Selected Physical and Chemical Properties of Commercial *Hypericum perforatum* Extracts Relevant for Formulated Product Quality and Performance

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ABSTRACT Objective. The complex composition-activity relationship of botanicals such as St John's Wort (SJW) presents a major challenge to product development, manufacture, and establishment of appropriate quality and performance standards for the formulated products. As part of a larger study aimed at addressing that challenge, the goals of the present study are to (1) determine and compare the phytochemical profiles of 3 commercial SJW extracts; (2) assess the possible impact of humidity, temperature, and light on their stability; and (3) evaluate several physical properties important to the development of solid dosage forms for these extracts. Methods. An adapted analytical method was developed and validated to determine phytochemical profiles and assess their stability. The extract physical properties measured were particle size (Malvern Mastersizer), flow (Carr's compressibility index; minimum orifice diameter), hygroscopicity (method of Callahan et al), and low-pressure compression physics (method of Heda et al). Results. The phytochemical properties differed greatly among the extracts and were extremely sensitive to changes in storage conditions, with marked instability under conditions of elevated humidity. All extracts exhibited moderate to free-flow properties and were very hygroscopic. Compression properties varied among the extracts and differed from a common use excipient, microcrystalline cellulose. Conclusions. Three commercial sources of SJW extracts exhibited different physical and chemical properties. Standardization to 1 or 2 marker compounds does not ensure chemical equivalence nor necessarily equivalent pharmacological activity. Flow and compression properties appear suitable for automatic

capsule-filling machines, but hygroscopicity and the moisture sensitivity of the phytochemical profile are concerns.

KEYWORDS: *Hypericum perforatum*, St John's Wort, Nutraceuticals

INTRODUCTION

Botanical dietary supplements are used extensively for disease treatment and prevention. *Hypericum perforatum* extract, commonly known as St John's Wort (SJW), is used in the treatment of mild to moderate depression, and is typically dosed at 300 mg standardized to 0.3% hypericins, taken 3 times daily. The reader is referred to Barnes et al for a recent, complete review¹.

Despite botanical supplements' growing popularity, there have been several examples of poor quality of these products²⁻⁵. Poor product quality may be due in part to the formidable challenges faced by formulators in developing botanical products. Botanicals may have poor flow, low bulk density, variable particle size distributions, and compression properties significantly different from general use excipients⁶. Also, the doses of botanicals may be very high, and the raw material may be in any form, ranging from crude powdered leaf or root to a finely powdered extract. Foremost among important physical properties are flowability, hygroscopicity, and compression properties. Poor fluidity may result in great difficulty in processing the material, especially on high-speed tableting or encapsulation

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equipment, leading to problems with fill weight variation and content uniformity. Hygroscopicity may contribute to poor fluidity, as well as adversely influence both physical and chemical stability. Compression properties are indicative of the processability of the material, which may be intended for compression into tablets under relatively high compressive forces or for filling into capsules using automatic fillers that form plugs under relatively low compressive forces. More commonly, such products are formulated as capsules. In addition to their challenging physical properties, botanicals are quite complex chemically. The complex phytochemical profile of SJW consists of several groups of phytochemicals including the phenolic acids (chlorogenic acid), flavonoids (rutin, hyperoside, isoquercitrin, quercitrin, quercetin), naphodianthrones (hypericin, pseudohypericin), and the phloroglucinols (hyperforin, adhyperforin). Pharmacologic activity has been attributed to several phytochemicals within SJW. The flavonoids may have some antidepressant activity⁷⁻¹⁰ as well as antioxidant activity¹¹. It has been proposed that the flavonoid antioxidant activity may increase overall extract efficacy by preventing oxidative degradation of other phytochemicals within the SJW matrix. Naphodianthrones are commonly and conveniently used as marker compounds for SJW standardization and, until recently, were thought to be primarily responsible for the antidepressant activity¹². The naphodianthrones have shown antiviral activity against human cytomegalovirus, influenza and other virus strains¹³. The phloroglucinols may be the most interesting group of phytochemicals within the matrix, as there is a great deal of compelling data suggesting anti-depressant activity. Hyperforin has been shown to inhibit the re-uptake of the neurotransmitters serotonin, norepinephrine, and dopamine¹⁴. In addition, there may be a dose-related response, although results are somewhat controversial^{15,16}. Melzer et al¹⁶ suggested that the activity of the SJW extract as a whole is greater than can be attributed to hyperforin alone. Therefore, either there are other active components within the SJW extract, or other constituents within the phytochemical matrix modulate the activity of hyperforin. Clearly, the complete phytochemical profile of commercial extracts is important, and not just one or two marker components that may not even be related to the antidepressant activity.

