prediction (SEP) of 0.64 and 0.71 µg/mL within a few seconds.[103] No data on the methods' abilities to distinguish among *Hypericum* species were provided.

Nichita et al. used spectroscopic (UV-VIS-NIR, FT-IR), chemical, and chromatographic techniques and managed to identify the presence of flavonoids in SJW. For the spectroscopic analysis, they used UV-VIS-NIR spectroscopy in the wavelength range of 190-2300 nm and FT-IR spectroscopy from 4000 to 400 cm^{-1}.[105]

Strzemski et al.,[79] compared data from chemometric evaluations of nine *Hypericum* spp., and four commercial St. John's wort samples by four analytical approaches including attenuated total reflectance infrared (ATR-IR). When compared to the results from quantitative determination of the main St. John's wort constituents by HPLC-UV/Vis, the IR data showed a relatively poor correlation, leading the authors to conclude that "the most reliable information can be obtained by HPLC analysis."

IR is a valuable tool for differentiating *Hypericum* species in whole, cut, or powdered form, but selecting the best mode is crucial for a successful analysis. NIR is an effective and fast method that can be used in the quantification of some constituents in SJW extracts. However, a second quantification method such as HPLC is required for cross-validation. No data are available on the ability of IR and NIR to detect adulteration with colorants. Additionally, its accuracy could be questioned in the analysis of low concentration molecules. IR or FTIR is fast, simple, and environmentally friendly and designed for monitoring the similarity of a group of materials. However, it is not appropriate for using as stand-alone method for the authentication of SJW extracts.

10.2.4 High-performance liquid chromatography (HPLC) and ultrahigh-performance liquid chromatography (UHPLC)

Methods described in the following literature were evaluated in this review: USP 2021,[91] Ph Eur 8.1,[17] AHP Monograph,[6] Ang et al.,[106] Božin et al.,[74] Brolis et al.,[107] Chandrasekera et al.,[108] Crockett et al.,[63] de los Reyes et al.,[109] Ganzera et al.,[110] Kladar et al.,[111] Napoli et al.,[70] Poutaraud et al.,[112] Smelcerovic et al.,[68,73] Tocci et al.,[58] Tolonen et al.,[113] and Zdunic et al.[57] Methods poorly explained or focused on the analysis of just one compound/class of compounds are not mentioned here. A comparison among the various HPLC methods is given in Table 3.

St. John's Wort
Hypericum perforatum
Photo ©2021 Steven Foster

Comments: In many studies, HPLC and UHPLC are used to compare the chemical constituents of SJW with other *Hypericum* species. In the study of Božin et al.,[74] chemical profiles of four *Hypericum* spp. (*H. maculatum* subsp. *immaculatum*, *H. olympicum*, *H. richeri* subsp. *grisebachii*, and *H. barbatum*), one commercial sample of SJW and five SJW samples collected from different localities were analyzed by HPLC-DAD.

A number of additional studies used HPLC with UV/Vis alone or in combination with MS detection to analyze *H. perforatum* and other *Hypericum* species. An assessment of these studies can be found in Table 3. [57,58,68,70,73,111]

Since the characteristic compounds of SJW are chemically diverse, different mobile phase systems and various column types (C18, C12, monolithic, phenyl-hexyl, polyethylene glycol, etc.) are reported in the literature for their efficient analysis. UV, diode array detectors (DAD), MS, and NMR are used for detection and quantification. The impact of extraction parameters (time, temperature, extraction solvent) and different extraction techniques (pre-extraction in Soxhlet to remove chlorophylls, sonication, shaking) have been investigated for optimization of the extraction method. Poutaraud[112] mentioned that extraction in the dark with water-ethanol (40:60) in a shaking water bath for 1 h at 80°C was the best method for extracting hypericins, but a 10% loss of total hyperforins was observed despite the precautionary measures. Extraction with methanol in the dark at room temperature for 2 hrs. on a shaker was found to be optimal for phloroglucinols. According to USP 2021, hyperforin (min 0.6%), hypericin, and pseudohypericin (min 0.04%) contents of the SJW flowering tops have to be determined. A single HPLC method (66 min) using oxybenzone as internal standard and gradient elution on a reversed phase column is prescribed. On the other hand, Ph Eur gives an additional specification for flavonoids (min 6.0% calculated as rutin) for the dried SJW extract and describes HPLC methods for the determination of hypericin (required content of 0.1-0.3% hypericins calculated as hypericin), and for hyperforin (required content of less than 6.0%) and flavonoids. Both pharmacopeial methods can be used for routine analysis.

There are some challenges in HPLC analysis of SJW extracts: the hyperforin signal is impacted by the pH of the mobile phase and hypericin may precipitate under acidic conditions (causing peak tailing) or during storage at cold temperatures depending on the solvent used. Additionally, sunlight, heat, and air affect the stability of hyperforin and adhyperforin. Thus, these factors are controlled in most cases. Mainly, gradient elutions are used, although the run times are relatively long (35-75 min) for the separation of different classes of chemical compounds in a single run.

Methods which have been the most thoroughly validated should be preferred. The method developed by Ganzera et al.[110] can be the best option for the analysis of flavonoids, naphthodianthrones, and the phloroglucinol derivative hyperforin. In a 35 min HPLC run, nine major compounds were identified, and baseline separated

Table 3. Comparison among different published HPLC methods for *H. perforatum* (continued on page 17)

