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Title

Elemental fingerprinting of *Hypericum perforatum* (St John's Wort) herb and preparations using ICP-OES and chemometrics

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Abstract

St. John's wort (SJW) (*Hypericum perforatum*) is a herbal remedy commonly used to treat mild depression. The elemental profiles of 54 samples (i.e., dry herbs, tablets and capsules) were evaluated by monitoring 25 elements using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The major elemental constituents in the SJW samples were Ca (300-199,000 µg/g), Mg (410-3,530 µg/g), Al (4.4-900 µg/g), Fe (1.154-760 µg/g), Mn (2.4-261 µg/g), Sr (0.88-83.6 µg/g), and Zn (7-64 µg/g). For the sixteen elements that could be reliably quantified, principal component analysis (PCA) was used to investigate underlying patterns in the data. PCA models identified 7 key elements (i.e., Ba, Ca, Cd, Mg, Mo, Ni and Y), which described 85% of the variance in the dataset in the first three principal components. The PCA approach resulted in a general delineation between the three different formulations and provides a basis for monitoring product quality in this manner.

Key Words

St. John's wort; ICP-OES; metal content; *Hypericum perforatum*; principal component analysis.

1. Introduction

In recent years, the global use of complementary and alternative medicines (CAM) has increased. In Asian and African countries, 80% of their population depends on traditional medicine as their primary form of healthcare, whilst 70-80% of people in the developed world have used some form of CAM (World Health Organisation, 2008). Herbal medicines, in particular, are a popular form of CAM and the UK alone spent £136 million on these products in 2009 (Mintel Oxygen, 2009). This is due, in part, to the view that herbal products are a 'more natural' and 'safer' treatment compared to synthetic medicines (Lynch & Berry, 2007). In actuality, herbal medications are chemically complex and challenges remain to reduce differences between products containing the same medicinal herb to ensure safety and similar therapeutic effects.

An area that is receiving more attention is the monitoring of elements for the quality control of medicines (US Pharmacopeia, 2013) including herbal products. Elements have a greater stability in comparison to molecular constituents and many herbs of medicinal interest are known accumulators or hyper-accumulators of metals (see e.g. Rascio & Navari-Izzo, 2011; Masorivicova, Kralova & Kummerova, 2010). The elemental profile of a herbal medicine can also be affected by processing and formulation. In recent years, a number of studies have reported on the presence of toxic elements such as As, Cd, Hg and Pb in herbal products (Ernst, 2002; Deng, 2002). Consequently, manufacturers are required to ensure concentrations of heavy metals in their products fall within recommended safety limits before they can be sold (US Department of Health and Human Services, 2004). However, the remaining elemental composition is largely overlooked and underutilised despite the metal accumulation properties of plants having a number of implications for product quality.

One herb that is of particular interest is *Hypericum perforatum,* commonly known as St John's Wort (SJW). SJW is widely used in the treatment of mild to moderate depression (Brattstrom, 2009) and is also noted for its anti-inflammatory and anti-bacterial effects (Medina, 2006). In Europe, SJW dry extract is standardised mainly according to the relative amounts of three groups of pharmacologically active constituents: hypericins, hyperforin and flavonoids (such as rutin) (British Pharmacopoeia, 2015). With respect to the elemental content in SJW, a number of studies (e.g. Tokalioglu, 2012; Bu, Cizdziel & Reidy, 2012; Gomez et al., 2004; Gomez et al., 2007; Jurca et al., 2011; Kalny et al., 2012; Razic et al., 2005; Yi, Liu & Li, 2004; Radanovic, Antic-Mladenovic & Jakovljevic, 2002) have investigated SJW plant and SJW products for the presence and relative concentrations of a variety of elements.

Although prior studies provide insight into elemental content, the number of samples, elements monitored and type of SJW product investigated tends to be limited. In addition, disparate sample preparation and analysis techniques have been utilised. Therefore these elemental concentrations cannot be used to provide definitive concentration ranges found in different types of SJW products. Thus, before the elemental profile of SJW products can be explored further, 'expected' concentration ranges for a selection of elements needs to be established using a uniform method with samples from different geographical origins.

Objective comparison of SJW samples containing a large number of elements is challenging. Chemometric approaches are extremely powerful for the analysis and interpretation of multidimensional data. The application of chemometric tools to data detailing the metal content of plant species has produced models which have been shown to accurately differentiate between species (Arceusz, Radecka & Wesolowski, 2010), manufacturer (Ni, Peng & Kokot, 2008) and origin (Moreda-Pineiro, Fisher & Hill, 2003). A few studies have used chemometrics to investigate the elemental content of plant material including SJW, yet these studies are focused either on a range of plant species or plants found in polluted areas (e.g. Moreno-Jimenez et al., 2009; Razic et al., 2005). To date there is a lack of comprehensive evaluation of the elemental profile of SJW and related products and the interpretation of such acquired data.