Unfortunately, the phytochemical profile does not lend itself to simple analysis. The Institute for Nutraceutical Advancement (INA) developed and validated INA method 104, *Constituent Identification of Hypericum perforatum Extracts*¹⁷, which borrows heavily from a method previously published by Brolis et al.¹⁸. The principle of the method is extraction of the SJW extract with ethanol:water (EtOH:H₂O) (1:1, vol/vol) at a concentration of 6 mg/mL, followed by separation using a Vydac 201TP54 C18 column (Vydac, Hesperia, CA) maintained at 30°C, using a complex ternary gradient composed of phosphoric acid: water (H₃PO₄:H₂O) 0.3:99.7 vol/vol buffer, acetonitrile, and methanol, at a flow rate of 1 mL/min, an injection volume of 10 µL, and a run time of 65 minutes. Detection is at λ = 270 nm with quantitation using rutin as an external standard. An adapted form of this method was used recently by Bilia et al, in which the mobile phase gradient is shifted to decrease run time to 30 minutes¹⁹. Limitations of the INA method 104/Brolis method include the absorbance of the buffer system at λ = 270 nm, in the same region the naphodianthrones elute, which may interfere with quantitation of hypericin and pseudohypericin at low SJW concentrations. In addition, the sample preparation technique calls for 6 mg/mL extractions of SJW in EtOH:H₂O (1:1, v/v). This extraction concentration is not feasible for this laboratory's applications; therefore, extraction concentration has been increased to a more useful unit dose concentration of 12 mg/mL SJW in solvent (eg 300 mg SJW/25 mL solvent). Also, EtOH:H₂O (1:1, vol/vol) may not be an optimal solvent system to use for extraction, especially with formulated product. There have been other methods published for the identification and quantification of naphodianthrones only (INA method 107)²⁰ or phloroglucinols only (INA method 112)²¹. These methods specify methanol (MeOH) as the extraction solvent, and also use reverse-phase chromatography, but with an isocratic mobile phase. The methods examining the naphodianthrones have wavelengths of detection of λ = 590 nm, with hypericin as an external standard. The sample preparation technique calls for extraction exposure to a specified wavelength of light for 30 minutes to facilitate complete conversion of protopseudohypericin and protohypericin to

pseudohypericin and hypericin, respectively. This light exposure step allows for good interlaboratory agreement, but may be detrimental to other photolabile phytochemicals within the SJW matrix. Methods examining hyperforin have wavelengths of detection of $\lambda = 270$ nm, with hyperforin as an external standard. Hyperforin reference standard may be difficult to obtain, is extremely expensive, and is often impure, containing adhyperforin. For routine analysis, the use of hyperforin reference standard is not feasible in many laboratories. The routine analysis of the phytochemical profile of neat and formulated extract may therefore be challenging.

In addition to the analytical challenges of these phytochemicals, several researchers have reported the instability of many of these compounds to heat, light, oxygen, and pH^{14, 20, 23-25}. Recently, Bilia et al published research demonstrating the challenge of simply translating International Conference of Harmonization (ICH) guidelines for long-term stability (25°C/60% relative humidity [RH]) and accelerated stability studies (40°C/75%RH) to the storage of two SJW capsule formulations. Overall, the classes of phytochemicals had variable stability under the different conditions¹⁹.

The objectives of this study were to assess the flow properties, hygroscopicity, and low-force compression physics relevant to encapsulation of several commercial SJW extracts; to use the adapted analytical method to evaluate samples for extractive stability and photostability during the extraction procedure of a commercial SJW extract; to use the adapted method to evaluate and compare the phytochemical profiles of several commercial SJW extracts; and to determine the influence of increased humidity, decreased humidity, and temperature on the phytochemical profile of SJW extract.

MATERIALS AND METHODS

Materials

SJW Commercial Samples

Hypericum perforatum powdered extract standardized to 0.3% hypericins was obtained from domestic and international suppliers (sources PWB, DNP, OPC).

Standards

Chlorogenic acid (phytochemical 1) and quercetin dihydrate (6) were purchased from Sigma Aldrich (St. Louis, MO); rutin trihydrate (2), hyperoside (3), isoquercitrin (4), quercitrin (5) and hypericin (8) were purchased from Indofine Chemical Company, Inc (Somerville, NJ); and pseudohypericin (7) and hyperforin (9) were purchased from Addipharma (Hamburg, Germany).

Solvents

Reagent grade phosphoric acid 85% (H₃PO₄) (JT Baker, Phillipsburg, NJ), HPLC-grade acetonitrile (ACN) (Burdick and Jackson, Muskegon, MI), and methanol (EM Science, Gibbstown, NJ) were used. All water (H₂O) was purified using an in-house Milli-Q System (Millipore, Milford, MA).

Methods

Physical Characterization

Flow Studies and Particle Size Analysis

Carr's compressibility index is an indirect measure of flow and may be determined from the tapped and bulk densities of the material (equation 1; all equations may be found in the Equations List at the end of the article)²⁵. The tapped and bulk densities were determined using a Stampf Volumeter (J Engelsman, Ludwigshaffen, a. Rh., distributed by Shandon Southern Instruments, Inc, Sewickley, PA) and the method of the United States Pharmacopeia²⁶. Densities and Carr compressibility indices were determined in triplicate.

Minimum orifice diameter studies were performed using a Flodex Powder Flowability Tester (Hanson Research Corporation, Northridge, CA) and the method of Gioia²⁷. The inverse of the minimum orifice through which a powder freely flows three times out of three is considered the flowability. A small minimum orifice and a corresponding large flowability are obtained for a freely flowing, non-cohesive powder.