Reference	Sample Set	Method	Analyte(s)	Isocratic (I)/ Gradient (G)	Pro	Con
USP 2021[91]	Flowering top, flowering top powder, dry crude extract	LC-UV/VIS	Hypericin, pseudohypericin, and hyperforin	G	Validated, relatively inexpensive, simple sample prep	Relatively long run time (66 min)
Ph Eur 8.1[17]	Dry crude extract	LC-UV/VIS	Total hypericins, hyperforin, and flavonoids (rutin, hyperoside, isoquercitrin, quercitrin, quercetin, and biapigenin)	I, G	Relatively inexpensive, simple sample prep	2 different methods have to be conducted, the extract has to be exposed to a xenon lamp for total hypericin analysis, hyperforin analysis has to be carried out protected from light
AHP[6]	Dry crude extract	HPLC-UV/VIS	Chlorogenic acid, rutin, hyperoside, isoquercitrin, quercitrin, quercetin, hypericin, pseudohypericin, and hyperforin	G	Relatively inexpensive, simple sample prep	The chromatogram contains some unresolved peaks
Ang[106]	Leaf/flower mixture (1), capsules (3), tea bags (2), puff (1), snack bar (1), drinks (3)	LC-UV LC-ESI-MS	Pseudohypericin, hypericin, hyperforin, and adhyperforin	I	Validated, short run time (16 min), good peak shapes, allows determination of SJW components in various products	No information about flavonoids, MS equipment is expensive
Božin[74]	SJW samples (5), commercial sample (1), 4 other *Hypericum* spp.	HPLC-DAD	Chlorogenic acid, caffeic acid, rutin, quercitrin, hypericin, and hyperforin	G	Short run time	Long extraction time (72 h), no validation
Brolis[107]	Crude extract	HPLC-DAD HPLC-ESI-MS	Chlorogenic acid, quercetin, quercitrin, isoquercitrin, rutin, hyperoside, I3,II8-biapigenin, pseudohypericin, hypericin, hyperforin, and adhyperforin	G	Validated, simple, accurate, specific method suitable for rutin analysis	Long extraction time (6 h), MS equipment is expensive
Chandrasekera[108]	Capsules (5) and tablets (6)	HPLC-ESI-MS	Hyperforin, hypericin, pseudohypericin, rutin, hyperoside, isoquercitrin, quercitrin, quercetin, and chlorogenic acid	G, I	Validated methods, specific, robust, applicable to alcoholic tinctures, capsules, tablets, and extracts	2 different methods have to be conducted, hyperoside and isoquercitrin peaks are not resolved well, hyperforin, pseudohypericin and hypericin peaks are tailing, MS equipment is expensive
Crockett[63]	Aerial parts of SJW (74 taxa of *Hypericum*)	HPLC-DAD	Rutin, hyperoside, isoquercitrin, quercitrin, quercetin, amentoflavone, hypericin, pseudohypericin, and hyperforin	G	Equipment common, relatively inexpensive, moderate run time (35 min)	Only qualitative analysis, some overlapping peaks are observed, drifting baseline problem, no validation

in methanolic extracts of commercial SJW products. It is a validated, moderately fast, and reliable method with a gradient mobile phase, composed of 10 mM ammonium acetate buffer (pH 5.0) and an acetonitrile/methanol mixture.[110] System suitability parameters (e.g., column efficiency, tailing factor, resolution) have been published only for the USP 2021 and Ph Eur methods. Additionally, none of these HPLC methods was assessed for its ability to detect the presence of synthetic colorants.

10.2.5 Gas chromatography (GC)

Methods described in the following literature were evaluated in this review: Maggi et al.,[115] Çırak et al.,[114] and Strzemski et al.[79]

Comments: In two of the investigations, essential oils obtained from *H. perforatum* from 10 regions in Turkey,[114] or from nine *Hypericum* taxa (including *H. perforatum* subsp. *veronense* and *H. perforatum* subsp. *perforatum*)[115] were analyzed by GC-FID and GC-MS, followed by statistical assessment with PCA or hierarchical cluster analysis (HCA). While the results permitted to distinguish among growing regions and species, authors of both papers noted a considerable variation in the essential oil profile of St. John's wort depending on the geographical origin of the samples. Therefore, basing *Hypericum* species identification on the essential oil profile does not represent a suitable approach.

Strzemski et al.[79] analyzed aqueous ethanol extracts of St. John's wort and eight other *Hypericum* species by GC-FID, and compared the results obtained from chemometric data (PCA and HCA) to those obtained by HPLC-UV/Vis, IR, ^{1}H NMR, and direct MS data (sections 10.2.3, 10.2.6, and 10.2.7). Results from PCA differed substantially depending on the analytical approach, and only the data using principal components 2 and 3 from the GC-FID analysis have a good correlation with quantitative data from the HPLC-UV/Vis analysis. Similarly, the results obtained with HCA were inconsistent, with *H. perforatum* being most similar to *H. hirsutum* when analyzed by IR and ^{1}H NMR; however, the GC-FID and direct MS data classified it most closely

Table 3 (continued). Comparison among different published HPLC methods for *H. perforatum*

Reference	Sample Set	Method	Analyte(s)	Isocratic (I)/ Gradient (G)	Pro	Con
de los Reyes[109]	Capsules	HPLC-Fluorescence and UV	Hypericin, pseudohypericin, and hyperforin	I	Validated, rapid method (10 min), simple sample prep, suitable for simultaneous analysis	No information about flavonoids
Ganzera[110]	SJW products (12)	HPLC-DAD HPLC-MS	Rutin, hyperoside, isoquercitrin, quercitrin, quercetin, I3, II8-biapigenin, hypericin, pseudohypericin, and hyperforin	G	Validated, moderate run time (35 min), simple and quick sample prep, compounds are baseline separated, good resolution	MS equipment is expensive
Kladar[111]	SJW tea samples (34)	HPLC-DAD	Caffeic acid, chlorogenic acid, gallic acid, *p*-hydroxybenzoic acid, quercetin, rutin, hypericin, and hyperforin	G, G	Moderate run time	Long extraction time (72 h), 2 different methods have to be conducted, no validation
Napoli[70]	Aerial parts of SJW and 10 other *Hypericum* spp.	HPLC-DAD-MS	Naphthodianthrones, acylphloroglucinols, cinnamic acids, flavonoids, and biflavones	G, G		Long extraction time (72 h), long analysis time, two different methods have to be conducted, no validation
Poutaraud[112]	Dried flowering tops (3), dried flowers (2), fresh flowers (1)	HPLC-DAD	Protopseudohypericin, adhyperforin, protohypericin, hypericin, hyperforin, adhyperforin	G	Validated, fast (17 min) and reliable method, good resolution	No information about flavonoids
Smelcerovic[68]	Aerial parts of SJW and other 16 *Hypericum* spp.	LC-MS/MS	Pseudohypericin, hypericin, hyperforin, rutin, hyperoside, quercitrin, and quercetin	G, G	Simple and quick sample prep	2 different methods have to be conducted, no data about calibration or validation, MS equipment is expensive
Smelcerovic[73]	Aerial parts of 6 *Hypericum* spp.	HPLC-DAD LC-MS/MS	Hyperoside, quercitrin, pseudohypericin, hyperforin, hypericin	G, G	Simple and quick sample prep	2 different methods have to be conducted, no validation, MS equipment is expensive
Tocci[58]	Aerial parts of SJW and 4 other *Hypericum* spp.	LC-MS	Benzoates, cinnamates, flavonols, flavones, flavones, chalcones, coumarins, flavan-3-ols, stilbenoids, phloroglucinols, and naphthodianthrones	G, G	Short run times	Long extraction time (3x24 h), 2 different methods have to be conducted, no data about calibration or validation parameters, MS equipment is expensive
Tolonen[113]	Freeze-dried SJW	HPLC-DAD HPLC-ESI/MS	Hypericin, protohypericin, pseudohypericin, protopseudohypericin, hyperforin, and adhyperforin	G	Validated and fast method (12 min), verified to function reliably in long-term use	No information about flavonoids
Zdunic[57]	Aerial parts of 7 *Hypericum* spp.	HPLC-UV LC-UV-MS	Cinnamic acids, flavonoids, biflavones, and naphthodianthrones	G		Long extraction time (3x24 h), relatively long run time, no validation, no information about hyperforin