In this study, the elemental profile was obtained for 54 SJW products including dry herb (n=22), tablets (n=20) and capsules (n=12). The presence and concentration of 25 elements (i.e., Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Hg, In, Mg, Mn, Mo, Ni, Pb, Pt, Sb, Se, Sr, V, Y and Zn) were established using inductively coupled plasma optical emission spectroscopy (ICP-OES). The elemental profiles were subsequently analysed using PCA to investigate underlying patterns in the data.

2. Method

2.1. Materials

Fifty-four SJW products were purchased from high street retailers and Internet sources (Supplementary Material). All labware was acid washed overnight with 4 M nitric acid prepared from 70% nitric acid (Fisher) and rinsed thoroughly with deionised water before use. High-purity nitric acid 70% (99.999% trace metal basis) (Sigma-Aldrich) was used for microwave digestion and preparation of 2% HNO_3 solutions. Elemental stock solutions (1000 ppm) of Al, As, B, Ba, Cd, Co, Pb, Mg, Mn, Mo, Ni, In and Hg (Fisher); Be and Pt (VWR); Ca, Cr, Cu, Fe, Sb, Se, Sr and Zn (Merck); V (Sigma-Aldrich); and Y (Acros organics) were used to prepare calibration standards.

2.2. Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES)

Elemental analysis was carried out using a 710 ICP-OES (Varian) axial spectrometer fitted with a Seaspray nebuliser and SPS3 autosampler. ICP-OES parameters for power, plasma flow, auxiliary flow, and nebuliser pressure were 1.4 kW, 15 L/min, 1.5 L/min and 180 kPa, respectively. The wavelengths used for each element are summarised in Table 1. Limits of detection (LOD) and quantification (LOQ) were calculated (i.e., LOD = standard deviation of the blank x 3; LOQ = standard deviation of the blank x 10) for each wavelength by analysis of a 2% HNO_3 blank. Element concentrations in the SJW samples were calculated using a calibration curve with a weighted regression.

2.3. Method Validation

Validation of the method was carried out using a NIST certified reference material (CRM), Polish tea (INCT-TL-1), and spike recovery methods. The NIST reference was certified for Al, B, Ba, Ca, Cu, Fe, Mg, Mn, Ni, Sr and Zn. To validate the additional metals used in the study the NIST reference samples were spiked with 0.5 ppm of As, Be, Cd, Co, Cr, Hg, In, Mo, Pb, Pt, Sb, Se, V and Y prior to acid digestion.

2.4. Sample preparation

Dry herb samples were ground using a Precelly's homogeniser (Bertin Technologies). The contents of capsule samples were removed from the capsule case (case discarded), and tablet samples were ground using an agate pestle and mortar then sieved (1 mm mesh) to remove any outer coating. All samples were dried (40 ^{0}C) overnight in an oven (8000 psi) and then stored in desiccators at room temperature before analysis. The samples (ca., 0.4 g) were weighed by difference and digested with 5 ml high purity nitric acid via a CEM MARS

Xpress microwave at 1600 W for 35 min to obtain complete digestion. The samples were then diluted 10:1 with deionised water, centrifuged for 45 min at 9000 RPM and filtered using a 0.22 µm syringe filter (Millipore) prior to analysis. All samples were prepared in triplicate.

2.5. Principal Component Analysis (PCA)

Principal component analysis (PCA) was carried out using Unscrambler 10.1 (CAMO) software. Prior to carrying out the PCA, elements with concentration values below the LOQ were removed from the dataset and the SJW data was ratio normalised for each element. Ratio normalization involves normalising the raw data for each individual element so that the maximum concentration of the element observed across the samples is always equal to 1, and the concentration of the element in the remaining samples is expressed as a proportion of this maximum value. This ascribes equal importance to each of the elements investigated independent of its relative abundance in the samples and guards against the analysis becoming skewed . A stepwise data reduction approach (see Section 3.3) was then applied to the remaining data to identify the minimal elemental fingerprint which illustrated delineation between dry herb and formulated samples. All of the data associated with a given element across all samples was concatenated to give a single point per element on the loadings plot. This gave 16 descriptors in total.

3. Results and Discussion

3.1. Method Validation

As a CRM for elements in St. John's wort was not commercially available, validation was carried out using the NIST polish tea CRM to verify accuracy of the method (Konieczynski & Wesolowski, 2007). The NIST CRM was spiked with known concentrations of As, Be, Cd, Co, Cr, Hg, In, Mo, Pb, Pt, Sb, Se, V and Y to include all elements of interest. The elements examined were selected based on those found in previous studies of SJW (e.g., Helmja et al., 2011; Radanovic, Antic-Mladenovic & Jakovljevic, 2002) and other essential and non-essential elements found in plant material. The concentrations obtained were compared to the certified values and spiked concentrations (Table 2). The elements As, B, Cd, Co, Cr, Cu, Fe, Hg, Ni, Sb, Sr, Y and Zn had recoveries ≥ 95%; Ba, Be, Ca, In, Mn, Mo, Pt, Se and V had recoveries between 91.5 – 94.5%; and Al, Mg and Pb had recoveries between 83.8 - 87%. The percent recovery values fall within those recommended for inorganic impurities by the U.S. Pharmacopeia (US Pharmacopeia, 2013).