As flowability is largely dependent on interparticulate interactions, particle size analysis was performed. The volume mean diameters of the commercial extracts were determined using a Malvern Mastersizer (Malvern Instruments Ltd., Malvern, UK). Each analysis was performed in triplicate.

Relative Hygroscopicity

Hygroscopicity was evaluated by the method of Callahan et al by determination of the equilibrium moisture content (EMC) of each commercial extract after storage at various relative humidities²⁸. Desiccators containing various saturated salt solutions and a 25°C precision temperature incubator were used to prepare chambers with humidities ranging from 9.5 to 88% RH was determined by a Cox Tracer Permanent Memory Internal Temperature and Humidity device (Cox Recorders, Belmont, NC). Drierite (WA Hammond Drierite Company Ltd, Xenia, OH) desiccant and water were used for 0% and 100% humidity chambers, respectively. Three hundred milligrams of the commercial extracts were placed in open containers within each storage chamber. To ensure adequate time for sample equilibration, samples were allowed to equilibrate for 1 month. An additional modification of Callahan's method was the use of Karl Fischer titration versus loss on drying to determine percent moisture, as volatiles may be present in the extracts. The amount of water present in each sample was used to calculate the EMC attained for each chamber, and the samples were classified accordingly (equation 2).

Compression Properties

Low-force compression properties were determined for commercial SJW powdered extracts, as well as for microcrystalline cellulose (Emcocel 90, Penwest, Patterson, NY) as reference material. A compaction simulator (Mand Testing Machines, Ltd, Stourbridge, UK) located at GlaxoSmithKline (King of Prussia, PA) was used. Since encapsulation is a common approach in formulating botanicals, the compaction simulator was equipped with custom-made, round, flat-faced, 5.71mm diameter tooling designed to match size number 1 tamping pins of a piston-tamp capsule machine. Calibration of the force and displacement transducers and other specifics of machine operation have been described previously²⁹. Single-ended saw-tooth displacement time waveforms were used to prepare powder plugs at constant punch speeds of 1 mm/sec and 50 mm/sec to examine the influence of punch speed on the plug formation process. Plugs with heights of 6 mm, 8 mm, and 12 mm were prepared to assess the axial transmission of loads through columns of powder. The die was lubricated prior to each

compression event with a saturated solution of magnesium stearate in acetone, and plug porosity was maintained within each extract source by adjusting fill weights. Plug breaking force was measured with a vertically mounted, motor-driven mechanical slide assembly (Unislide Model B4009P20J, Velmex Inc, Bloomfield, NJ) described previously by Shah³⁰. The true density of each material was determined by helium pycnometry using a 1305 Multivolume Helium Pycnometer (Micromeritics, Norcross, GA). Low-pass Fourier transform filtering was performed on the upper and lower force data to maximize the signal to noise ratio using IGOR Pro software (WaveMetrics, Lake Oswego, OR). For Heckel analysis, the most linear portion was determined from the second derivative.

The data were fit to various compression models. The coefficient of lubrication, *R*, is simply the ratio of the peak applied lower punch force to the peak upper punch force (equation 3). In a perfectly lubricated system, the applied force will be transmitted completely through the powder plug, resulting in *R* equal to unity³¹. The Shaxby-Evans' equation (equation 4) is used to describe the exponential decay in the applied force through a column of powder in a single-ended compression³². The Heckel Pressure -Density relationship (equation 5), which assumes first-order kinetics of densification with applied pressure³³, may be used to determine the mean yield pressure of the material. Kawakita's pressure-volume relationship (equation 6) addresses the relationship between the applied pressure and volume reduction³⁴.

Chemical Characterization

Instrumentation

HPLC analysis was performed on a ThermoQuest Finnigan Mat Spectra system (Thermo Separation Products, San Jose, CA) consisting of a quaternary pump, autosampler, and photodiode array detector. Data was processed with ChromQuest Chromatography software (ThermoQuest, Inc, San Jose, CA). Quantitation was performed using rutin as an external standard for phytochemicals 1-6 and 9, and hypericin was used as an external standard for components 7 and 8. All injections were performed in triplicate unless otherwise stated.

Analytical Conditions- Adapted Method

Column: Vydac 201TP54, C18, 5mm, 4.6 x 250 mm

Column Temperature: 30°C

Flow rate: 1 mL/min

Detector: Scans 250-600 nm, discrete 270 nm (1-6, 9) and 590 nm (7, 8)

Injection volume: 10 μ L

Mobile phase: H₂ O:H₃ PO₄ (99.7:0.3 vol/vol), ACN, MeOH

Ternary gradient: ¹⁹

Run time: 65 minutes

Sample Extraction Preparation

To determine if the extractions could be prepared at a more reasonable concentration, they were prepared at both 6 mg/mL and 12 mg/mL SJW. Either 150 mg or 300 mg SJW was weighed directly into a 25 mL volumetric flask covered with aluminum foil. Twenty milliliters of solvent (MeOH or EtOH:H₂ O [1:1 vol/vol]) were added to the flask, and then sonicated for 30 minutes. The samples were diluted to volume, filtered through a polypropylene syringe-filter (0.45 μ m) and injected.