to *H. calycinum/H. hookerianum* and *H. maculatum*, respectively. While the use of a GC-FID fingerprint in combination with multivariate statistics should in principle provide useful data for species distinction, the available information does not provide sufficient evidence that it is suitable to authenticate St. John's wort herb, or extracts made thereof.

10.2.6 Nuclear magnetic resonance (NMR)

The following methods were included in this review: Bilia et al.,[116] Porzel et al.,[45] Scotti et al.,[67] and Strzemski et al.[79]

NMR metabolomics have been successfully used to identify the metabolome of SJW and to cluster crude materials and commercial finished products, based on the presence and the concentration of certain metabolites. Bilia et al. evaluated the composition of a commercial SJW extract by using one-dimensional (1D) and two-dimensional (2D) NMR techniques. The ^{1}H NMR spectra obtained revealed signals in four main regions (9.0–6.0 ppm, 5.5–4.5 ppm, 4.5–3.0 ppm, and 2.7–0.7 ppm), which were assigned to flavonols, phloroglucinols, naphthodianthrones, polyphenols, chlorogenic acid, lipids, and sucrose. A large number of SJW constituents, including hypericins, was identified.[116] Porzel et al. used NMR-based metabolomics coupled to PCA and HCA (hierarchical cluster analysis) to investigate the differences in the chemistry of seven *Hypericum* species, including *H. perforatum*. The clustering of the species occurred mainly due to qualitative and quantitative differences in patterns of hyperforins, lipids, and phenolic acids, while hypericins could not be detected in NMR based PCA. The HCA showed that *H. polyphyllum*, *H. tetrapterum,* and *H. perforatum* grouped, indicating that the two species could be possibly used to substitute SJW based on this method of identification.[45] Scotti et al. used ^{1}H NMR-based PCA comparing nine *Hypericum* species, and showed that SJW is easily distinguishable from the other species except from *H. ascyron*. However, only *H. patulum* is known as a potential adulterant of SJW among the nine species analyzed in this study.[67] In the comparison of ^{1}H NMR fingerprints of nine *Hypericum* species by

Strzemski et al, St. John's wort clustered most closely to *H. hirsutum* with both PCA and HCA.[79] However, the spectral features of *Hypericum* metabolites were less prominent in this case since all the spectra were dominated by a signal at ca. 3.4 ppm, possibly due to the presence of residual water in the samples.

Comments: NMR experiments are fast, nondestructive, and versatile to provide a fingerprint of a SJW extract. NMR is useful for the identification of similarities and differences among species, and easily allows evaluation of the results obtained from large pools of samples. In all the methods evaluated above, the relatively small number of botanically authenticated samples may have prevented a more meaningful assessment. Samples have to be freeze-dried before analysis in order to avoid large water signals in the spectra. A restriction of the spectral window for chemometric evaluations may be necessary for a clearer distinction among species. NMR has lower sensitivity and precision (depending on the analyte signal) than the other methods; in addition, the equipment costs and maintenance expenses are high. While the universal detection mode of ^{1}H NMR should be able, in principle, to detect the presence of undeclared dyes in SJW, evidence to prove this is lacking.

10.2.7 Direct mass spectrometry (MS)

Only one method by Strzemski et al.[79] was available for review.

As discussed before (section 10.2.5), the direct MS data showed a poor correlation to data obtained by quantitative HPLC-UV/Vis of the main constituents in St. John's wort. Nevertheless, it was the only chemometric approach where the St. John's wort sample clustered most closely to the four commercial extracts that were analyzed together with the nine *Hypericum* species in the PCA based model. Therefore, it may hold some promise as a means to distinguish among *Hypericum* species. However, more data are necessary to assess its usefulness to authenticate St. John's wort herb and its extracts.

Comments: Like NMR and IR approaches, direct (without prior separation) MS followed by multivariate statistics is a fast and environmentally friendly method for obtaining SJW extract fingerprints. MS has excellent sensitivity, but the signal is less stable over time than, e.g., with an NMR; in addition, the equipment costs are high. As with other methods relying on multivariate statistics, the results are only as good as the model that is used to analyze the samples, and care needs to be taken to include a sufficient number of samples from various geographical locations to account for the chemical variability within a particular species.