3.2. SJW Samples

The concentrations of 25 elements were determined for 54 SJW samples (Table 3). Sixteen elements had concentrations above the calculated LOQs (i.e., Al, B, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pt, Sr, Y and Zn), four elements had some concentrations below the determined LOQs but were detectable (i.e., Be, Co, Pb and V), and five elements were consistently below determined LODs (i.e., As, Hg, In, Sb and Se).

3.2.1. SJW Dry Herbs

The six elements found in highest average concentration for the dry herbs were Ca, Mg, Al, Fe, Mn, and Zn (i.e., 4,900 - 40 µg/g). The results for Mg, Fe, Mn, Al and Zn were similar to those determined in previous studies (see e.g. Chizzola, Michitsch & Franz, 2003; Gomez et al., 2004; Konieczyński & Wesołowski, 2007). For Ca, the results obtained (9,500 - 2,600 µg/g) agreed with those determined by Ražić et al., but in general were higher (up to 1000 times higher) than previous studies (Gomez et al., 2004; Hussain et al., 2010; Yi, Liu & Li, 2004). The smaller sample sizes used by Ražić et al. or the samples being collected from a specific geographic area in the other studies may contribute to these differences. High concentrations of Ca are often present in plant cells as it is used in numerous functions including alleviation of toxic metal effects (Brady et al., 1993). The elements found in concentrations (on average) from 28 to 13 µg/g were Ba, Cu, Sr, Pt, and B. In general, the concentrations for these elements agreed with prior studies (see e.g. Gomez et al., 2004; Helmja et al., 2011; Konieczyński & Wesołowski, 2007). One study reported Ba concentrations up to 74 µg/g (Yi, Liu & Li, 2004); however, these samples were obtained from the Xinjiang region of China where Ba content in soils can be high (Kabata-Pendias, 2010). The elements found in the lowest average concentrations (2 – 0.2 µg/g) were Y, Cd, Mo, Cr, and Ni. Concentrations of Cr, Cd, and Ni agreed with levels found in SJW in prior studies (see e.g. Gomez et al., 2007; Helmja et al., 2011; Jurca et al., 2011).

3.2.2 SJW Capsules and Tablets

Manufactured SJW products (i.e., capsules and tablets) were analysed to establish notable changes to the elemental profile as a result of processing and formulation. The six elements found in highest average concentration were Ca, Mg, Fe, Al, Mn and Zn for capsules (i.e., 9700 – 40 µg/g), and Ca, Mg, Fe, Al, Zn and Sr for tablets (i.e., 69,000 – 22 µg/g). On average, an increase in the amount of Ca was observed for the formulated products when compared to the dry herb. This is likely due to the addition of calcium carbonate and di-calcium phosphate to the capsule and tablets as stated on their label claim (supplementary information). Values obtained for Ca are again higher than those seen in previous studies for tablets and capsules (Bu, Cizdziel & Reidy, 2012; Gomez et al., 2004), but values from these prior studies were for only one sample, which may explain the discrepancy. A small increase was observed in the average amount of Mg in the tablet form (1700 µg/g) when compared to the dry herbs (1500 µg/g) and capsules (1400 µg/g). This may also be due to the addition of excipients, such as magnesium stearate and magnesium silicate, for tablets (supplementary information). Results agreed with those found by Bu et al. (2012) for Mg in SJW capsules. An increase in the average Fe concentration was also observed for the tablets and capsules (173 & 170 µg/g respectively) when compared to the dry herb (140 µg/g). The Fe levels observed agreed with reported studies for tablets (Gomez et al., 2007; Kalny et al., 2012) and capsules (Bu, Cizdziel & Reidy, 2012). Iron oxides are sometimes used as colouring agents for tablet and capsule coatings, and although great care was taken to remove these during sample processing, some transfer may have occurred. The average concentration for capsules and tablets of Al, Zn, and Mn agreed with the majority of values cited in previous studies (Bu, Cizdziel, & Reidy, 2012; Gomez et al., 2004; Gomez et al., 2007). However, Zn concentrations found by Kalny et al. (2012) were four times those found in this study, but the reported value was obtained from a single SJW sample. The next four elements found in decreasing average concentrations in the formulated SJW products were B, Cu, Pt, and Sr for capsules (20 – 7 µg/g); and Mn, B, Pt, and Cu (18 – 9 µg/g) for tablets. The five elements found in the lowest average concentrations were Ba, Cr, Ni, Cd, and Y for capsules (5 – 0.33 µg/g); and Ba, Cr, Ni, Y, and Cd for tablets (2 – 0.49 µg/g). Reported concentrations available for these elements in capsules and tablets agreed with previous studies (see e.g. Bu, Cizdziel, & Reidy, 2012; Gomez et al., 2004; Kalny et al., 2012). To the authors' knowledge, this is the first time Y and Pt have been consistently identified in *Hypericum perforatum* products. Considered to be non-essential elements, Y and Pt have been observed with typical concentrations in plants ranging from 0.01 to 3.5 µg/g (Kabata-Pendias & Mukherjee, 2007) and ≤ 0.6 µg/g (Ravindra, Bencs & van Grieken, 2004), respectively. Pt and Y are often used in alloys for machinery, which may be a potential source of these elements in our SJW samples.