Extraction Stability

Because the run time is long, and in anticipation of several analyses to be performed routinely, it is possible that the extractions may be prepared and yet not be analyzed for several hours. Therefore, one-day stability of SJW extractions was determined. An extraction of SJW in MeOH (12 mg/mL) was prepared and analyzed. The sample was subsequently protected from light, and stored at 5°C. After 24 hours, the sample was injected again and the contents of each phytochemical of interest were compared.

Photostability During Extraction Procedure

To ascertain the stability of components to light exposure during sample preparation, an extraction of SJW in MeOH (12 mg/mL) was prepared following the aforementioned sample extraction preparation procedure. A portion of the sample was immediately filtered and injected. The foil wrap was removed, and the remainder was exposed to a Phillips Lamp (F20T12D, CRI = 79) at a distance of 10 cm for 30 minutes, filtered, and injected per INA method 107,

and compared with the sample prepared without light exposure.

Extraction from Excipient Matrix

To determine the most appropriate solvent system and demonstrate suitability of the method for a wet granulation type formulation, extraction studies from an excipient matrix were performed. The excipient matrix consisted of 89% lactose monohydrate, NF (JT Baker, Phillipsburg, NJ); 3% povidone, USP (Plasdone K-29/32, ISP Technologies, Wayne, NJ); 2% hydrogenated vegetable oil, type I, NF (Lubritab, Penwest Pharmaceuticals, Patterson, NY); and 6% croscarmellose sodium, NF (AcDiSol, FMC, Newark, DE). The blend was prepared consisting of a 1:3 excipient matrix:SJW extract. This ratio represents a realistic 400 mg dosage form containing the typical dose of 300 mg SJW, leaving 100 mg available for excipients. Samples were prepared using both MeOH and EtOH:H₂ O (1:1, vol/vol) and injections were made of neat SJW extract, SJW plus excipient matrix, and excipient matrix alone.

Identification

To identify peaks in chromatograms, retention times of chromatographic peaks were compared with those of the nine reference standards.

Linearity

Calibration curves were prepared by serial dilution for the external standards rutin (0.0012-0.16 mg/mL) and hypericin (0.068-0.00034 mg/mL), at a minimum of seven levels of concentration. Linearity of the nine phytochemicals within SJW extract was also determined at five levels of concentration ranging from 3.2 mg/mL to 16 mg/mL by comparison with external standard rutin for compounds 1-6 and 9 and with external standard hypericin for components 7 and 8.

Limit of Detection (LOD) and Limit of Quantitation (LOQ) for Rutin and Hypericin

The average baseline noise (BLN) was measured on five chromatograms of reference solutions. The LOD and LOQ were defined as 3xBLN and 10xBLN, respectively.

Reproducibility and Repeatability

As a part of routine system suitability, standard methanolic solutions of rutin and hypericin were

Table 1. Flowability of Commercial Extracts

St John's Wort Source	Mean Bulk Density-Loose (g/mL)	Mean Bulk Density-Tapped (g/mL)	Mean Carr Index (%)	Mean Flowability Index (mm ⁻¹)
OBC	0.457	0.507	9.71	0.0333
PWB	0.573	0.706	18.8	0.0357
DNP	0.578	0.677	14.6	0.0357

injected five times and the relative standard deviations of the integrated areas were calculated.

Five solutions of SJW extract were prepared at different concentrations (3.2-16 mg/mL) and injected in triplicate. The quantity of phytochemicals 1-9 was determined from the calibration curves, and the overall mean relative standard deviation (RSD) was calculated for components 1-9.

Stability of Standard Solutions

Standard solutions of rutin (0.06 mg/mL) and hypericin (0.068 mg/mL) in methanol were stored at 5°C and protected from light. Stability of standard solutions was determined routinely prior to each SJW analysis.

Calculation of % wt/wt

Equation 7 was used to calculate the percentage of each phytochemical within the extract.

Comparison of Phytochemical Profiles of Commercial Extracts

Extractions of the three sources of commercial SJW extracts (OBC, PWB, DNP) were prepared, and phytochemical profiles were determined using the adapted method.

Storage Stability

PWB SJW was initially stored at 24°C/50% RH for 5 months, protected from light. A 70% RH chamber was prepared using a saturated salt solution of CaCl₂ maintained at 25°C in a precision temperature incubator. RH was checked by a Cox Tracer Permanent Memory Internal Temperature and Humidity device (Cox Recorders, Belmont, NC). These conditions are designed to represent possible transport and storage conditions of extracts. As a baseline condition, a 0 % RH chamber was prepared utilizing Drierite desiccant, and maintained at 5°C in a refrigerator (Environmental Growth Chambers, Chagrin Falls, OH). A portion of PWB SJW was placed in each chamber, protected from light and

under a nitrogen purge. Phytochemical profiles were determined routinely in a 3-month stability study.

Statistical Analysis

The data are expressed as mean $\pm$ % RSD. Where appropriate, the 2-tailed, unpaired student's t-test was used to compare the data. The data were considered to be significant when $p < .05$.

