Determining hyperforin and hypericin content in eight brands of St. John's wort

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Hypericum perforatum, also known as St. John's wort, is commonly used to treat mild to moderate depression. In clinical studies, St. John's wort extract was found to be superior to placebo and appeared to be as effective as commonly prescribed antidepressants while producing fewer adverse effects.[1] With its growing popularity, there is concern about the quality assurance procedures used when manufacturing this herbal product. For example, Liu and coworkers[2] recently showed that there were significant variations in the amounts of major components among five different brands of St. John's wort. Generally, most pharmaceutical manufacturers standardize their products on the basis of pseudohypericin and hypericin content (Figure 1).[3] However, relying on hypericin and pseudohypericin content as the only marker to establish the potency of St. John's wort has raised strong skepticism, primarily because studies have not correlated the effects of the extract with the pure or synthetic form of hypericin. Likewise, hypericins isolated from the extract have been shown to be virtually inactive as psychotropic agents in animal models.[4,5]

Recent literature indicated that hyperforin is not only an active antidepressant in biochemical and behavioral models but is also easily bioavailable and therapeutically active in humans (Figure 1).[6-8] Moreover, in vitro data suggest that hyperforin is the ingredient responsible for most of St. John's wort's interactions with drugs that involve induction of drug metabolism. Despite these pharmacologic and clinical findings, hyperforin is not extensively used for the routine standardization of St. John's wort products, mainly because of its tendency to rapidly degrade under ambient conditions, especially in the presence of light and oxygen.[9,10]

We recently developed and validated a simple, rapid, reliable, and reproducible high-performance liquid chromatography (HPLC) assay for the simultaneous determination of hypericins and stabilized hyperforin in extract solutions of St. John's wort.[11] We report the results of our study that used this method to determine hypericins and hyperforin content in eight commercial brands of St. John's wort.

Methods. Eight commercial St. John's wort preparations (one fine powder, four caplets, and three capsules) were randomly sampled from various commercial sources and assayed for hyperforin and hypericins using an HPLC method described previously.[11] This method was specifically developed to ensure that the stability of hyperforin is maintained during the extraction and assay. The extraction medium was carefully selected to ensure that the extraction of other major components was not compromised.

The following chemicals were obtained: stock standards of hyperforin,[a] >90% HPLC purity; pseudohypericin,[b] 85.62% HPLC purity; hypericin,[c] >85% HPLC purity; American Chemical Society (ACS)-

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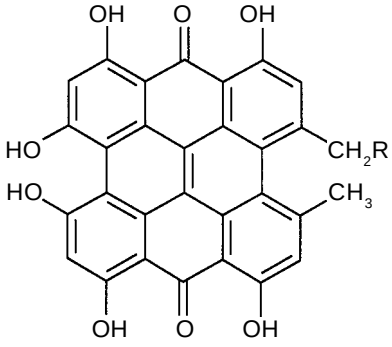
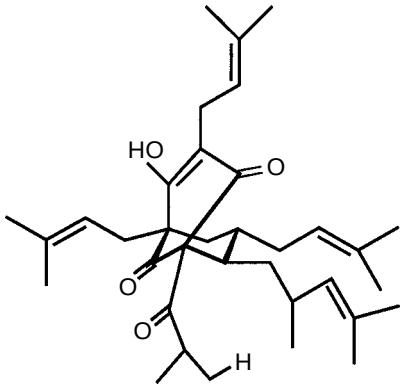
Figure 1. The chemical structure of hypericin ($C_{30}H_{16}O_8$), pseudohypericin ($C_{30}H_{16}O_9$), and hyperforin ($C_{35}H_{52}O_4$). R = H in hypericin model and OH in pseudohypericin model.



Hypericin and pseudohypericin



Hyperforin

grade anhydrous methyl alcohol[d]; ACS-grade acetonitrile[d]; and ACS-grade *o*-phosphoric acid, 85%.[e] The HPLC system consisted of a pump,[f] an autoinjector,[g] an ultraviolet-light spectrophotometric detector[h] set at 273 nm, a fluorescence spectrometer,[i] and a laboratory computing integrator.[j] During the HPLC assay, the samples were maintained at 0 °C by using a thermostat.[k] Hyperforin, hypericin, and pseudohypericin were separated on a C_{18} reverse-phase column.[l] The mobile phase consisted of acetonitrile and 0.3% phosphoric acid (90:10). The medium was adjusted to pH 2.5 using 1 *N* phosphoric acid. The solution was degassed by suction–filtration through a nylon membrane.[m] The extraction solvent consisted of 80% methanol in water, with 5% hydroxypropyl-β-cyclodextrin, adjusted to pH 2.5 with 85% *o*-phosphoric acid.

