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assessment of their authenticity and quality**

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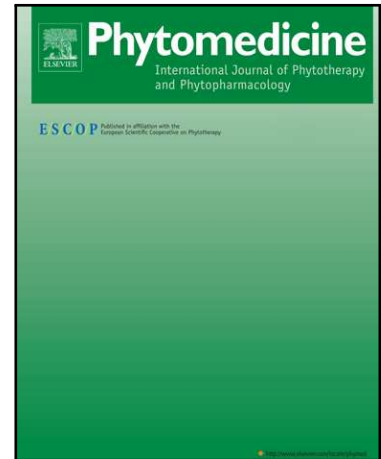
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St John's wort (*Hypericum perforatum*) products – an assessment of their authenticity and quality

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ABSTRACT

Background: St John's wort products (*Hypericum perforatum* L.) are widely available for sale in many countries including the UK via the internet. In the UK, these products are required to hold either a marketing authorisation or Traditional herbal registration (THR) to be sold legally. The THR and other regulatory schemes help to ensure product safety and quality providing an example of best practice but there is a risk if both regulated and un-regulated products continue to be available to consumers.

Aims: the project is embedded in a larger study aiming to investigate the quality of different herbal medicinal products along diverse value chains. Here we focus on a comparison of the quality of the finished products and assess phytochemical variation between registered products (THRs) and products obtained from the market without any registration.

Methods: 47 commercial products (granulated powders and extracts) were sourced from different suppliers. We analysed these samples using high performance thin layer chromatography (HPTLC) and ¹H-NMR spectroscopy coupled with multi-variate analysis software following a method previously developed by our group.

Results: the consistency of the products varies significantly. Adulteration of the products (36%), possibly with other *Hypericum* species obtained from China or use of chemically distinct *H. perforatum* cultivars or chemotypes, and adulteration of the products (19%) with food dyes (tartrazine, amaranth, brilliant blue, sunset yellow) were the principle findings of this study.

Conclusions: There is significant compositional variation among commercial finished products and two main causative quality problems were identified as adulteration by incorrect species or adulteration with food dyes. Generally, food supplements and unlicensed products were found to be of poorer quality than the regulated ones like registered THRs.

Keywords

Quality control, value chains, Traditional Herbal Registration (THR), avicularin, St John's wort, *Hypericum perforatum*

Abbreviations

ADC-automatic developing chamber, AV-avicularin, BRM-botanical reference material, DCHA-dicyclohexylamine, DCM-dichloromethane, DER-drug extract ratio, EP-European pharmacopoeia, EtOAc-ethyl acetate, FS-food supplement, HPTLC-high performance thin layer chromatography, MS-mass spectrometry, NMR-nuclear magnetic resonance, NP-natural products, PCA-principal component analysis, PEG-polyethylene glycol, SJW-Saint John's wort, STD-reference standard, THMPD-Traditional Herbal Medicinal Products Directive, THR-Traditional Herbal Registration, TMS-tetramethylsilane, UCL-University College London, USP-United States Pharmacopoeia

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Introduction

Hypericum perforatum L. Hypericaceae (St John's wort) (SJW) products are widely used for the treatment of low mood and mild to moderate depression (Barnes *et al.*, 2001). In many European Union countries including the UK, products are registered as traditional herbal medicines for indications of slightly low mood and mild anxiety. On the other hand, in Germany, for example, these products are licenced medicines, whereas in the USA and some other countries, products containing SJW are classified as dietary (botanical) supplements.

Various licensing schemes including the European Traditional Herbal Registration (THR) and voluntary self-regulation have contributed towards the establishment of better quality products in some markets. However, SJW is a prime example of a group of products where regulated and unregulated products coexist in the same market place. Therefore, it is essential to better understand how regulation impacts on the quality of products and consequently on the safety of consumers.

Previous work by our group has shown that herbal food supplements used with a medical or preventive claim are often of poor quality or adulterated. This is may be due to poor control of production processes but in some cases there appears to have been deliberate adulteration, including the addition of therapeutically irrelevant marker substances like food dyes in an attempt to make these products pass routine analytical procedures, especially ones defined in a pharmacopoeia or in routine analytical procedures used as industrial standards (Booker *et al.* 2016a, Booker *et al.* 2016b).

Although it is of course important to identify adulterated products through routine analysis (Agapouda *et al.* 2016), quality control in isolation cannot provide a lasting solution. A greater emphasis needs to be directed towards understanding what constitutes best practice along the value chains and what measures can be introduced to minimise problems along these chains (Heinrich, 2015).

SJW is a plant which requires extra caution when prescribed or consumed due to its well defined pharmacological actions, as well as the interaction potential with other medications, making it even more essential that commercial SJW products are of adequate quality (Schmidt and Butterweck 2015). This study thus forms the first part of a project investigating the quality of SJW and SJW products along the value chains from the primary production to final products.

Aims and objectives

To investigate the quality of regulated SJW products compared to food supplements (botanicals) and of un-registered herbal medicines available from the internet.

- To investigate the compositional diversity among finished SJW products available chiefly on the UK market.
- To test the consistency of commercial products with their label claims.
- To identify adulteration and examine the possible causes.