RESULTS

Physical Properties

Carr compressibility indices and minimum orifice diameter measurements indicate that the commercial SJW extracts tested have flowabilities suitable to solid dosage form development (**Table 1**). Compressibility indices less than 15% are indicative of free-flowing powders; indices greater than 40% usually correspond to very poor flow²⁵. The order of compressibility indices from highest to lowest is PWB > DNP > OBC. Source PWB exhibited a compressibility index only slightly greater than 15%, and, in general, the SJW samples tested overall appear to be moderate to free-flowing. Their minimum orifice diameters reveal a similar ordering of flowability: OBC $\cong$ DNP > PWB. Particle size analysis supports the trends with average volume mean diameter (μ m) $\pm$ %RSD for each extract assuming the same order: OBC (77.3 $\pm$ 3.7) > DNP (63.1 $\pm$ 4.1) > PWB (30.8 $\pm$ 0.96).

Hygroscopicity

The EMC data (**Figure 1**) reveal the hygroscopic nature of the commercial extracts tested. At 100% RH, all samples deliquesced. According to the hygroscopicity classification described by Callahan et al, the extracts would be ranked as Class IV materials, or very hygroscopic, where moisture content increases at RH as low as 40% to 50% and where more than 30% increases in moisture content occur above 90% RH²⁸.

Figure 1. Hygroscopicity of commercial St John's Wort samples.

Figure 2. Shaxby-Evans' relationship.

Low-Force Compression Properties

Most modern automatic capsule-filling machines employ a compression (or tamping) principle in which plugs of the powder formulation are formed in dosing tubes or dosing discs under low compression forces (often 50 N to 150 N) and then ejected into the

capsule bodies³⁵. Thus, the compression behavior of SJW powdered extracts under low-force compression is relevant to their potential development as capsule products. The modified compaction simulator employed in this research provided a unique opportunity to study such

important compression parameters as force transmission, volume reduction, lubrication requirements and apparent mean yield pressure at punch speeds, compression forces and plugs heights representative of automatic filling machines²⁹.

Force transmission through the column of powder (plug) provides information relevant to lubrication requirements. The data clearly demonstrate the exponential decay of applied force through the powder column, as described by the Shaxby-Evans' relationship (Figure 2). In addition, there was a significant decrease in the coefficient of lubrication,

R , with increased plug heights at both speeds tested (Figure 3). This trend indicates that more of the applied force is being lost to the die wall to overcome friction as the plug length increases. Overall, punch speed did not significantly influence R values. In general, the relatively low values of R suggest that formulations containing these SJW powdered extracts would benefit from the inclusion of typical die wall lubricants, such as magnesium stearate.

The Heckel equation provides information related to compressibility and plug formation. In general, the data fit the Heckel relationship well (Figure 4).

Figure 3. Coefficient of lubrication R .

Figure 4. Heckel equation.

As Heda et al proposed previously, the mean yield pressure in such low-pressure studies is best regarded as an apparent mean yield pressure (*AMYP*), since the forces generated are probably not sufficient to induce substantial permanent deformation, but more likely represent a resistance to densification by particle rearrangement and packing²⁹. There were significant increases in *AMYP* s with increased punch speed at all plug heights and for all materials tested. With increased punch speed, the powders have less time to assume more favorable packing arrangements, and may therefore offer increased resistance to deformation. There was no clear relationship between plug height and *AMYP*. Microcrystalline cellulose consistently exhibited higher apparent mean yield pressures compared with those of the extracts tested.

The Kawakita relationship was used to determine the compressibility constant *a*, as well as the inclination towards volume reduction, *b*³⁴. The compressibility constant, *a*, which in the case of piston compression may be interpreted as powder bed initial porosity³⁴, decreased significantly with increased plug height. This trend, which suggests greater densification and packing down of the powder as die cavity depth increases, may be in part justified by the moderate flowability of the extracts, but also may in part be attributable to experimental technique owing to the difficulty in filling powders into small-diameter die

cavities. Microcrystalline cellulose exhibited a significantly greater compressibility constant compared with those of the extracts tested. The inclination towards volume reduction, *b*, decreased significantly with increased plug height at both speeds tested for all materials tested (**Figure 5**). A decrease in *b* indicates increased resistance to densification and should correlate with an increase in *AMYP*. In the present study, *AMYP* did increase with punch speed, but no clear relationship was found between *AMYP* and plug height.

Plug breaking force data combined with compression data may be used as an indication of compactibility. The SJW extracts tested exhibited dramatically different plug breaking forces when formed under identical conditions. For 12 mm plugs prepared at a punch speed of 12 mm/sec and at the same compression force (198-200N), the mean breaking forces (N) ± %RSD were: PWB (0.44 ± 7.9) = MCC (0.40 ± 0.9) > DNP (0.14 ± 4.4) > OPC (N/A). Source OPC did not form coherent plugs with measurable plug breaking force.