11. Conclusion

Macroscopic assessment of the whole plant material may provide the first indication for adulteration such as the absence of the characteristic black hypericin glands on the stems, leaves, and petals and presence of the opposing ridges on the stem, which is diagnostic for *H. perforatum* in Europe. In the absence of systematic anatomical studies of *Hypericum perforatum* and related species, macroscopic test methods are most appropriate for identification of relatively whole material in which diagnostically relevant characteristics are intact but are obviously inadequate to detect adulteration of cut or powdered raw material, extracts, or commercial products. A color assessment test (as described in section 8) and/or a genetic approach combined with chemical identification methods is recommended for these materials. HPTLC methods can be used for detecting adulterated raw material and extracts, and they are also a good option for examining the presence of synthetic dyes. Appropriate HPTLC methods include the methods described in references.[91-93] Suitable HPLC methods include the methods described in Table 3. HPLC-UV/Vis methods can be used as both qualitatively and quantitatively. The conditions by Ganzera et al.[110] are appealing due to its validation, easy sample preparation, run time, and its usefulness to assess hypericins, hyperforin, and flavonoids in one run. IR spectroscopy,[102] direct MS,[79] and NMR spectroscopy[45,67,116] may be used as orthogonal methods for detecting adulteration. Although not widely used in the herbal medicine and dietary supplement industry, ^{1}H NMR spectroscopy (with or without chemometric data analysis) provides a suitable option for those companies with access to an NMR instrument. Differentiating among closely related species of SJW and detecting mixtures of SJW with other *Hypericum* species is more challenging. Authentication of *Hypericum* species solely based on the presence of marker compounds is insufficient unless a thorough statistical evaluation (*e.g.*, with HPLC, direct MS, or NMR) is performed.

12. References

1. Anonymous. The 23rd Annual General Meeting of the American Pharmaceutical Association Report of the Committee on Adulterations and Sophistications. Sherman & Co. Printers; 1875:501.
2. McCutcheon A. Adulteration of *Hypericum perforatum. Botanical Adulterants Prevention Bulletin*. 2017:1-9.
3. Costa J, Campos B, Amaral JS, Nunes ME, Oliveira MBPP, Mafra I. HRM analysis targeting ITS1 and matK loci as potential DNA mini-barcodes for the authentication of *Hypericum perforatum* and *Hypericum androsaemum* in herbal infusions. *Food Control*. 2016;61:105–114.
4. World Health Organization. *Herba Hyperici*. Vol 2. *WHO Monographs on Selected Medicinal Plants*. World Health Organization; 2004.
5. Invasive Species Compendium. Center for Agriculture and Bioscience International (CABI) Website. Accessed April 19, 2021. https://www.cabi.org/isc/datasheet/28268#tosummaryOfInvasiveness
6. Upton R, Graff A, Swisher D. *American Herbal Pharmacopeia and Therapeutic Compendium: St. John's wort.* Hypericum perforatum *L. Quality Control, Analytical, and Therapeutic Monograph*. Santa Cruz, CA: American Herbal Pharmacopoeia; 1997.
7. Pyrka I, Stefanaki A, Vlachonasios KE. DNA barcoding of St. John's wort (*Hypericum* spp.) growing wild in North-Eastern Greece. *Planta Med*. 2021;
8. Bergonzi M, Bilia A, Gallori S., Guerrini D, Vinvieri F. Variability in the content of the constituents of *Hypericum perforatum* L. and some commercial extracts. *Drug Dev Ind Pharm*. 2001;27(6):491–497.
9. Eroglu Ozkan E, Aksu B, Mat A. An overview on *Hypericum* species of Turkey. *J Pharmacogn Phytother*. 2013;5(3):38–46.

Table 4. Comparison among the different techniques to characterize/authenticate SJW

Method	Applicable to	Pro	Con
Macroscopic	Whole or cut plant	No solvents required Quick Inexpensive No reference material needed	No systematic anatomy studies exist No automation/statistics Difficult or impossible for finely cut and sifted materials
Microscopic	Whole, cut, or powdered plant	Quick Inexpensive Few solvents required No reference material needed	No automation/statistics Not many distinctive characteristic elements currently known
Organoleptic	Whole, cut, or powdered plant, extracts	Quick Inexpensive Color of the methanol extract can give an idea about adulteration	Cannot detect adulteration by other *Hypericum* species
Genetic	Whole, cut, or powdered plant, some extracts[a]	Able to distinguish closely-related species Reliable Able to detect small amounts of adulterants Only method for botanical blend No reference material needed when database established	Labor-intensive sample preparation and analysis Expensive equipment Cannot distinguish among plant parts Cannot detect adulteration with chemical compounds such as undeclared dyes DNA in certain processed materials cannot be detected
UV Spectrometry	Whole, cut, or powdered plant,[b] extracts	Quick Basic systems affordable for smaller labs	Lack of specificity Occurrence of adulteration with dyes and other *Hypericum* species cannot be detected Method only suitable for quantitation of a class of compounds
TLC/HPTLC	Whole, cut, or powdered plant,[b] extracts	Quick Basic systems affordable for smaller labs Able to detect small amounts of adulterants Can be used to detect the presence of synthetic dyes	No statistics High-end equipment somewhat expensive Need for authenticated botanical reference materials
IR, NIR	Whole, cut, or powdered plant,[c] extracts	No sample preparation needed Short analysis time State-of-the-art statistical evaluation	Accuracy and precision for low-concentration compounds insufficient Limited quantitative information Large number of authenticated samples needed for statistics
HPLC-UV	Whole, cut, or powdered plant,[b] extracts	Standard equipment in laboratories Able to detect small amounts of adulterants Both quantitative for chemical profiling and quantitative for marker compounds Suitable for routine analysis	Equipment somewhat expensive Often no statistics applied (although software is available)
HPLC-MS and HPLC-MS/MS	Whole, cut, or powdered plant,[b] extracts	Able to detect small amounts of adulterants Qualitative and quantitative	Equipment expensive Quality of data depends on the ability to ionize analytes
GC-MS	Whole, cut, or powdered plant,[b] extracts, essential oil	Able to detect small amounts of adulterants Qualitative and quantitative	Equipment expensive Mainly for volatile constituents, other compounds need derivatization prior to analysis
NMR	Whole, cut, or powdered plant,[b] extracts	Short analysis time Reliable and highly reproducible State-of-the-art statistical evaluation possible Basic NMR methods are independent of analyst's expertise after the method is set up Qualitative and quantitative	Equipment and maintenance very expensive Substantial space requirements for high magnetic strength instruments Initial setup of parameters complex Large number of authenticated samples needed for statistics Not currently used for routine analysis
Direct MS	Whole, cut, or powdered plant,[b] extracts	Short analysis time Sensitive State-of-the-art statistical evaluation possible	Equipment expensive Quality of data depends on the ability to ionize analytes Large number of authenticated samples needed for statistics