Standard solutions containing hyperforin (10, 25, and 50 µg/mL), hypericin (0.5, 1.0, and 2.5 µg/mL), and pseudohypericin (0.35, 0.7, and 1.6 µg/mL) were prepared by serially diluting stock solutions with the extraction medium.

The contents of 10 capsules or 10 caplets of each product were weighed and triturated in a glass mortar and pestle. An accurately weighed amount of the fine powder, approximately equivalent to 300 mg of the herb or extract, was transferred into a 100-mL volumetric flask containing about 70 mL of the extraction solvent. The mixture was sonicated for 15 minutes and filtered through cellulose paper into another volumetric flask. The extraction flask was rinsed with the solvent, and the washings were passed through the same filter paper to bring the filtrate up to volume. A 1:4 dilution of the preparation was used in the HPLC assay.

A 50-µL sample of the standard or test solution was injected into the HPLC column. The mobile phase was eluted isocratically at a flow rate of 1.5 mL/min at room temperature. The effluent was monitored using ultraviolet-light detection at 273 nm and fluorescent-light detection at 315 and 590 nm (excitation and emission, respectively). The retention times were about 3.0, 6.5, and 9.0 minutes for pseudohypericin, hyperforin, and hypericin, respectively. Quantification of the chromatogram was performed from standard curves constructed by plotting the peak height of each analyte versus concentration.

Results and discussion. Table 1 lists the brand names, product descriptions, manufacturers, lot numbers, expiration dates, labeled hypericin and pseudohypericin content, and the assayed hypericin, pseudohypericin, and hyperforin content for the various St. John's wort products tested. The assayed potencies of each product were calculated by using this general formula: % marker ingredient = 100 $(C \times V \times D)/W$ in which C is the concentration of the marker ingredient in the sample preparation obtained from the standard curve (in milligrams per milliliter), V is the final volume of the sample preparation (100 mL), D is the dilution factor (4′), and W is the sample weight (in milligrams).

Hyperforin is now regarded as the primary active constituent of St. John's wort and is mainly responsible for the herb's drug interactions that are associated with the induction of drug metabolism. We found that the concentration of hyperforin varied among brands, ranging from 0.01% to 1.89%.

The formulation of St. John's wort that has been clinically proven to treat depression contains 1–6% hyperforin[12]; however, two (Hyperifin and Brite-Life) of the eight products we tested contained more than 1% hyperforin.

Except for the product distributed by Vitamin Classics, all products tested claimed to contain 0.15%, 0.2%, or 0.3% hypericin and pseudohypericin. The assays we conducted revealed that the actual hypericin content in the products ranged from 0.03% to 0.29%. This concentration range corresponded to 56.6–130.0% (mean ± S.D., 93% ± 23%) of the label claim.

Hyperifin, a pure extract powder, had the lowest variance of hypericins from the label claim and the highest hyperforin content. In addition, our assay results for YourLife and Brite-Life closely agreed with the claimed potencies for hypericins (95% and 110% of label claim, respectively). On the other hand, Nature's Balance, the only product in this study with no label claim for hypericin content, exhibited the smallest amount of hypericins and hyperforin. This product had hypericin and pseudohypericin content below the minimum required by the *United*

Table 1.
Claimed Versus Assayed Potencies of Eight St. John's Wort Products

Brand Name (Product Description)	Manufacturer or Distributor (Lot No.)	Expiration Date	Claimed Potency of Hypericins (%)	Mean ± S.D.		
				Assayed Potency[a] of Hypericins (%)	% of Label Claim	Assayed Potency[a] of Hyperforin (%)
Hyperifin (pure extract powder)	Finzelberg, Birkenweg, Germany (834322AA)	. . .[b]	0.30	0.29 ± 0.01	98.3 ± 1.6	1.89 ± 0.120
PNC (300 mg herb per capsule)	Pharmacists' Nutrition Center, Wilsonville, OR (P4311)	Aug 2001	0.15	0.12 ± 0.02	80.9 ± 10.1	0.20 ± 0.003
Brite-Life (150 mg extract per capsule)	Bergen Brunswig, Orange, CA (KA11503)	Nov 2001	0.20	0.22 ± 0.03	110.0 ± 15.4	1.16 ± 0.015
ShopKo (150 mg extract per capsule)	ShopKo Stores, Green Bay, WI (8E02223)	Apr 2000	0.20	0.26 ± 0.07	130.0 ± 35.0	0.05 ± 0.005
Shurfine (300 mg extract per caplet)	Shurfine International, Northlake, IL (8H00627)	Sep 2000	0.30	0.17 ± 0.04	56.6 ± 12.3	0.29 ± 0.006
YourLife (300 mg extract per caplet)	Leiner Health Products, Carson, CA (8G01233)	Aug 2000	0.30	0.28 ± 0.03	95.0 ± 9.0	0.19 ± 0.003
Nature's Balance (100 mg herb per caplet)	Vitamin Classics, Calabasas, CA (Y947)	Dec 2002	. . .[b]	0.03 ± 0.02	. . .[b]	0.01 ± 0
Natrol (300 mg extract per caplet)	Natrol, Chatsworth, CA (936622)	Jan 2002	0.30	0.25 ± 0.02	82.4 ± 5.1	0.48 ± 0.018