Chemical Properties

Concentrations and Solvents

The sample preparation technique may be suitably scaled up from 6 mg/mL to 12 mg/mL (**Figure 6**). However, sample preparation involving extraction with MeOH versus EtOH:H₂O (1:1, vol/vol) results

Figure 5. Kawakita relationship-inclination towards volume reduction *b*.

in significantly higher yields of several phytochemicals (**Figure 7**).

Extraction Stability

Phytochemical profiles of extractions prepared at either 6 mg/mL or 12 mg/mL in MeOH and stored at 5°C and protected from light remained stable for 24 hours (**Figure 8**). There were no statistically significant decreases in any phytochemical of interest. It may be surmised that samples may be analyzed within 24 hours without losing the integrity

of the profile, as long as they are stored appropriately (5°C, protected from light).

Photostability of Phytochemical Profile

Several phytochemicals demonstrated poor photostability during the extraction procedure, especially hyperforin, which exhibited the most dramatic decrease in percent wt/wt (**Figure 9**). The photo-instability of hyperforin is well documented and was expected. All phytochemicals (except hypericin and pseudohypericin) demonstrated

Figure 6. Sample concentration.

Figure 7. Extraction solvents (*significant difference $p < .05$).

Figure 8. Extraction stability (no significant difference between day 0 and day 1).

Figure 9. Photostability during sample preparation (*significant difference $p < .05$).

decreases in percent wt/wt. Accordingly, during sample preparation the samples must be protected from light.

Extraction from Excipient Matrix

Excipient matrix: SJW (1:3) blends extracted with MeOH had significantly higher yields of hyperforin, hypericin, quercetin and rutin compared to the blend extracted with EtOH:H₂O (1:1, vol/vol). Based on these results, and previous results comparing extraction of neat material with either solvent system, methanol was the extraction solvent of choice.

Identification, Linearity and LOD/LOQ

Retention times (minutes $\pm$ %RSD) for the 9 standards may be found in **Table 2**. Rutin and hypericin calibration curves were linear, with correlation coefficients of 0.999 and 0.997, respectively. All phytochemicals of interest within the SJW extract were linear, with correlation coefficients of at least 0.97. LOD and LOQ for rutin were 0.0207 μ g/mL and 3.42 μ g/mL, and for hypericin, 0.269 μ g/mL and 0.732 μ g/mL, respectively.

Table 2. Retention Times (RT) of Phytochemicals

Compound	Avg. RT (min) $\pm$ %RSD
Chlorogenic Acid	10.4 $\pm$ 0.087
Rutin	15.1 $\pm$ 0.53
Hyperoside	15.4 $\pm$ 0.24
Isoquercitrin	15.7 $\pm$ 0.29
Quercitrin	18.1 $\pm$ 0.19
Quercetin	26.7 $\pm$ 1.88
Pseudohypericin	40.1 $\pm$ 0.071
Hypericin	42.6 $\pm$ 0.025
Hyperforin	45.6 $\pm$ 0.11

Reproducibility and Repeatability

Routine analysis of reproducibility as part of system suitability typically results in RSD lower than 1% for hypericin and rutin. The method had good repeatability, with the mean RSD for all 5 concentrations of components 1-9 less than 1%.

Standard Stability

Methanolic solutions of rutin and hypericin stored at 5°C and protected from light remained stable for 7 weeks and to date (9 weeks), respectively (**Figure 10**).

Figure 10. Standard stability.

A. Rutin standard stability; rutin remained stable about 5 weeks.

B. Hypericin standard stability; hypericin remained stable almost 9 weeks.

Figure 11. Comparison of phytochemical profiles (different commercial sources have vastly different profiles).

Figure 12. Stability (5°C/0% relative humidity). By day 28, significant decrease in hyperforin content. By day 85, contents of all phytochemicals decreased significantly ($p < .05$).

Comparison of Phytochemical Profiles

The three commercial sources of SJW extract had markedly different phytochemical profiles (Figure 11). Source DNP had much lower levels of phytochemicals 1-5, 7, and 9 compared with those of sources OPC and PWB. Sources OPC and DNP had significantly greater levels of the flavonoid, quercetin, than did source PWB.

Stability

The phytochemical profile of dry SJW extract stored at reduced temperature and humidity (5°C/0% RH) and protected from light and oxygen, is maintained up to 8 weeks (Figure 12). By week 4, hyperforin had a significant decrease in content. However, the remaining 8 phytochemicals were seemingly

unaffected. Week 12 data reveals a statistically significant decrease in all phytochemicals of interest.

The degradation was more dramatic when the humidity was increased from 50% to 70% (Figure 13). By week 2, the contents of all phytochemicals analyzed, except chlorogenic acid and quercitrin, had significantly decreased.

DISCUSSION

Physical Properties

The data suggested that all SJW powdered extracts tested would flow well. This observation is in sharp contrast to a previous study in which some *Ginkgo biloba* extracts were found to exhibit exceedingly poor flow properties⁵. Though the SJW extracts may inherently possess properties that favor good flow, it is more likely that the techniques and excipients (eg sugars, silicas) used to prepare the powdered extracts from the SJW crude extract were better optimized to produce free-flowing products.