Materials and methods

Sample Collection

47 finished SJW products were purchased from the internet, pharmacies and stores, mainly in Central London, but also Germany and the United States. . The products were formulated as tablets (22), capsules (24), and powder (1), and they claimed to contain either plant extract (24) or ground aerial parts of the plant (17), while six claimed to contain a combination of plant extract and crude ground material. Nine products were THR products, registered in the UK under the European Directive 2004/24/EC for Traditional Herbal Medicinal Products (THMPD) and obtained mainly from pharmacies, and the rest of the products were marketed as food/herbal/botanical/dietary supplements and were mainly obtained from the internet. The THR extracts claimed Ethanol 60 – 68% as the extraction solvent, information regarding the extraction of the food supplements was lacking.

Most of the products claimed to contain solely SJW, while three of the samples (13, 26, 30) claimed to contain additional medicinal plants, including *Griffonia* seed extract, *Rosmarinus officinalis*, spirulina, *Melissa officinalis*, and *Avena sativa*. SJW raw material was also obtained for comparison. A Table of the sample details can be found in the supplementary material (S1).

Reference standards

Sample 1, a registered and quantified product, containing plant extract, was used for the method development of NMR and sample 2, also a registered and quantified product of the same manufacturer (but higher dose), was used to generate a reference fingerprint for HPTLC. SJW dry extract (European Pharmacopoeia, EP, Reference Standard 01131, code: Y0001050, batch: 2.0) was obtained from Sigma-Aldrich, hyperforin (For NMR PCA). DCHA was obtained from Apidogen AG, (LOT A00082/B), rutin (L10815B002) and hypericin (L12069B001) from Adooq Bioscience. Although commonly regarded as an important quality marker for SJW products, hyperforin was not assessed in this study as few of the products investigated made any claims regarding their hyperforin content. Powdered spirulina (LOT SAP1), powdered rosemary (LOT SRO1) and rosmarinic acid (LOT FOM076) were obtained from USP, the USP SJW extract standard (United States Pharmacopoeia, USP, Lot F0G245), and the food dyes tartrazine (CAS No.1934-21-0, E102), brilliant blue (CAS No.3844-45-9, E102), amaranth (CAS No.915-67-3, E123), sunset yellow (CAS No.2783-94-0, E110) were provided by Capsugel.

¹H-NMR spectroscopy

500 MHz NMR Bruker Avance spectrometer equipped with a QNP multi-nuclear probe head with z-gradient/5mm cryoprobe head and operating at a proton frequency of 500.13 MHz was used for spectra acquisition. The acquisition parameters were: size of the spectra 64k data points, line broadening factor = 0.16 Hz, pulse width = 30° and the relaxation delay d1 = 1 s, 256 scans. The acquisition temperature was 298 K. Topspin software version 3.2 was used for spectra acquisition and processing.

Sample Preparation for NMR analysis

Preliminary experiments revealed that 50 mg of SJW finished product, dissolved in 1 ml of deuterated methanol, was the optimum weight in order to produce the clearest spectra. Sample 1, used for the optimisation of the method contained SJW extract. This quantity was found to correspond to 35 mg of extract. Subsequently, all samples (capsules/tablets/extracts) were prepared to contain 35 mg of extract per ml of solvent.

Each commercial product was of different strength. Some (24) contained SJW extract, others (17) contained SJW ground raw material and some (6) products contained a combination. For the products that did not contain plant extract, 50 mg of powder of the ground tablets/capsules was used for the analysis. All samples were stored in sealed, light proof containers in a freezer.

The solutions were shaken on a rotary mixer, sonicated for 5 min at room temperature, centrifuged at 13000 rpm for 5 min and 600 μ l of the supernatant was transferred to NMR tubes. The samples were subjected to NMR immediately to avoid degradation of sensitive compounds (e.g. hyperforin).

Principal Component Analysis of Data

The tetramethylsilane (TMS) peak was calibrated to zero and the ^1H -NMR spectra were zeroed to TMS. Autophase correction and automatic baseline correction were applied to the spectra. AMIX Bruker Biospin multivariate analysis software version 3.9.14 was used for converting spectra to an ASCII file. Only positive intensities were used. A number of integrated regions (buckets) of the data set were created through AMIX and the size of the buckets was 0.04 ppm with no scaling. The samples (including commercial products, crude batches and a SJW standards) were numbered from 1 to 47 (Standards labelled 55 and 56) and the data set was introduced to Microsoft Excel. Sample 34 was selected to be prepared and analysed twice as a control for the Principle Component Analysis (PCA). PCA was conducted using SIMCA software version 14.0. After running some statistical models in PCA

to investigate which of those brought the two samples labelled 34 together, it was concluded that the no scaling in AMIX and no scaling in SIMCA is the statistical model that produces the best clustering of samples and brings the two samples closest together.

HPTLC

CAMAG HPTLC equipment was used, consisting of the visualizer, automatic TLC sampler 4 (ATS4), automatic developing chamber 2 (ADC2), chromatogram immersion device III for derivatization, TLC plate heater III and TLC scanner coupled to visionCATS version 2.1 software. Centrifuge EBA21 (Hettich, Tuttlingen, Germany), ultrasonic bath SW 3H (Sonoswiss) and analytical balance MS 205 DU (Mettler-Toledo Beaumont Leys, Leicester) were used during the sample preparation.

Reference Standards

The standards hyperoside, rutin, hypericin, and rosmarinic acid were prepared at the concentration of 1 mg/ml in methanol and were sonicated for 10 min at 60 °C. The USP and mixture standards were prepared at a concentration of 50 mg/ml in methanol. Rosemary and spirulina were prepared at a concentration of 100 mg/ml in methanol.