A single method has been successfully applied to evaluate 25 elements in 54 SJW samples, including different formulations, which is the largest study to date for SJW. The average concentrations of Ca and Mg in the SJW formulated products increased or remained constant when compared to the dry herb samples. In contrast, elements such as B, Ba, and Mn decreased in concentration by between 29 and 85% with a higher decrease for the tablet than for the capsule. In addition, Mo was found in three dry herb samples above the LOQ but not in the capsules and tablets. Observed decreases in elements due to formulation may be rationalised as follows. Firstly, the majority of the formulated products in this study contained the dry alcoholic extract of SJW and not the dry herb (see e.g. Anand et al., 2005). This means that only those elements that are released from the bulk plant material in a soluble form would be extracted (Suliburska & Kaczmarek, 2012). The elemental concentration in the dried extract used in the products would therefore be dependent on the extraction conditions and amount of extract recovered; and for most elements this would result in a reduction in element concentration compared to the dry herb (Helmja et al., 2011; Suliburska & Kaczmarek, 2012). Secondly, the addition of excipients when formulating a product (e.g., bulking agents in tablets and capsules) can act as a diluent and decrease relative element concentrations, except for elements that may be present in the excipient itself (i.e., Ca and Mg). These differences caused by processing (e.g., extraction) and formulation can potentially be exploited for classification.

3.3. Application of chemometrics to SJW elemental profiles

The elemental profiles of the 54 SJW samples were subjected to principal component analysis (PCA). From the initial dataset of 25 elements, those elements with concentrations below the LOQ for all samples (i.e., As, Be, Co, Hg, In, Pb, Sb, Se, and V) were removed. A PCA was subsequently carried out using the remaining 16 elements. The samples were identified using an alphanumeric code (Supplementary Material): the first letter of the code denotes whether or not the sample was a dry herb ("H"), tablet ("T") or capsule ("C"). The number following the letter was used to delineate between samples of the same type.

This initial scores plot (Figure 1) showed that the first two principal components accounted for 57% of the variance within the dataset. As a quality control measure, a 95% confidence interval ellipse, calculated using the Unscrambler software, was applied to the results of the PCA. Despite the samples being of varying formulation and from a number of geographic areas, 49/54 (91%) of the SJW samples were within the 95% confidence limit. Five samples (9%) fell outside the ellipse indicating that they were significantly different to the other samples in the dataset. These samples comprised one dry herb sample (H15), two tablets (T5 and T19) and two capsules (C1 and C2). In an attempt to understand the differences of these samples from the remaining dataset, these outliers were examined in closer detail. In the case of T19, the label claim reported each tablet contained 99% SJW extract. However, the tablet content was visually much whiter than that of the other tablets, suggesting that the label claim was fallacious, and a large proportion of the product was excipient. The label claim of T5 (i.e., 6% extract) also indicated a large proportion of excipient similar to that suggested for T19. The other three outliers (H15, C1 and C2) possessed significantly higher concentrations of Al, B, Fe, Mn, Ni, Sr and/or Pt compared to the other samples. All three samples were composed of raw herb only from a similar geographic area (i.e., Bulgaria and/or Eastern Europe and Balkans), which may explain the difference in element composition from the majority of samples. These outliers were removed from the dataset.. The remaining data was re-normalised (Section 2.5) once the outliers had been removed.

A PCA was then carried out on the remaining 49 SJW samples. A 3D scores plot (Figure 2) of this PCA model using the first three principal components represented 65% of the total variance of the dataset and showed delineation between the dry herb and formulated products. Additionally, there is a general trend for further delineation between tablets and capsules, although some overlap between these two categories is apparent. The separation observed is primarily along PC1 which has high positive loadings for B, Ba, Cd, Mn, Ni and Zn and high negative loadings for Ca, Cr and Y. This shows that the herb samples have higher values for B, Ba, Cd, Ni, Zn and Mn and lower values for Ca, Cr and Y in comparison to the processed samples. As mentioned previously, formulated products often contain excipients containing calcium such as calcium carbonate (or talc) and di-calcium phosphate, which may contribute to these differences. On PC2 there is a positive loading for Al, Cu, Fe, Ni and Mo and strong negative loadings for Ca, Cr, Y and Sr. The loadings on principal component three (PC3) included a high positive loading for Al, Fe, Mg, Ni and Pt.