[a] The success in accurate identification of extracts by genetic means depends on the processing and on the genetic method used (see section 9)

[b] Whole, cut, and powdered material only suitable for analysis after extraction

[c] With NIR, whole, cut, and powdered material can be analyzed without prior extraction, for IR, prior extraction is necessary

10. *Hypericum perforatum* L. Kew Names and Taxonomic Backbone. The International Plant Names Index and World Checklist of Selected Plant Families 2021. KEW. Accessed April 17, 2021. http://powo.science.kew.org/taxon/433719-1#source-KB
11. International Plant Names Index (IPNI) Database. Accessed April 19, 2021. https://www.ipni.org/n/433719-1
12. Dauncey EA, Irving JTW, Allkin R. A review of issues of nomenclature and taxonomy of *Hypericum perforatum* L. and Kew's Medicinal Plant Names Services. *J Pharm Pharmacol*. 2019;71(1):4–14.
13. Robson N. And then came molecular phylogenetics—Reactions to a monographic study of *Hypericum* (Hypericaceae). *Phytotaxa*. 2016;255(3):181–198.
14. Robson N. Studies in the genus *Hypericum* L. (Guttiferae) 4(2). Section 9. *Hypericum sensu lato* (part 2): Subsection 1. *Hypericum* series 1. *Hypericum*. *Bulletin of the British Museum (Natural History) Botany*. 2002;32(02):61–123.
15. Robson N. The genus *Hypericum*. In: Ernst E, ed. *Hypericum: The Genus Hypericum*. London, UK: Taylor and Francis; 2003
16. *Hypericum perforatum* L. Missouri Botanical Garden. Accessed April 19, 2021. http://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?kempercode=e198#:~:text=Hypericum%20perforatum%2C%20St.,western%20Asia%20and%20northern%20Africa.
17. St. John's wort (1438) and St. John's wort dry extract, quantified (1874). *European Pharmacopoeia (Ph Eur).* 8th ed. Strasbourg, France: Council of Europe; 2014.
18. Berghöfer R, Hölzl J. Johanniskraut (*Hypericum perforatum* L.) Prüfung auf Verfälschung. *Dtsch Apoth Ztg*. 1986;126:2569–2573.
19. Mitra SK, Kannan R. A note on unintentional adulterations in Ayurvedic herbs. *Ethnobot Leafl*. 2007;11:11–17.
20. Czygan F-C, Frohne D., Hiller K., et al. *Herbal Drugs and Phytopharmaceuticals: A Handbook for Practice on a Scientific Basis.* 3rd ed. Boca Raton, FL: CRC Press; 2004:305-308.
21. Giuliani C, Pellegrino RM, Tirillini B, Bini LM. The role of secreting structures position on the leaf volatile organic compounds of *Hypericum androsaemum*. *Nat Prod Commun*. 2010;5(1):1934578X1000500126.
22. Raclariu AC, Paltinean R, Vlase L, et al. Comparative authentication of *Hypericum perforatum* herbal products using DNA metabarcoding, TLC and HPLC-MS. *Sci Rep*. 2017;7(1):1291.
23. Huck-Pezzei VA, Bittner LK, Pallua JD, et al. A chromatographic and spectroscopic analytical platform for the characterization of St John's wort extract adulterations. *Anal Methods*. 2013;5(3):616–628.
24. Frommenwiler DA, Reich E, Sudberg S, Sharaf MH, Bzhelyansky A, Lucas B. St. John's Wort versus counterfeit St. John's wort: an HPTLC study. *J AOAC Int*. Sep 2016;99(5):1204-1212.
25. Booker A, Agapouda A, Frommenwiler DA, Scotti F, Reich E, Heinrich M. St John's wort (*Hypericum perforatum*) products – an assessment of their authenticity and quality. *Phytomedicine*. 2018;40:158–164.
26. Plants of the World Online. Kew Science. Accessed April 20, 2021. http://powo.science.kew.org/
27. Tropicos.org. Accessed April 20, 2021. http://www.tropicos.org
28. Plants For A Future. Accessed April 28, 2021. https://pfaf.org/
29. Plants Database. US Department of Agriculture Natural Resources Conservation Service Accessed April 28, 2021. https://plants.usda.gov/
30. American Herbal Product Association. *Hypericum perforatum* (flowering tops). American Herbal Product Association. Accessed April 22, 2021. http://www.botanicalauthentication.org/index.php/Hypericum_perforatum_(flowering_tops)
31. Rahfeld B. Johanniskraut – Hyperici herba. *Mikroskopischer Farbatlas Pflanzlicher Drogen*. Heidelberg, Germany: Spektrum Akademischer Verlag; 2009:94–95.