Sample Preparation for HPTLC analysis

The HPTLC association method for extraction and analysis of SJW powdered drug was used (HPTLC 2016). All samples, regardless of their content either in extract or in raw material, were converted to raw material content. An equivalent of 500 mg of raw material from each sample was weighed and mixed with 5 ml of methanol. The mixture was sonicated for 10 minutes at 60 °C, centrifuged and the supernatant was used for the analysis. For the samples which contained extracts, the average drug extract ratio (DER) declared on their labels was used for the calculation.

The THR products consisting of plant extract (except for sample 34) and included information about the raw material of SJW used for each tablet/capsule and DER (3,5-6:1). For the non-THR products that contained extracts and did not include information about the

amount of raw material used for each tablet/capsule, the extract content was converted into crude material, based on an average drug extract ratio of 4.75:1. For the products (capsules/tablets) which contained only crude material, the weight of raw material without excipients was used for the calculation. Five samples contained both extract and crude material. For those the extract content was converted to crude material, and then added to the crude material content.

HPTLC Analysis

All HPTLC parameters were adjusted according to USP general chapter 203 (USP 39-NF34 (2016) <203> High-Performance Thin-Layer Chromatography Procedure for Identification of Articles of Botanical Origin, U.S. Pharmacopoeial Convention, Rockville, MD).

Table 1 HPTLC methods used for the analysis of, flavonoids, hypericins and dyes

	Analysis of flavonoids and hypericins	Analysis of dyes
Stationary phase	20x10cm Si 60 F254 plates (Merck)	20x10cm Si 60 RP-18 W plates (Merck)
Application (CAMAG ATS 4)	2 μ l	4 μ l
Mobile phase (freshly prepared)	EtOAc:DCM:H ₂ O:HCOOH:CH ₃ COOH (100:25:11:10:10 v/v/v/v/v)	methanol: 5% aqueous sodium sulfate (3:4 v/v)
Development (CAMAG ADC 2)	20 min saturation, 10 min MgCl _{2sat} conditioning at 33% relative humidity, 70 mm distance from lower edge, room temperature = 23-29 °C	
Documentation prior to derivatisation (coupled to visionCATS 2.1)	white light, UV 254 nm, UV 366 nm	
Derivatisation (CAMAG chromatogram immersion device)	Plate heating at 100 °C for 3min, derivatisation in NP and then PEG, speed 3 s, dwell time 0s	N/A

Documentation after derivatisation (coupled to visionCATS 2.1)	white light, UV 366 nm	N/A
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The derivatization solvents were prepared as described below:

Natural products (NP) reagent: 1 g of 2-aminoethyl diphenylborinate was dissolved in 200 ml of ethyl acetate

Polyethylene glycol (PEG) reagent: 10 g of polyethylene glycol 400 (macrogol) were dissolved in 200 ml of dichloromethane

The plates were visualised under white light and under UV 254 nm before application of samples in order to correct the background of the plate in later steps.

UV Assay for measuring the total hypericin content

A UV assay was conducted as described in the EP (PhEur 2014) one time with 800 mg of sample 36 (which is a SJW powder containing sample) and a second time with sample 36 (793 mg) to which a mixture of the dyes tartrazine (0.3 mg), amaranth (1.7 mg), sunset yellow (3.0 mg) and brilliant blue (1.6 mg) had been added. Quantities were taken from Frommenwiler *et al.* (2016). Each sample was extracted with 60 ml of water:tetrahydrofuran 20:80 v/v in a round-bottom flask. A magnetic stirrer was added and the mixture was refluxed at 70 °C for 30 min. After centrifugation (1000 rpm for 2 min) the supernatant was separated and the residue was transferred back into the round-bottom flask. Extraction was repeated with another 60 ml of water:tetrahydrofuran 20:80 (v/v). After centrifugation (1000 rpm for 2 min) the supernatant was separated and combined with that from the first extraction. After evaporation to dryness at 40 °C, using a rotary evaporator (for 40 min) followed by freeze drying at -20 °C (for 12 h) the residue was dissolved in 15 ml of methanol under sonication. The solution was transferred to a 25 ml volumetric flask and made up to volume with methanol. The mixture was then centrifuged

and 10 ml of it were filtered through a syringe filter (0.2 μm). The first 2 ml of filtrate were discarded. The next 5 ml were placed into a 25 ml volumetric flask and made up to volume with methanol. The absorbance of both sample 36 and sample 36 with the dyes solutions was measured at 590 nm and compared with methanol as reference. The percentage of total hypericins was calculated with the formula below:

$$A \times 125m \times 870$$

A = absorbance of solution at 590 nm and m = mass of herbal drug to be examined (0.8 g in both cases).