Those samples that were not clearly separated on the scores plot into their expected category of dry herb, tablet or capsule were investigated to determine the cause, if any, for their “mis-classification”. C10 and T6, which grouped closely to the dry herb samples, were found to be composed of ground herb with some excipients. Therefore their position close to the dry herb samples is to be expected. C7 was found to contain a mixture of ground herb and alcoholic extract, therefore explaining why this sample is positioned between the capsule and dry herb clusters. C5 is clustered closely with the tablets; the levels of Ca, Mg and Sr for this capsule are more comparable to those of the tablets than the other capsules. In contrast, both T7 and T14 clustered more closely to the capsules as T7 had the lowest concentration values for Ca, Fe, Mg, and Sr for all tablets analysed and T14 had the second lowest Ca concentration. Previously, it has been demonstrated that the elemental profile can be used to discriminate between botanical families (Arceusz, Radecka & Wesolowski, 2010) and origin (Moreda-Pineiro, Fisher & Hill, 2003; Ni, Peng & Kokot, 2008) when evaluating dry herbal material. The results from this study suggest that by monitoring the elemental profile, not only can the quality of the SJW product be assessed based on its elemental composition, but this can also be used to decipher whether or not processing has taken place. This is key when evaluating herbal material used for medicinal products as in many cases the product will be in a processed form.

Any method to assess the nature and quality of unknown SJW products based on elemental profiling would be feasible only if a small number of elements were required to establish such information. As such, it was of interest to determine the identity of the minimum number of elements required from the initial analysis in conjunction with PCA to achieve an accurate delineation of products according to their formulation type (i.e., dry herb, tablet or capsule). This small group of elements which results in delineation of formulation type in the PCA was named as a “discriminative elemental fingerprint”. In any robust PCA the first principal component (PC1) explains the greatest amount of the variance in the dataset. Two elements with similar values on PC1 in the loadings plot are therefore explaining the variance in the dataset with respect to PC1 in very similar ways i.e. there is redundancy in the dataset. It is therefore feasible to remove one of these elements from the dataset whilst retaining delineation of SJW products according to their formulation type in the PCA scores plot. The decision as to which of the two elements should be removed comes from examining their respective loading values on the second principal component (PC2). PC2 represents the principal component that explains the second greatest amount of variance with respect to the dataset as a whole. It is also orthogonal to PC1. Therefore, by retaining the element which has the highest absolute loading value on PC2, and rejecting the other, the descriptor which is contributing most to explaining the variance of the dataset as a whole is retained. In cases where the absolute loading values for PC2 were similar to the loading values of the elements on the third principal component (PC3), these were compared to determine which of the elements to retain. After removal of an element, a new PCA model was generated using the reduced dataset. An increase in the total percentage variance explained by the first three principal components and a concomitant retention of the delineation of products in the scores plot according to product type was an indication that noise had been removed from the dataset and that the overall model had not broken down.

In total nine elements were removed from the dataset. Attempts to reduce the number of elements any further resulted in the model breaking down with respect to orthogonality of the principal components. This manifested as a distinct linear correlation between the data points on principal components 1 and 2 of the loadings plot where no such correlation should exist. This arises when there is insufficient variance in the descriptors in the dataset to generate discrete, orthogonal principal components, thus indicating a minimum number of elements has been reached. The values for the seven elements that had been retained in the dataset (i.e., Ba, Cd, Ca, Fe, Ni, Sr and Y) were then used to produce a final PCA model (Figure 3). The first three principal components of this model accounted for 85% of the total variance in the dataset, an increase from 65% for the original model. Clear delineation existed in the scores plot between SJW dry herb products and formulated products, and those samples which had been investigated due to their “mis-classification” in the original analysis (i.e., C10, C7, C5, T6, T7 and T14) remained in their original groupings. Although there was slight bleeding of the clusters representing capsules and tablets into one another distinct groups for tablets and capsules were still discernible, which is promising for future application of the technique. Hence, a seven-element fingerprint for SJW products has been established for this dataset. In contrast to previous studies (e.g., Arceusz, Radecka & Wesolowski, 2010; Ni, Peng & Kokot, 2008), a data reduction approach for the selection of “key” elements could provide better discrimination between samples of interest.

4. Conclusions

This study has determined an ‘expected’ range for 16 elements (i.e., Al, B, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pt, Sr, Y and Zn) in SJW dry herb and processed preparations. The consistent presence of Y in SJW samples has not previously been reported. The application of PCA to the elemental profiles for the SJW samples clearly differentiated the dry herb samples from the processed samples with additional differentiation between tablets and capsules. A reduction in the average concentration of B, Ba, Cd, Ni, and Mn occurred post formulation and it has been postulated that this could be due to factors such as the extraction process and/or powder dilution. Higher levels of Ca and Mg found in processed SJW products were identified as expected, but higher levels of Cr, Y, and Sr were also found, which could be due to contamination from metal alloys used in the manufacturing process. A PCA model identified a 7-element fingerprint (i.e., Ba, Ca, Cd, Fe, Ni, Sr and Y) capable of differentiating between the three categories of SJW product investigated. Results indicated sample forms (i.e., herb, tablet and capsule) were differentiated by a change in the elemental profile contributed by excipient addition, dilution, and/or the extraction process.