Although the extracts had good flow properties, they were strongly hygroscopic. Source DNP consistently exhibited the highest EMC, with sources OPC and PWB behaving similarly to each other. This observation could be due to differences in the proprietary extract processing conditions. Whereas source DNP is extracted using a water-only process,

sources OPC and PWB utilize hydroalcoholic extraction procedures, and therefore have slightly less water associated with the extracts. In addition, the hygroscopic tendencies of excipients used in processing of the powdered extracts may well influence the hygroscopic nature of the final extract powder.

Overall, there are ordered differences in their compression properties and compaction properties, with extracts generally behaving significantly differently from the general use excipient, microcrystalline cellulose. The results for 12 mm plugs prepared at 50 mm/sec are as follows: *R* (MCC > DNP OPC > PWB); mean AYP (MCC > DNP $\equiv$ OPC > PWB); Kawakita a (MCC > PWB > DNP $\equiv$ OPC); and Kawakita b (MCC < PWB < DNP $\equiv$ OPC). Plug breaking force data reveal a source of concern for the formulator. PWB exhibited plug breaking forces similar to MCC and is likely to form coherent plugs that allow quantitative transfer to the capsule shell. In contrast, source DNP plugs exhibited a significantly lower breaking force, and source OPC did not form coherent plugs. Poor compactibility may be a concern when a dosator type capsule-filling machine is used, as the compressed plug is unsupported during transfer, and a stable arch must be formed by the fill material over the orifice of the dosator for quantitative plug transfer.

Figure 13. Stability (25°C/70%relative humidity). By day 14, significant decrease in contents except chlorogenic acid and isoquercitrin. By day 28, all phytochemical contents decreased significantly ($p < .05$).

Nonetheless, the data suggest that any of the SJW powdered extracts tested here have suitable compressibility and flow properties for running on plug-forming automatic capsule filling machines. However, adequate lubrication will need to be provided. Disintegrants (eg sodium starch glycolate, croscarmellose) to promote plug disintegration and dispersion and surfactants (eg, sodium lauryl sulfate, Tween 80) to promote wetting and liquid uptake into the plug, as well as dissolution should also be explored to ensure delivery of active components. Poor compactibility may be addressed by the use of direct compression filler-binders (eg MCC, Fast Flo Lactose). In all cases, the excipient compatibility of the SJW extract key components needs to be assessed. The strongly hygroscopic nature of the extracts suggests that traditional gelatin capsules should be used with caution, since hygroscopic fill materials can remove physically bound moisture from shells on storage, thereby causing them to become brittle^{36, 37}. A sorption-desorption moisture transfer model can be useful in selecting initial loss on drying values for empty gelatin shells and the formulation that may ensure that brittleness does not occur³⁶. However, as will be discussed later, exposure to moisture may adversely affect the stability of important phytochemicals in the extract. Shells composed of hydroxypropylmethyl cellulose (Shionogi Qualicaps, Whitsett, NC; Capsugel, Greenwood, SC) may also be considered.

It is interesting to note that although sources DNP and OPC behave differently in terms of their EMC, they are quite similar in terms of their compression properties, compaction properties, flow properties, and particle size analysis. Although extraction of the crude material may have involved different types of solvents or conditions, further processing of the powdered extract may have imparted specific physical properties.

Chemical Properties

Simple adaptations to existing analytical methods allowed adequate separation, identification, and quantitation of the 9 phytochemicals of interest. Photoinstability of the extractive highlights the need for protection from light during sample preparation, as well as the necessity of a standardized analytical method for evaluating the extract. Although the conversion of protopseudohypericin and protohypericin to pseudohypericin and hypericin,

respectively, with a light exposure step after the extraction procedure is desirable for good interlaboratory agreement (INA 107), the rest of the phytochemical matrix may be adversely affected. Accordingly, it may be difficult to compare results from the adapted method across laboratories, as even with the most diligent sample preparation, some light exposure may be possible, resulting primarily in varying levels of pseudohypericin and hypericin. The analyst must be certain to adhere to careful sample preparation technique.

The phytochemical profiles of commercial SJW extracts varied greatly. This difference may be due to the fact that for source DNP, the crude SJW is extracted with a water-only, low-temperature extraction method, whereas sources OPC and PWB are extracted by a hydro-alcoholic method. Also, proprietary processing procedures following crude extraction, such as spray drying with excipients to facilitate handling, may influence the phytochemical profiles of the extracts. The levels of hypericin for all extracts tested are not as variable for each source, presumably because they are standardized to equal quantities of hypericin. Results from this analytical method suggest the total level of hypericins (pseudohypericin and hypericin) was less than the 0.3% standardization claimed by manufacturers, with percent wt/wt of 0.19, 0.17, and 0.27 for sources OPC, DNP, and PWB, respectively. However, because a light conversion step was not used to convert protopseudohypericin and protohypericin to pseudohypericin and hypericin, respectively, a lower total hypericins level is obviously expected and does not imply that the extracts do not meet manufacturers' standardization claims.