Results and discussion

HPTLC

In order to investigate the quality of SJW products sold in different channels, 47 samples were prepared and analysed according to the method published by the International Association for the Advancement of High Performance Thin Layer Chromatography (HPTLC Association) for the identification of SJW, which was adopted in USP 38/NF33 in 2015. Samples 1-8 and 34 are THR products while samples 9-33 and 35-47 are licensed products (Germany) and food supplements. Among the latter, samples 36-38 are products from the German market, and 39-42 from the American market. The phenolic compound fingerprint after derivatisation with NP and PEG (Fig. 1) of the samples were compared to the USP monograph description/HPTLC association images. Fingerprints visualised under white light prior to derivatisation can be found in the supplementary material (S3). The samples were grouped according to their fingerprint similarities (Fig. 1) and a summary of the groups' characteristics is given in Table 2. Samples of group 1 (26 samples, tracks 1 – 26) show fingerprint similar to that of SJW, as specified in the USP monograph description and HPTLC association images. Among those, 8 are THR products (tracks 3, 5, 7-11 and 21); samples of group 2 (11 samples, tracks 27 – 37) show a fingerprint similar to these of group 1 but present a yellow zone at R_f 0.49 and no yellow zone below chlorogenic acid; samples of group 3 (10 samples, tracks 38 – 47) show either a faint fingerprint with and without the

additional yellow zone at R_F 0.49 (8 samples, tracks 38-45) or a very faint fingerprint (2 samples, tracks 46 and 47).

According to USP, SJW samples exhibit two yellowish-orange fluorescent bands at R_F corresponding to the rutin and hyperoside; a blue fluorescent band directly below the band due to hyperoside, corresponding to the chlorogenic acid; two red fluorescent bands at R_F corresponding to the pseudohypericin and hypericin; and two to three yellowish-orange fluorescent bands in the middle third section of the chromatogram. Based on this description only two samples, from group 3, which show no zone due to rutin, chlorogenic acid, hyperoside and hypericins (tracks 46 and 47) and another sample from group 3, which shows no yellow zones above hyperoside (track 38), are not compliant with USP's description of SJW.

However, according to the HPTLC association images, no samples of *H. perforatum* herb show this yellow zone R_F 0.49 (as in the samples from group 2) except the sample of *H. undulatum* (supplementary material S4). However, this species shows no zone due to rutin, which is present in samples from group 2. Based on this description, only the samples from group 1 are in compliance with the HPTLC association acceptance criteria. The samples of group 3 show an overall weak fingerprint and their intensities don't comply with the amount of extract/crude drug declared on their labels.

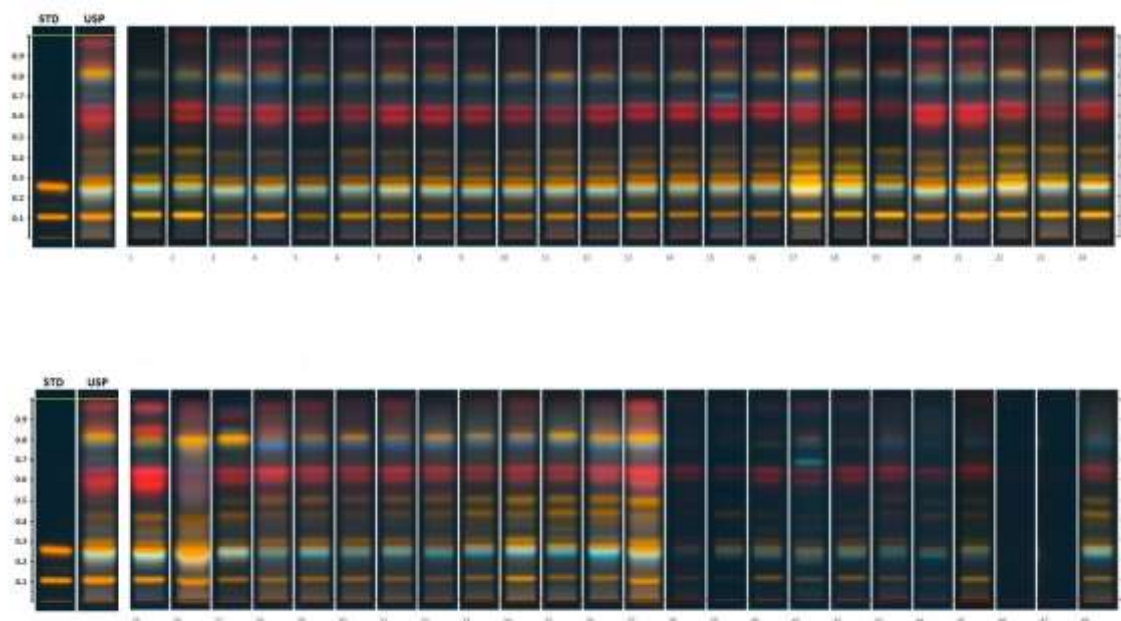


Fig. 1 Fingerprints of 47 St. John's wort samples (commercial products) organised by similarities in the fingerprint under UV 366 nm after derivatisation with NP and PEG. The standards rutin ($R_f = 0.1$) and hyperoside ($R_f = 0.25$) are shown in the STD track, next to USP reference standard, and the rest of the tracks contain samples. Tracks 1-47: samples 44, 47, 1, 33, 2, 10, 5, 6, 3, 4, 7, 9, 20, 25, 26, 27, 36, 43, 45, 32, 34, 38, 39, 37, 29, 12, 8, 16, 18, 19, 21, 22, 40, 41, 42, 28, 17, 24, 14, 15, 30, 35, 23, 31, 46, 11 and 13, respectively. Track 48: sample 13, 10-fold concentrated.

As reported by Frommenwiler *et al.* and Huck-Pezzei *et al.* (2013), the fingerprint of some samples coming from China and labelled as SJW show the same features as samples from group 2, in contrast with the typical European *H. perforatum* samples (as in group 1). In our study, we evaluated 4 crude cultivated samples collected in China by one of the authors in collaboration with Prof. Xiaofei Shang (Lanzhou Institute of Husbandry and Pharmaceutical Sciences, Chinese Academy of Agricultural Science, Lanzhou) and two BRM's acquired in China. All samples acquired in China (Fig. 2) show the same features as those samples from group 2, supporting the theory that samples of SJW maybe a different chemotype of the same species or another species of *Hypericum*.