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Figure Captions

Figure 1. A two-dimensional PCA plot (PC1 *vs.* PC2) using the concentration of 16 elements (i.e., Al, B, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pt, Sr, Y and Zn) from 54 SJW samples with a 95% confidence ellipse applied (squares = herbs, circles = tablets, triangles = capsules).

Figure 2. A 3D scores plot using the concentration of 16 elements (i.e., Al, B, Ba, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pt, Sr, Y and Zn) from 49 SJW samples (squares = herbs, circles = tablets, triangles = capsules). Samples, which cluster contrary to their type (C5, C10 C7, T6, T7 and T14), are highlighted in solid black.

Figure 3. A 3D scores plot using the seven-element fingerprint (i.e., Ba, Ca, Cd, Mg, Mo, Ni and Y) for 49 SJW samples (squares = herbs, circles = tablets, triangles = capsules) accounting for 85% of the variance in the dataset. Samples, which cluster contrary to their type (C5, C10 C7, T6, T7 and T14), are highlighted in solid black.

Tables

Table 1. Wavelengths used to monitor selected elements using ICP-OES.

Element	Wavelength (nm)
Al	396.152
As	188.980
B	249.772
Ba	455.403
Be	234.861
Ca	370.602
Cd	214.439
Co	228.615
Cr	267.716
Cu	327.395
Fe	238.204
Hg	184.887
In	230.606
Mg	278.142
Mn	257.610
Mo	202.032
Ni	231.604
Pb	220.353
Pt	203.646
Sb	217.582
Se	196.026
Sr	407.771
V	292.401
Y	371.029
Zn	213.857

Table 2. NIST polish tea (INCT-TL-1) and spiked recovery values

Element	Certified value or spike amount	Experimental value[1]	Recovery %
Al	0.229 ± 0.028 wt%	0.192 ± 0.001	83.8 ± 0.4
As	Spiked with 0.5 ppm	0.506 ± 0.003	101.1 ± 0.6
B	26 mg/kg	25 ± 2	95 ± 7
Ba	43.2 ± 3.9 mg/kg	40.2 ± 0.1	93.1 ± 0.3
Be	Spiked with 0.5 ppm	0.464 ± 0.003	92.8 ± 0.7
Ca	0.582 ± 0.052 wt%	0.534 ± 0.003	91.8 ± 0.6
Cd	Spiked with 0.5 ppm	0.480 ± 0.003	95.9 ± 0.7
Co	Spiked with 0.5 ppm	0.482 ± 0.003	96.4 ± 0.6
Cr	Spiked with 0.5 ppm	0.496 ± 0.003	99.2 ± 0.6
Cu	20.4 ± 1.5 mg/kg	23 ± 3	110 ± 10
Fe	432 mg/kg	410 ± 10	96 ± 3
Hg	Spiked with 0.5 ppm	0.49 ± 0.01	98 ± 2
In	Spiked with 0.5 ppm	0.460 ± 0.003	91.9 ± 0.5
Mg	0.224 ± 0.017 wt%	0.428 ± 0.003	85.7 ± 0.6
Mn	0.157 ± 0.011 wt%	0.206 ± 0.002	91.8 ± 0.8
Mo	Spiked with 0.5 ppm	0.144 ± 0.001	91.5 ± 0.6
Ni	6.12 ± 0.52 mg/kg	0.489 ± 0.004	97.8 ± 0.8
Pb	Spiked with 0.5 ppm	5.3 ± 0.1	87 ± 2
Pt	Spiked with 0.5 ppm	0.466 ± 0.003	93.3 ± 0.6
Sb	Spiked with 0.5 ppm	0.53 ± 0.01	106 ± 3
Se	Spiked with 0.5 ppm	0.472 ± 0.001	94.5 ± 0.3
Sr	20.8 ± 1.7 mg/kg	0.52 ± 0.02	103 ± 4
V	Spiked with 0.5 ppm	19.14 ± 0.08	92.0 ± 0.4
Y	Spiked with 0.5 ppm	0.495 ± 0.003	99.1 ± 0.6
Zn	34.7 ± 2.7 mg/kg	0.490 ± 0.004	98.0 ± 0.7

[1] Unit same as certified or spiked unit.