32. Crockett SL, Douglas AW, Scheffler BE, Khan IA. Genetic profiling of *Hypericum* (St. John's wort) species by nuclear ribosomal ITS sequence analysis. *Planta Med*. 2004;70(10):929–935.
33. Howard C, Bremner PD, Fowler MR, Isodo B, Scott NW, Slater A. Molecular identification of *Hypericum perforatum* by PCR amplification of the ITS and 5.8S rDNA region. *Planta Med*. 2009;75(8):864–869.
34. Howard C, Socratous E, Williams S, et al. PlantID – DNA-based identification of multiple medicinal plants in complex mixtures. *Chin Med*. 2012;7(1):18.
35. Kazi T, Hussain N, Bremner P, Slater A, Howard C. The application of a DNA-based identification technique to over-the-counter herbal medicines. *Fitoterapia*. 2013;87:27–30.
36. Scotti F, Mali P, Masiero E, et al. *Hypericum perforatum*–a comparison of commercial samples using DNA-barcoding and chemical approaches. *Planta Med Int Open*. 2017;4(S 01):We-SL-04.
37. Howard C, Hill E, Kreuzer M, et al. DNA authentication of St John's wort (*Hypericum perforatum* L.) commercial products targeting the ITS region. *Genes (Basel)*. 2019;10(4):286.
38. Howard C, Lockie-Williams C, Slater A. Applied barcoding: the practicalities of DNA testing for herbals. *Plants (Basel)*. 2020;9(9):1150.
39. Parveen I, Gafner S, Techen N, Murch SJ, Khan IA. DNA barcoding for the identification of botanicals in herbal medicine and dietary supplements: strengths and limitations. *Planta Med*. 2016;82(14):1225–1235.
40. European Medicines Agency Evaluation of Medicines for Human Use Committee on Herbal Medicinal Products. *Assessment report on Hypericum perforatum L., herba*. 2009. https://www.ema.europa.eu/en/documents/herbal-report/assessment-report-hypericum-perforatum-l-herba_en.pdf
41. Nahrstedt A, Butterweck V. Biologically active and other chemical constituents of the herb of *Hypericum perforatum* L. *Pharmacopsychiatry*. 1997;30(S 2):129–134.
42. ESCOP. Hyperici herba – St. John's Wort. In: Roberta Hutchins SM, ed. *ESCOP Monographs Online Series*. European Scientific Cooperative on Phytotherapy; 2018.
43. Baugh SF. Simultaneous determination of protopseudohypericin, pseudohypericin, protohypericin, and hypericin without light exposure. *J AOAC Int*. 2005;88(6):1607–1612.
44. Kurth H, Spreemann R. Phytochemical characterization of various St. John's wort extracts. *Adv Ther*. 1998;15(2):117–128.
45. Porzel A, Farag M, Mülbradt J, Wessjohann L. Metabolite profiling and fingerprinting of *Hypericum* species: a comparison of MS and NMR metabolomics. *Metabolomics*. 2013;10(4):574–588.
46. Greeson J, Sanford B, Monti D. St. John's wort (*Hypericum perforatum*): A review of the current pharmacological, toxicological, and clinical literature. *Psychopharmacology*. 2001;153:402–414.
47. Maisenbacher P, Kovar K-A. Adhyperforin: a homologue of hyperforin from *Hypericum perforatum*. *Planta Med*. 1992;58(3):291–293.
48. Bilia AR, Bergonzi MC, Morgenni F, Mazzi G, Vincieri FF. Evaluation of chemical stability of St. John's wort commercial extract and some preparations. *Int J Pharm*. 2001;213(1-2):199–208.
49. Fuzzati N, Gabetta B, Strepponi I, Villa F. High-performance liquid chromatography–electrospray ionization mass spectrometry and multiple mass spectrometry studies of hyperforin degradation products. *J Chromatogr A*. 2001;926(1):187–198.
50. Orth H, Rentel C, Schmidt P. Isolation, purity analysis and stability of hyperforin as a standard material from *Hypericum perforatum* L. *J Pharm Pharmacol*. 1999;51(2):193–200.
51. Lazzara S, Napoli E, Carrubba A. *Hypericum* spp.: a resource from wild Mediterranean flora for the treatment of mild depression. *Bioactive phytochemicals—perspectives for modern medicine*. 2015;3:337–354.
52. Berghöfer R, Hölzl J. Biflavonoids in *Hypericum perforatum*1; part 1. Isolation of I3,II8-biapigenin. *Planta Med*. 1987;53(02):216–217.
53. Tusevski O, Krstikj M, Stanoeva JP, Stefova M, Gadzovska Simic S. Phenolic profile and biological activity of *Hypericum*