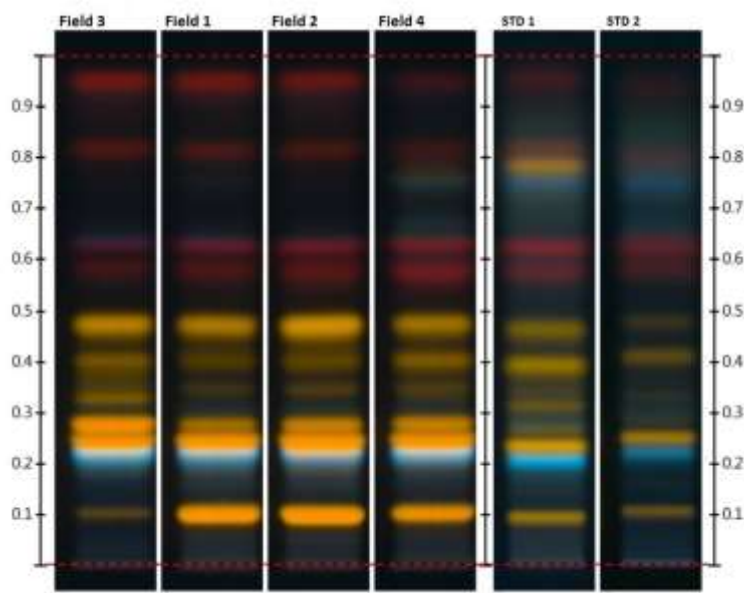


Fig. 2 Fingerprint of six *H. perforatum* herb samples coming from China. Tracks 1-4: cultivated samples collected in the fields in China. Tracks 5-6, BRM samples obtained in China; Image under UV 366 nm after derivatisation with NP and PEG.

The identity of the yellow zone at R_F 0.49 was investigated by comparing the R_F s and colours after derivatisation of flavonoids standards with the Chinese BRM sample on tracks 5 and 6 (Fig. 2). After finding that this zone corresponds in R_F and colour to the compound avicularin, a flavonol isolated from several plant species, including *H. perforatum* (Zhou et al. 2004, Wu et al. 2002, Wei et al. 2009), an HPTLC-MS experiment was performed to confirm its identity. The MS spectra of the zone corresponding to the standard solution avicularin and the same zone in the sample solution show a similar pattern (the major ion m/z 433, and the ions m/z 301 and 447; see supplementary material S2). Jürgenliemk and Nahrstedt, 2002, 2003 and Tuseveski et al., 2016 reported that guajaverin, a compound with the same molecular weight and fragmentation pattern as avicularin, was also isolated from *H.*

perforatum. Therefore we assumed that the yellow zone at R_F 0.49 can be either avicularin or guaijaverin.

Frommenwiler *et al.* (2016) also mentioned a correlation between the presence of the yellow zone in the flavonoid fingerprint, identified as avicularin, and the adulteration with the dyes amaranth, tartrazine, sunset yellow and brilliant blue. To investigate the presence of dyes, another test with the atypical samples was conducted using the method described in Table 1.

In Fig. 3, under white light the dyes brilliant blue (E133, blue zone at $R_F = 0.10$), sunset yellow (E110, yellow zone at $R_F = 0.34$), amaranth (E1023, pink zone at $R_F = 0.57$) and tartrazine (E102, faint yellow zone at R_F 0.62) are seen in tracks 5, 6 and 7 with similar intensities, while tracks 1, 2, 3, 4 and 8 present the same dyes except for tartrazine and their intensities are lower than these of tracks 5, 6 and 7. The sample in track 9 shows very faint zones due to brilliant blue, sunset yellow and amaranth, which is better visualised after sample concentration (Track 10).

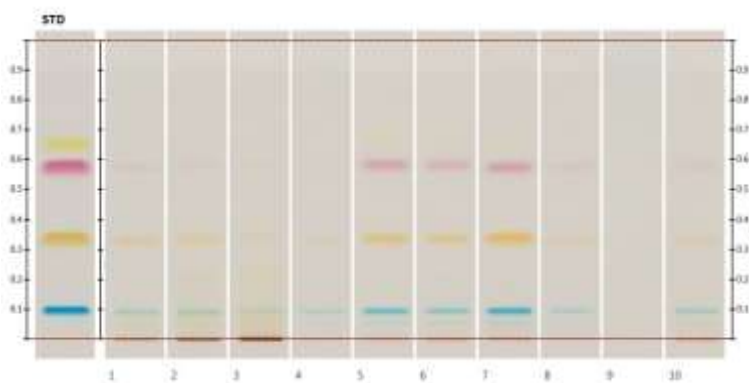


Fig. 3 Fingerprint of the samples 40, 28, 17, 31, 24, 23, 46, 11, 13 and 13C (tracks 1-10, respectively), under white light, after development with the method for detection of dyes. The STD track contains brilliant blue, sunset yellow, amaranth and tartrazine in ascending order.