Table 3. Element concentrations found in SJW herb and preparations reported as average (range low – high) *median*.[1,2]

Element	Dry herb (µg/g) (n=22)	Capsule (µg/g) (n=12)	Tablet (µg/g) (n=20)
Al	100 (20–370) *92*	75 (4.4 – 399) *30*	92 (BLQ – 900) *41.7*
As	BLD	BLD	BLD
B	28 (16 – 47) *26*	20 (BLD – 42) *15*	14 (BLQ – 37) *13.7*
Ba	13 (2.7 – 22) *12*	5 (0.3 – 17.4) *0.82*	2 (0.51 – 5.7) *1.06*
Be	BLD	BLD	BLQ/BLD
Ca	4900 (2600 – 9500) *4500*	9700 (410 – 93000) *600*	69000 (300 – 199000) *79600*
Cd	0.8 (BLQ – 1.73) *0.72*	1 (BLD – 1.78) *1.2*	0.49
Co	BLQ/BLD	BLQ/BLD	BLQ/BLD
Cr	1 (BLD – 1.4) *1.0*	2 (BLD – 2.42) *1.6*	2 (BLD – 5) *2.04*
Cu	14 (4.64 – 120) *9.6*	19 (9 – 83) *13*	9 (BLQ – 20) *9.0*
Fe	140 (38 – 760) *110*	173 (17.8 – 750) *46*	170 (1.154 – 630) *77*
Hg	BLD	BLD	BLD
In	BLD	BLD	BLD
Mg	1500 (790 – 1870) *1570*	1400 (949 – 2330) *1240*	1700 (410 – 3530) *1590*
Mn	110 (59.1 – 261) *102*	53 (4.4 – 240) *16.3*	18 (2.4 – 84.5) *14.33*
Mo	1 (BLD – 1.47) *1.41*	BLQ/BLD	BLQ/BLD
Ni	2 (BLD – 5.37) *1.88*	2 (BLQ – 2.9) *2.3*	2 (BLD – 3.2) *1.572*
Pb	BLQ/BLD	BLQ/BLD	BLD
Pt	17	16 (BLD – 18.7) *16*	11 (BLD – 14.6) *11*
Sb	BLD	BLD	BLD
Se	BLD	BLD	BLD
Sr	15 (9.29 – 30.33) *12.9*	7 (0.9 – 21.2) *1.8*	22 (0.88 – 83.6) *17.5*
V	BLQ/BLD	BLQ/BLD	BLQ/BLD
Y	0.2 (BLD – 0.26) *0.18*	0.33	0.6 (BLD – 0.92) *0.47*
Zn	40 (23 – 64) *36*	40 (17.2 – 60) *42.3*	27 (7 – 56.6) *24.9*

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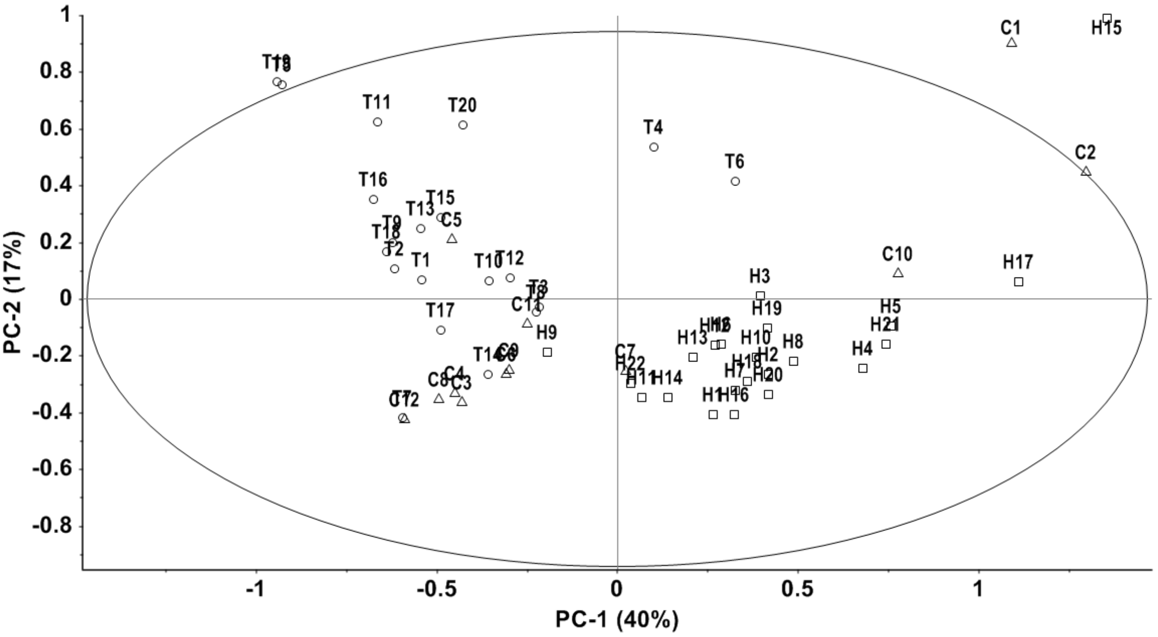
[1] BLQ = below limits of quantification; BLD = below limits of detection

[2] Samples were run in triplicate with typical uncertainties of ≤8% RSD.