[a]Minimum of 3 high-performance liquid chromatography injections. All samples were analyzed in May 2000.
[b]Not specified on label.

States Pharmacopeia (no less than 0.04% hypericins for the powdered herb).[13] Moreover, at the time of the assay (more than two years before the labeled expiration date), a consumer would receive only 15% of the amount of hypericins recommended in a single dose to produce a therapeutic effect.

Conclusion. Assays for hyperforin, hypericin, and pseudohypericin content should be included in the standardization protocol for St. John's wort products.

[a]Addipharma, Hamburg, Germany, batch RHF 00698.
[b]Addipharma, batch RPH 00297.
[c]Sigma-Aldrich, St. Louis, MO, lot 099H1231.
[d]Mallinckrodt, Paris, KY.
[e]Fisher Scientific, Springfield, NJ.
[f]LC-410 pump, PerkinElmer, Shelton, CT.
[g]ISS-100 autoinjector, PerkinElmer.
[h]LC 90 UV spectrophotometric detector, PerkinElmer.
[i]LS-4 fluorescence spectrometer, PerkinElmer.
[j]LCI-100 laboratory computing integrator, PerkinElmer.
[k]MGW Lauda RM 6 thermostat, Brinkman Instruments, Los Angeles, CA.
[l]Discovery C_{18} reverse-phase column, 150 × 4.6 mm inner diameter, 5-µm particle size, Supelco, Bellefonte, PA.
[m]Nylon 100 membrane, Alltech, Deerfield, IL.

References

1. Linde K, Ramirez G, Mulrow CD et al. St John's wort for depression–an overview and meta-analysis of randomised clinical trials. *BMJ*. 1996; 313:253-8.
2. Liu F, Ang C, Heinze T et al. Evaluation of major active components in St. John's Wort dietary supplements by high-performance liquid chromatography with photodiode array detection and electrospray mass spectrometric confirmation. *J Chromatogr A*. 2000; 888:85-92.
3. Upton R, ed. St. John's wort: quality control, analytical and therapeutic monograph. Santa Cruz, CA: American Herbal Pharmacopeia; 1997:14.
4. Butterweck V, Wall A, Lieflander-Wulf U et al. Effects of the total extract and fractions of Hypericum perforatum in animal assays for antidepressant activity. *Pharmacopsychiatry*. 1997; 30(suppl 2): 117-24.
5. Butterweck V, Petereit F, Winterhoff H et al. Solubilized hypericin and pseudohypericin from Hypericum perforatum exert antidepressant activity in the forced swimming test. *Planta Med*. 1998; 64: 291-4.
6. Chatterjee SS, Bhattacharya SK, Wonnemann M et al. Hyperforin as a possible antidepressant component of hypericum extracts. *Life Sci*. 1998; 63:499-510.
7. Laakmann G, Schule C, Baghai T et al. St. John's wort in mild to moderate depression: the relevance of hyperforin for the clinical efficacy. *Pharmacopsychiatry*. 1998; 31(suppl 1):54-9.
8. Bhattacharya SK, Chakrabarti A, Chatterjee SS. Activity profiles of two hyperforin-containing hypericum extracts in behavioral models. *Pharmacopsychiatry*. 1998; 31(suppl 1):22-9.
9. Orth HC, Rentel C, Schmidt PC. Isolation, purity analysis and stability of hyperforin as a standard material for Hypericum perforatum L. *J Pharm Pharmacol*. 1999; 51:193-200.
10. Orth HC, Schmidt PC. Stability and stabilization of hyperforin. *Pharm Industrie*. 2000; 62:60-3.
11. 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; 26:959-65.
12. Chatterjee SS, Noldner M, Koch E et al. Antidepressant activity of hypericum perforatum and hyperforin: the neglected possibility. *Pharmacopsychiatry*. 1998; 31(suppl 1):7-15.
13. St. John's wort monograph. The United States pharmacopeia and the national formulary, 9th suppl. Rockville, MD: The United States Pharmacopeial Convention; 1998:4709-10.