Table 2 Conclusions for each sample regarding the HPTLC fingerprints results.*FS = food supplement

N° of samples	Group	Type	Track number and description of the classification
26	1 (equivalent to SJW)	THR (8)	3, 5, 9, 10, 7, 8, 11, 21
		FS* (18)	12, 6, 26, 13,, 14-16, 25, 20, 4, 17,24,22,23, 18, 1, 19 and 2
11	2 (presence of avicularin/guaijaverin)	THR (1)	Contain avicularin/guaijaverin in the flavonoids fingerprint: 27
		FS* (7)	Contain avicularin/guaijaverin in the flavonoids fingerprint: 28, 29, 30, 31, 32, 34 and 35
		FS* (3)	Contain dyes and avicularin/guaijaverin in the flavonoids fingerprint: 37, 36 and 33
10	3 (weak fingerprint with or without avicularin/ guaijaverin)	FS* (4)	Very faint flavonoid fingerprint but no avicularin/guaijaverin or dyes: 39, 40, 41 and 42
		FS* (3)	Faint flavonoid fingerprint, presence of avicularin/guaijaverin and dyes: 43, 44 and 45
		FS* (2)	Very faint flavonoid fingerprint and presence of dyes: 46 and 47/48
		FS* (1)	No zone between pseudohypericin and hyperoside in the flavonoid fingerprint but presence of dyes: 38

UV assay for measuring hypericin content

EP (PhEur 2014) describes an assay for the detection of hypericin using its absorbance at UV 590 nm. After analysing sample 36 alone and sample 36 containing the four dyes in proportions detected by Frommenwiler *et al.* (2016), it was found that the absorption of sample 36 alone was 0.236 giving a calculated value of 'total hypericins, expressed as hypericin' of 0.042%. The absorption of sample 36 containing the dyes was found 0.786 meaning that resulting in an alleged value of 'hypericins expressed as hypericin' of 0.141%. Therefore, there is an indication that the dyes may mimic the absorbance of hypericins in SJW samples and amplify their content 3.36 times. This is the first time that a possible explanation is given to why producers may add food dyes in their samples. The 3.36 fold enhancement of hypericin absorbance implies that producers could use one third of the SJW

amount in their products and still pass the requirements of the assay in the relevant Pharmacopoeia.

$^1\text{H-NMR}$ spectroscopy coupled to PCA-Metabolomics investigation

NMR based metabolomics coupled to Principle Component Analysis was applied to the samples in order to investigate the metabolite variation between the samples. Visual observation of the $^1\text{H-NMR}$ spectra produced by the samples indicates what we already observed in HPTLC, that the variation is significant.

The commercial products were numbered as 1-47; sample 34 was selected to be analysed twice in order to check the reliability and accuracy of the PCA multivariate analysis. A chemical shift range of 6-8 ppm was chosen in order to exclude product excipients and baseline noise.

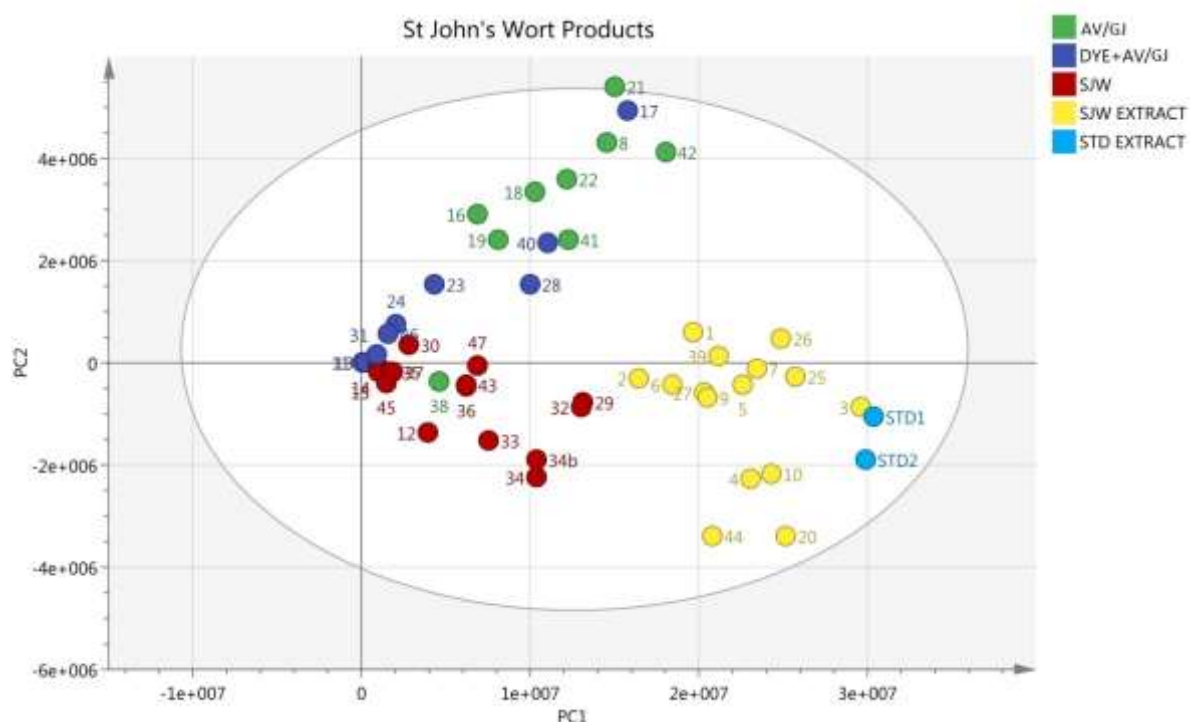


Fig. 4 SIMCA scores plot of all the commercial samples and reference standards. AV/GJ = contains avicularin/guaijaverin, DYE + AV/GJ = contains dye and avicularin/ guaijaverin, SJW = powdered crude material, SJW EXTRACT = extract of SJW, STD EXTRACT = standard reference material