perforatum L.: Can roots be considered as a new source of natural compounds? *S Afr J Bot*. 2018;117:301–310.
54. Jürgenliemk G, Nahrstedt A. Phenolic compounds from *Hypericum perforatum*. *Planta Med*. 2002;68(1):88–91.
55. Wei Y, Xie Q, Dong W, Ito Y. Separation of epigallocatechin and flavonoids from *Hypericum perforatum* L. by high-speed counter-current chromatography and preparative high-performance liquid chromatography. *J Chromatogr A*. 2009;1216(19):4313–4318.
56. Çırak C, Radušienė J, Karabük B, Janulis V. Variation of bioactive substances and morphological traits in *Hypericum perforatum* populations from Northern Turkey. *Biochem Syst Eco*. 2007;35(7):403–409.
57. Zdunic G, Godjevac D, Savikin K, Petrovic S. Comparative analysis of phenolic compounds in seven *Hypericum* species and their antioxidant properties. *Nat Prod Commun*. 2017;12(11):1934578X1701201.
58. Tocci N, Perenzoni D, Iamonico D, Fava F, Weil T, Mattivi F. Extracts from *Hypericum hircinum* subsp. *majus* exert antifungal activity against a panel of sensitive and drug-resistant clinical strains. *Front Pharmacol*. 2018;9:382.
59. Sarrou E, Giassafaki L-P, Masuero D, et al. Metabolomics assisted fingerprint of *Hypericum perforatum* chemotypes and assessment of their cytotoxic activity. *Food Chem Toxicol*. 2018;114:325–333.
60. Ploss O, Petereit F, Nahrstedt A. Procyanidins from the herb of *Hypericum perforatum*. *Die Pharmazie*. 2001;56(6):509–511.
61. Tusevski O, Stanoeva J, Markoska E, Brndevska N, Stefova M, Gadzovska Simic S. Callus cultures of *Hypericum perforatum* L. a novel and efficient source for xanthone production. *Plant Cell Tissue Organ Cult*. 2016;125(2):309–319.
62. Kladar N, Mrđanović J, Anačkov G, et al. *Hypericum perforatum*: synthesis of active principles during flowering and fruitification—novel aspects of biological potential. *Evid Based Complementary Altern Med*. 2017;2017:2865610.
63. Crockett SL, Schaneberg B, Khan IA. Phytochemical profiling of new and old world *Hypericum* (St. John's wort) species. *Phytochem Anal*. 2005;16(6):479–485.
64. Maggi F, Ferretti G, Pocceschi N, Menghini L, Ricciutelli M. Morphological, histochemical and phytochemical investigation of the genus *Hypericum* of the Central Italy. *Fitoterapia*. 2004;75(7):702–711.
65. Sagratini G, Ricciutelli M, Vittori S, Öztürk N, Öztürk Y, Maggi F. Phytochemical and antioxidant analysis of eight *Hypericum* taxa from Central Italy. *Fitoterapia*. 2008;79(3):210–213.
66. Filippini R, Piovan A, Borsarini A, Caniato R. Study of dynamic accumulation of secondary metabolites in three subspecies of *Hypericum perforatum*. *Fitoterapia*. 2010;81(2):115–119.
67. Scotti F, Löbel K, Booker A, Heinrich M. St. John's wort (*Hypericum perforatum*) products–how variable is the primary material? *Front Plant Sci*. 2019;9:1973.
68. Smelcerovic A, Zühlke S, Spiteller M, Özen T. Phenolic constituents of 17 *Hypericum* species from Turkey. *Biochem Syst Ecol*. 2008;36:316–319.
69. Çırak C, Radusiene J, Jakstas V, Ivanauskas L, Seyis F, Yayla F. Altitudinal changes in secondary metabolite contents of *Hypericum androsaemum* and *Hypericum polyphyllum*. *Biochem Syst Ecol*. 2017;70:108–115.
70. Napoli E, Siracusa L, Ruberto G, et al. Phytochemical profiles, phototoxic and antioxidant properties of eleven *Hypericum* species — a comparative study. *Phytochemistry*. 2018;152:162–173.
71. Bruňáková K, Bálintová M, Henzelyová J, et al. Phytochemical profiling of several *Hypericum* species identified using genetic markers. *Phytochemistry*. 2021;187:112742.
72. Bektas N, Ersoy E, Boga M, et al. Cytotoxic and apoptotic effects of *Hypericum androsaemum* on prostate adenocarcinoma (PC-3) and hepatocellular carcinoma (Hep G2) cell lines with identification of secondary metabolites by LC-HRMS. *Turk J of Chem*. 2021;45:1621–1638.
73. Smelcerovic A, Verma V, Spiteller M, Ahmad SM, Puri SC, Qazi GN. Phytochemical analysis and genetic characterization of six *Hypericum* species from Serbia. *Phytochemistry*. 2006;67(2):171–177.
74. Božin B, Kladar N, Grujić N, et al. Impact of origin and biological source on chemical composition, anticholinesterase and antioxidant properties of some St. John's wort species (*Hypericum* spp., Hypericaceae) from the Central Balkans. *Molecules*. 2013;18(10):11733–11750.
75. Calie PJ, Schilling EE, Webb DH. Flavonoid chemistry of the generic segregates *Ascyrum* and *Crookea* of *Hypericum*. *Biochem Syst Ecol*. 1983;11(2):107–109.
76. Gitea D, Vicas S, Gitea MA, et al. HPLC screening of bioactives compounds and antioxidant capacity of different *Hypericum* species. *Rev Chim*. 2018;69(2):305–309.
77. Çırak C, Radusiene J, Jakstas V, Ivanauskas L, Seyis F, Yayla F. Secondary metabolites of seven *Hypericum* species growing in Turkey. *Pharm Biol*. 2016;54(10):2244–2253.
78. Kladar N, Rat M, Gavaric N, et al. Chemical characterization and chemotaxonomy of *Hypericum hirsutum* L. 1753 from Vojvodina (Serbia). *Matica Srpska J Nat Sci Novi Sad*. 2016;(131):167–175.
79. Strzemski M, Dresler S, Sowa I, et al. Direct spectroscopic and GC profiling combined with chemometric analysis as an alternative approach to investigate *Hypericum* species: Is it possible to replace HPLC? *Ind Crops Prod*. 2020;157:112930.
80. Umek A, Kreft S, Kartnig T, Heydel B. Quantitative phytochemical analyses of six *Hypericum* species growing in Slovenia. *Planta Med*. 1999;65(04):388–390.
81. Toiu A, Vlase L, Drăgoi C, Vodnar D, Oniga I. Phytochemical analysis, antioxidant and antibacterial activities of *Hypericum humifusum* L. (Hypericaceae). *Farmacia*. 2016;64(5):663–667.
82. Béjaoui A, Ben Salem I, Rokbeni N, M'Rabet Y, Boussaid M, Boulila A. Bioactive compounds from *Hypericum humifusum* and *Hypericum perfoliatum*: Inhibition potential of polyphenols with acetylcholinesterase and key enzymes linked to type-2 diabetes. *Pharm Biol*. 2017;55(1):906–911.
83. Radušienė J, Bagdonaiu E, Kazlauskas S. Morphological and chemical evaluation on *Hypericum perforatum* and *H. maculatum* in Lithuania. *Acta Hortic*. 2004:55–62.
84. Mártonfi P, Repčák M, Mihoková L. *Hypericum maculatum* Crantz subsp. *maculatum* X *H. perforatum* L. (Hypericaceae): Corroboration of natural hybridization by secondary metabolite analysis. *Folia Geobot Phytotax*. 1996;31(2):245–250.
85. Kladar N, Srđenović B, Grujić N, et al. Ecologically and ontogenetically induced variations in phenolic compounds and biological activities of *Hypericum maculatum* subsp. *maculatum*, Hypericaceae. *Braz J Bot*. 2015;38(4):703–715.
86. Rainha N, Koci K, Coelho A, Lima E, Baptista J, Fernandes-Ferreira M. HPLC–UV–ESI-MS analysis of phenolic compounds and antioxidant properties of *Hypericum undulatum* shoot cultures and wild-growing plants. *Phytochemistry*. 2013;86:83–91.
87. Agapouda A, Booker A, Kiss T, Hohmann J, Heinrich M, Csupor D. Quality control of *Hypericum perforatum* L. analytical challenges and recent progress. *J Pharm Pharmacol*. 2019;71(1):15–37.
88. *Pharmacopeia Helvetica.* 8th ed. Bern, Switzerland: Department Federal de l'Interieur; 1997.
89. DAB. *Monographie Johanniskraut* vol 3rd Supplement, 1991. German Drug Codex; Deutscher Arzneimittel Codex 86. 1986.
90. Gitea D, Monica Ş, Tămaş M, Pasca MB. The analysis of alcoholic extracts of *Hypericum* species by UV/VIS spectrophotometry. *Analele Univ din Oradea Fasc Biol*. 2010;TOM XVII
91. St John's wort, St John's wort powder, and St John's wort extract. *USP43–NF38.* Rockville, MD: United States Pharmacopeial Convention; 2021.