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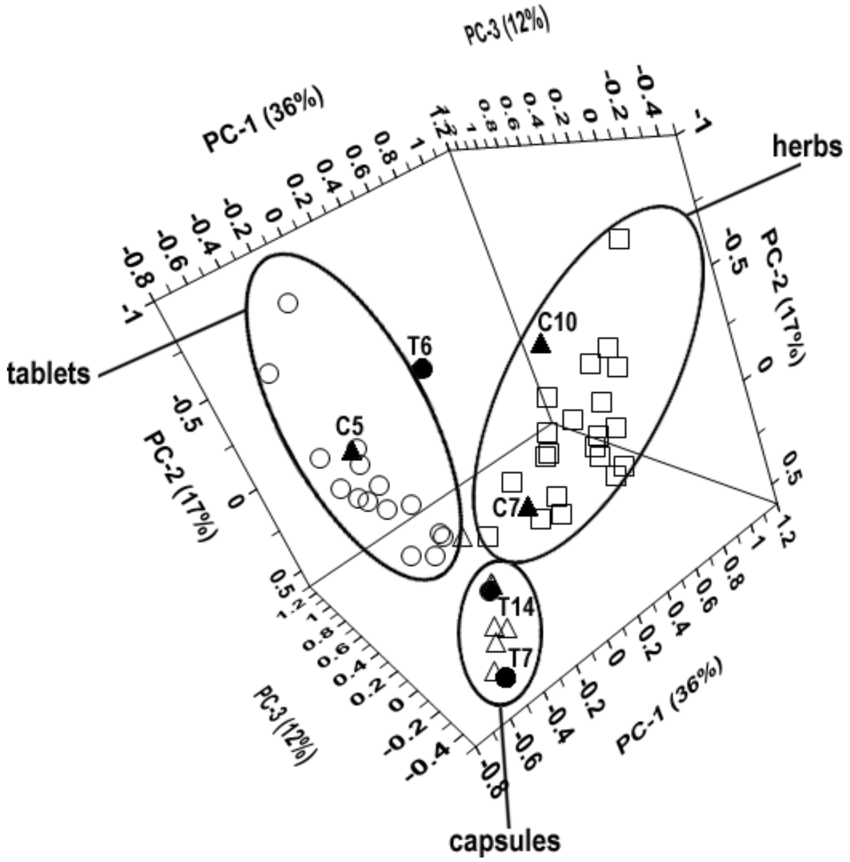

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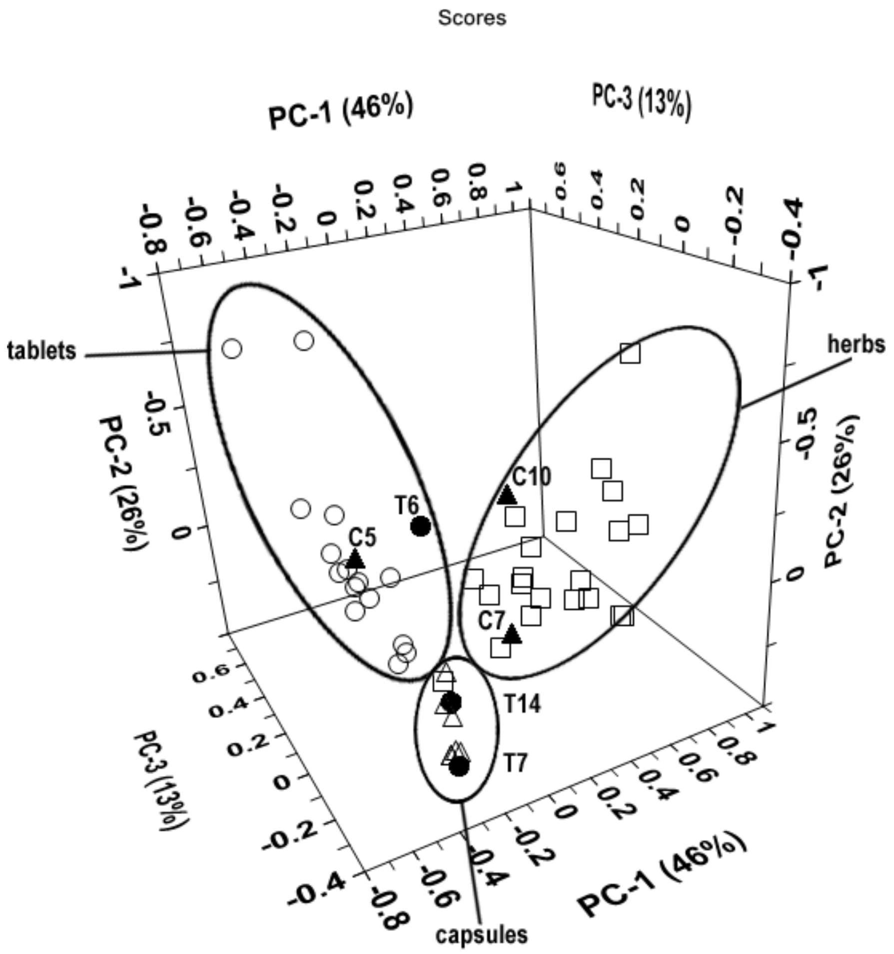

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