The PCA illustrates a high variance of metabolites between samples but also some discrete clustering (Fig. 4). The green samples have the extra yellow zone (at $R_f = 0.49$ in the HPTLC analysis, Fig. 1) which has been positively identified by mass spectrometry as avicularin/guaijaverin (AV/GJ), the dark blue samples contain avicularin/guaijaverin as well dyes (DYE + AV/GJ). The red samples are commercial products that contain ground crude material (SJW) and the yellow samples contain a SJW extract (SJW EXTRACT). Samples 34 and 34b are samples prepared twice. They appear close together in the scores plot indicating that the method is reproducible and that the correct scaling has been used to process the data. The botanical reference material extracts are shown as light blue spots (STD EXTRACT).

Samples containing dyes and avicularin/guaijaverin (dark blue) cluster together and show an upward trend towards PC2. They are close to the SJW samples containing an extra yellow zone but no dyes (green) and some of the Dye + AV/GJ containing samples that cluster with the AV/GJ containing samples (e.g. samples 17 and 21 Fig. 4).

The THR extract products (1-8,) are distributed close to the samples made of SJW extract. All THR products, except for 34, contain SJW extract. Sample 34 contains crude material and is found closer to other crude material containing samples. The THR product 8 groups with the avicularin containing samples.

The extract containing reference samples STD1 (SJW standard provided by CAMAG) and STD2 (the EP reference material) correctly grouped with the other extract containing samples.

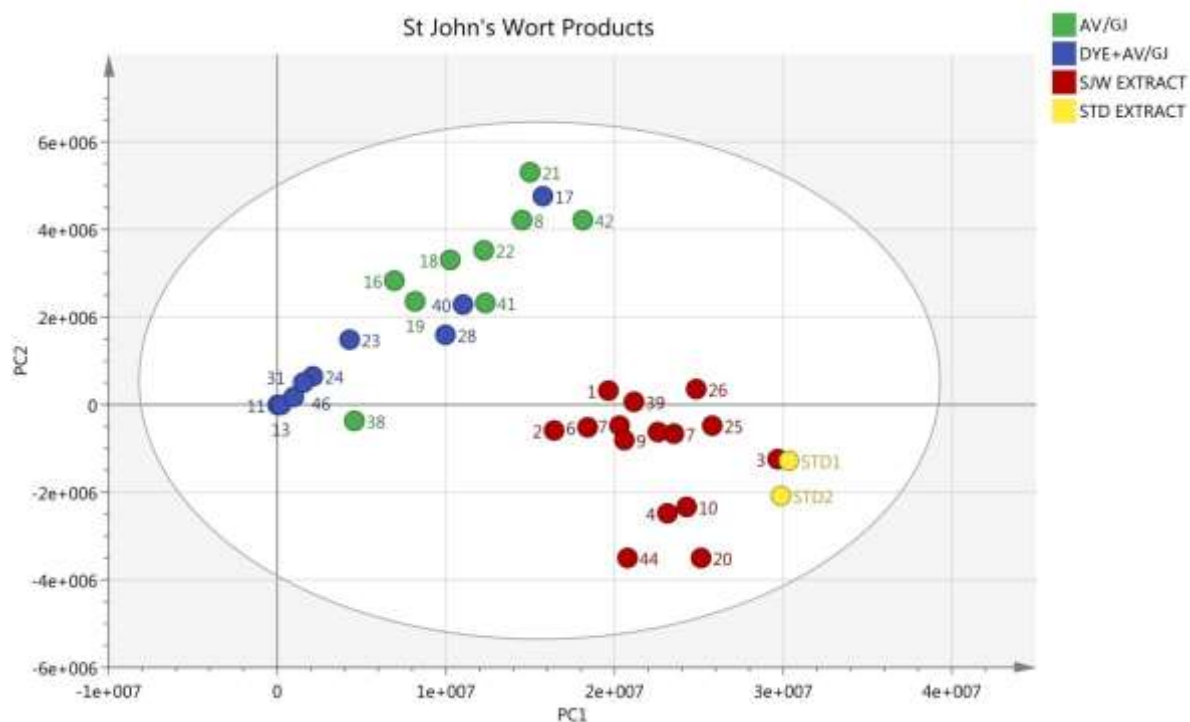


Fig. 5 SIMCA scores plot of the dye and avicularin/guaijaverin containing samples and reference standards. AV/GJ = contains avicularin and/or guaijaverin, DYE + AV/GJ = contains dye and avicularin/guaijaverin, SJW EXTRACT = extract of SJW, STD EXTRACT = standard reference material

The Dye + AV/GJ samples, and other extract containing samples, form two discrete clusters (blue + green samples and red + yellow samples, Fig. 5). The Dye + AV/GJ and AV/GJ samples cluster in one group and are distributed towards PC2. The other extract containing samples (including most of the THRs, except sample 8) cluster in a second group and are distributed towards PC1.

Conclusions

Although there were possible differences in the chemical composition for one of the THR products analysed, all of the regulated products tested were of comparable concentration, free from adulteration by dyes and compliant to their label claims.