Overall, source PWB had the greatest content of most constituents. Of the phytochemicals of interest, the contents of hyperforin(9) and the flavonoids(2-6) differed the most among the extracts tested. While these constituents are not used routinely as markers, they have demonstrated some relationship (direct or indirect) to the antidepressant activity of SJW extract. Although source PWB had significantly higher levels of several phytochemicals, this does not guarantee it is a superior extract. The type of phytochemical profile that results in the most efficacious preparation of SJW is unknown at this time. The variability in the phytochemical profile

may have a profound effect on the overall stability and quality control of the neat and formulated material and, quite possibly, efficacy outcomes in clinical trials. Clearly, standardization to 1 or 2 marker compounds does not imply chemically equivalent products, or necessarily equivalent pharmacologic activity. The data also highlight the great difficulty of storage and handling the extract. Storage of the extract under humid conditions, even at moderate temperatures (25°C/70% RH) and protected from light and oxygen, results in the rapid degradation of several phytochemicals. However, even if precautions are taken to remove excess humidity and decrease the temperature substantially (5°C/0%RH), the levels of the phytochemicals of interest eventually begin to decrease. Orth recommends long-term storage of isolated hyperforin at -70°C, under nitrogen²³. Although the isolated hyperforin does not contain the beneficial antioxidant influence of flavonoids¹¹ within the matrix, resulting in a worst-case scenario, obviously -70°C is not feasible for the storage of large quantities of neat extract. Extract storage and transport conditions should be a primary concern for manufacturers of botanical products.

CONCLUSION

Overall, the commercial SJW extracts tested were moderate to free-flowing and very hygroscopic in nature. Although source DNP had the greatest amount of moisture associated with it, it did not exhibit the worst flow properties, highlighting that many factors contribute to powder flowability. Low-force compression and compaction data reveal that extracts from various sources exhibit rank order differences in their compression and compaction properties. In addition, their compression properties were significantly different from the general use excipient, microcrystalline cellulose. However, the compactibility of one source was quite similar to that microcrystalline cellulose in the compression force range studied.

The adapted analytical method provides adequate separation, identification, and quantitation of 9 phytochemicals of interest for both neat and formulated SJW extract, and it is well suited to a larger, ongoing project where routine analysis of the phytochemical profile is critical. Using this method to compare phytochemical profiles of various commercial extracts revealed significant differences

in the contents of constituents related either directly or indirectly to the antidepressant activity of SJW, although the content of the traditional marker compound (hypericin) was similar. Therefore, standardization to marker components does not necessarily guarantee the same product, or possibly, the same potency. This fact may have a profound effect on raw material quality control, stability testing, and the quality of the formulated product, as well as clinical trial outcomes.

The adapted method was also used to assess the stability of neat SJW extract stored under various conditions. Increasing humidity from 50% to 70% has an adverse effect on the phytochemical profile, with a significant decrease in the contents of almost all compounds by 2 weeks. Storage under conditions of reduced humidity and temperature (5°C/0% RH) resulted overall in much better stability. However, by 12 weeks, all phytochemicals had significantly degraded. These observations raise concern about attaining stability adequate to ensure potency over a reasonable product shelf-life.

In conclusion, a primary concern of manufacturers should be the storage of the neat extract, and care should be taken to avoid not only oxygen and light but humidity as well. In addition, it is recommended that the neat extract be placed in the lowest temperature storage facility available. Finally, chemical extraction of the crude material as well as further processing may significantly influence the physical and chemical characteristics of the powdered commercial extract. Therefore, it is very important for the supplement manufacturer to perform a complete physico-mechanical and chemical characterization when extracts are purchased from multiple suppliers.

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APPENDIX

Equations

Equation 1. Carr's Compressibility Index, C

$$C = \left(\frac{\rho_t - \rho_o}{\rho_t} \right) \times 100$$

ρ_t = tapped bulk density; ρ_o = loose bulk density

Equation 2. Equilibrium Moisture Content (EMC)

$$EMC = \left(\frac{P}{P+100} \right) \times 100$$

P = % moisture (dry basis)

Equation 3. Coefficient of Lubrication, R

$$R = \frac{F_l}{F_u}$$

F_l = Peak lower punch force; F_u = Peak upper punch force

Equation 4. Shaxby-Evan's Relationship

$$\frac{F_l}{F_u} = \exp\left(-\frac{4HK}{D}\right)$$

H = plug height; K = material specific constant; D = plug diameter

Equation 5. Heckel Equation

$$\ln\left[\frac{1}{1-D}\right] = KP + A$$

$1-D$ = porosity pressure, P ; K = slope of linear region; K^{-1} = yield pressure; A = Heckel number

Equation 6. Kawakita Equation

$$\frac{P}{C} = \frac{P}{a} + \frac{1}{ab}$$

$$C = \frac{(V_o - V)}{V_o}$$

P = applied pressure; V = volume at pressure, P ; V_o = initial volume, a = constant (compressibility), b = constant (inclination towards volume reduction)

Equation 7.

$$\%w/w = \frac{Cx FV \times 100}{W}$$

C = concentration (mg/mL) of each phytochemical determined from either rutin (1-6, 9) or Hypericin (7, 8) calibration curves FV = final volume of the sample W = weight of SJW (mg) used in sample preparation

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