92. HPTLC Association. *Hypericum perforatum* – Flowering top. International Association for the Advancement of High Performance Thin Layer Chromatography; 2018. https://www.hptlc-association.org/media/7D5EVO4W/Hypericum_perforatum_Flowering_top_HPTLC_Association_USP40-NF35_V1.pdf
93. HPTLC Association. St. John's wort (*Hypericum perforatum*) Test for food dyes. International Association for the Advancement of High Performance Thin Layer Chromatography; 2018. Accessed May 19, 2021. https://www.hptlc-association.org/media/F280A6Q6/Hypericum_perforatum_Food_dyes_adulteration_HPTLC_Association_V1.pdf
94. CAMAG. Application note: adulteration of St. John's wort products: an HPTLC-MS investigation. 2016:1–4. Accessed May 19, 2021.
95. Abdel-Tawab M, Husch J, Schubert-Zsilavecz M, Dingermann T. Freiverkäufliche Johanniskrautpräparate unter der Lupe. *Pharm Ztg*. 2011. https://www.pharmazeutische-zeitung.de/ausgabe-172011/freiverkaeufliche-johanniskraut-praeparate-unter-der-lupe/
96. Jesionek W, Majer-Dziedzic B, Choma IM. Separation, identification, and investigation of antioxidant ability of plant extract components using TLC, LC-MS, and TLC-DPPH•. *J Liq Chromatogr Relat Technol*. 2015;38(11):1147–1153.
97. Kırmızıbekmez H, Atay İ, Yeşilada E. Determination of four major flavonoids in the methanolic extract of *Hypericum perforatum* by HPTLC-densitometry. *Planta Med*. 2008;74(09):PC72.
98. Kitanov GM. Hypericin and pseudohypericin in some *Hypericum* species. *Biochem Syst Ecol*. 2001;29(2):171–178.
99. Maleš Ž, Plazibat M, Vundać VB, Žuntar I, Pilepić KH. Thin-layer chromatographic analysis of flavonoids, phenolic acids, and amino acids in some Croatian *Hypericum* Taxa. *JPC – J Planar Chromat – Modern TLC*. 2004;17(4):280–285.
100. Marrelli M, Conforti F, Toniolo C, Nicoletti M, Statti G, Menichini F. *Hypericum perforatum*: Influences of the habitat on chemical composition, photo-induced cytotoxicity, and antiradical activity. *Pharm Biol*. 2014;52(7):909–918.
101. Mulinacci N, Bardazzi C, Romani A, Pinelli P, Vincieri FF, Costantini A. HPLC-DAD and TLC-densitometry for quantification of hypericin in *Hypericum perforatum* L. extracts. *Chromatographia*. 1999;49(3–4):197–201.
102. Kokalj M, Kolar J, Trafela T, Kreft S. Differences among *Epilobium* and *Hypericum* species revealed by four IR spectroscopy modes: transmission, KBr tablet, diffuse reflectance and ATR. *Phytochem Anal*. 2011;22(6):541–546.
103. Huck CW, Abel G, Popp M, Bonn GK. Comparative analysis of naphthodianthrone and phloroglucine derivatives in St. John's wort extracts by near infrared spectroscopy, high-performance liquid chromatography and capillary electrophoresis. *Anal Chim Acta*. 2006;580(2):223–230.
104. Rager I, Roos G, Schmidt PC, Kovar KA. Rapid quantification of constituents in St. John's wort extracts by NIR spectroscopy. *J Pharm Biomed Anal*. 2002;28(3):439–446.
105. Nichita C, Giurginca M, Bazdoaca C, Parvu L, Meghea A. Vegetal antioxidants obtained from *Hyperycum perforatum* species. *Rev Chim*. 2007;58(9):910.
106. Ang CY, Cui Y, Chang HC, et al. Determination of St. John's wort components in dietary supplements and functional foods by liquid chromatography. *J AOAC Int*. 2002;85(6):1360–1369.
107. Brolis M, Gabetta B, Fuzzati N, Pace R, Panzeri F, Peterlongo F. Identification by high-performance liquid chromatography–diode array detection–mass spectrometry and quantification by high-performance liquid chromatography–UV absorbance detection of active constituents of *Hypericum perforatum*. *J Chromatogr A*. 1998;825(1):9–16.
108. Chandrasekera DH, Welham KJ, Ashton D, Middleton R, Heinrich M. Quantitative analysis of the major constituents of St John's wort with HPLC-ESI-MS. *J Pharm Pharmacol*. 2005;57(12):1645–52.
109. de los Reyes GC, Koda RT. Development of a simple, rapid and reproducible HPLC assay for the simultaneous determination of hypericins and stabilized hyperforin in commercial St. John's wort preparations. *J Pharm Biomed Anal*. 2001;26(5-6):959–965.
110. Ganzera M, Zhao J, Khan I. *Hypericum perforatum*—Chemical profiling and quantitative results of St. John's wort products by an improved high-performance liquid chromatography method. *J Pharm Sci*. 2002;91(3):623–630.
111. Kladar N, Anačkov G, Srđenović B, et al. St. John's wort herbal teas—Biological potential and chemometric approach to quality control. *Plant Foods Hum Nutr*. 2020;75:390–395.
112. Poutaraud A, Lobstein A, Girardin P, Weniger B. Improved procedure for the quality control of *Hypericum perforatum* L. *Phytochem Anal*. 2001;12(6):355–362.
113. Tolonen A, Hohtola A, Jalonen J. Fast high-performance liquid chromatographic analysis of naphthodianthrones and phloroglucinols from *Hypericum perforatum* extracts. *Phytochem Anal*. 2003;14(5):306–309.
114. Çırak C, Bertoli A, Pistelli L, Seyis F. Essential oil composition and variability of *Hypericum perforatum* from wild populations of northern Turkey. *Pharm Biol*. 2010;48(8):906–914.
115. Maggi F, Cecchini C, Cresci A, et al. Chemical composition and antimicrobial activity of the essential oils from several *Hypericum* taxa (Guttiferae) growing in central Italy (Appennino Umbro Marchigiano). *Chem Biodivers*. 2010;7(2):447–466.
116. Bilia AR, Bergonzi MC, Mazzi G, Vincieri FF. Analysis of plant complex matrices by use of nuclear magnetic resonance spectroscopy: St. John's wort extract. *J Agric Food Chem*. 2001;49(5):2115–2124.