There is significant compositional variation among commercial finished products and two main causative quality problems were identified:

- 1) Adulteration of the products (36%), possibly with other *Hypericum* species obtained from China or use of chemically distinct *H. perforatum* cultivars or chemotypes and
- 2) Adulteration of the products with food dyes (19%) (tartrazine, amaranth, brilliant blue, sunset yellow) were the principle findings of this study.

This adulteration with food dyes is a possible means whereby poor quality material is able to pass certain spectroscopic analytical tests and so it is likely to be a driving force for material gain.

The reasons behind the adulteration of the value chain with *Hypericum* species from China which are not acceptable based on the European Pharmacopoeia and the label claims on food supplements or the use of chemically distinct *H. perforatum* chemotypes (or infraspecific taxa) are more complex. It is unclear whether this involves deliberate adulteration or accidental substitution. Some actors within the industry claim that the use of Chinese sourced SJW is a legitimate choice and it should not be deemed adulteration. Certainly further work is required in this area to ascertain an accurate chemical composition and variability of the material sourced from China and its accurate systematic status and taxonomic designation.

Generally, food supplements and unlicensed products were found to be of poorer quality than the registered THRs as 53% of the former did not comply with the reference standards fingerprint, because of an extra yellow zone identified as avicularin/guaijaverin, the addition of food dyes or an overall very low quantity of SJW in the preparations (when compared visually to the reference standards or THR products). However, food supplements are under no obligation to comply with pharmacopoeial requirements, and in countries where SJW is legally classified as a dietary supplement (e.g. USA) these products could not be described as poor quality providing they contain what is stated on the label and are free from adulteration. On the other hand, important suppliers in the food supplement industry have demonstrated their capability of managing supply chains with some success and this is particularly evident with products that are registered as being organic. Organic certification requires companies to have the necessary framework in place to show that they can trace their products back to farms where the raw material is cultivated and that comply with the required standards. Consequently, based on the outcomes of this study one needs to call for

a better self-regulation of the sector, especially for products of such importance as SJW and for a better management of the value chains from producer to consumer..

HPTLC is a robust and reliable technique for the detection of adulteration (dyes) and can detect compositional differences between products; it can also detect adulteration with incorrect species and potential chemotypes within the same species. However, NMR-based metabolomics is the technique of choice for grouping of products according to their overall composition of metabolites, some of which can remain undetected when using a single HPTLC method.

This project has shown that registered products have a good quality profile but poor quality and adulterated SJW products are readily available on the internet, even though any product with a medical claim needs to comply with the regulation of medicinal products, and, therefore, their sale as food supplements is - under European and UK law - illegal.

Furthermore we have shown that adulteration is once again being used to circumvent regulatory testing that would normally safeguard quality. This obviously represents a risk to consumers of these products but also helps to explain how sub-standard starting and intermediate material may enter the value chain.

It is evident that there are examples of good practice and ethical trading in both the areas of THR herbal medicinal products and also within enclaves of food supplement manufacture. Future strategies should be developed to harmonise these two separate but complementary areas of medicinal plant commerce in order to further consolidate the reputation of THR products and improve market confidence surrounding the quality of food supplements sourced by UK consumers.

Conflict of interest

Francesca Scotti's postdoctoral position is funded through a charitable donation by Dr. Willmar Schwabe GmbH & Co. KG, Karlsruhe, Germany, who has had no input into the

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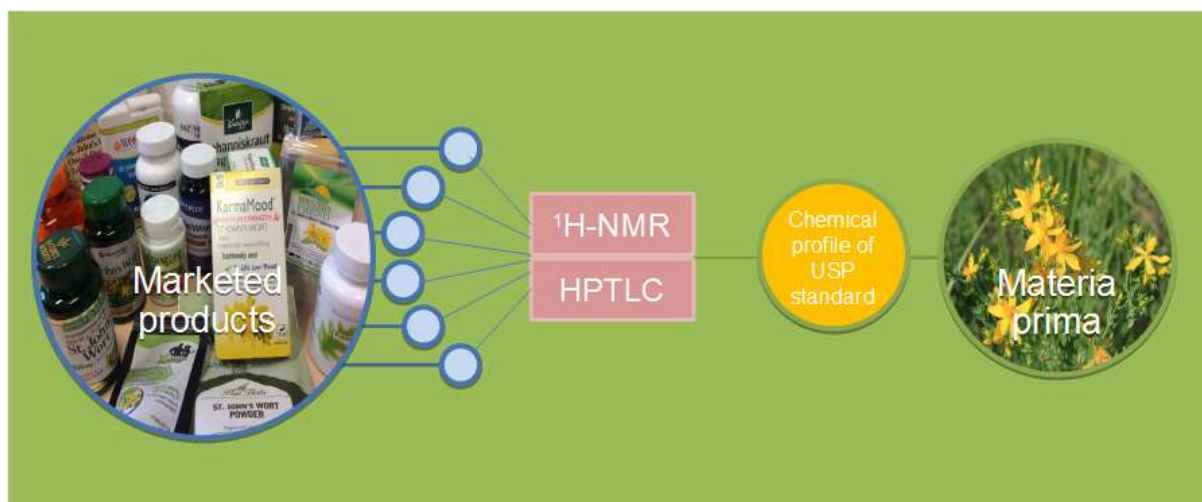
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Graphical abstract



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