Identity issues surrounding American skullcap (Scutellaria lateriflora) and an optimised high performance liquid chromatography method to authenticate commercially available products

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Identity issues surrounding American skullcap (*Scutellaria lateriflora*) and an optimised High Performance Liquid Chromatography method to authenticate commercially available products.

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Abstract

Traditional use of American skullcap (*Scutellaria lateriflora*) for anxiety and related conditions is well documented. There is evidence of flavonoid instability in *S. lateriflora* and a high rate of substitution with other skullcap species or adulteration with potentially hepatotoxic germanders (*Teucrium* spp). It is therefore essential for the identity, quality and safety of a commercial *S. lateriflora* product to be verified prior to clinical use.

The objective was to review the literature relating to substitution and adulteration of *S. lateriflora* and to present a simple, optimised high performance liquid chromatography (HPLC) method to verify the absence of adulterants in a commercial sample of *S. lateriflora,* by comparing its chromatographic profile with that of authenticated *S. lateriflora*.

S. lateriflora reference material and a freeze-dried commercial sample were extracted with methanol and water (80:20 v/v) and compared by HPLC analysis. The commercial sample showed reproducible retention times (RTs) of the flavonoid biomarkers baicalin (RT = 14.8 min; mean ± SD = 11.71 ± 1.16 mg/g); baicalein (RT = 20.4 min; 7.67 ± 0.89 mg/g); wogonin (RT=23.7 min; 0.65 ± 0.06 mg/g). It appeared to be free from adulteration with germander (verbascoside was not detected; RT= 9.1 minutes) and its phytochemical profile was consistent with that of the *S. lateriflora* reference material.

Conclusions: It is crucial that commercial products are adequately identified prior to use. The reported HPLC method has shown the potential to compare non-authenticated *S. lateriflora* samples with authenticated voucher specimens - essential when conducting any phytochemical analysis of the herb.

Key words: *Scutellaria lateriflora*; Skullcap; Flavonoid biomarkers; HPLC; Voucher specimen; Adulteration; Substitution; Identification

1. Introduction

Scutellaria lateriflora (Figure 1) is a perennial herb belonging to the *Lamiaceae* (mint) family and is one of 360 known *Scutellaria* species worldwide (Malikov and Yuldashev, 2002). It grows on wetlands and is indigenous to North America and Canada where it is widely distributed (U.S.D.A., 2012) and is grown commercially worldwide (Wills and Stuart, 2004). It has been in traditional use for centuries by Native Americans for a variety of ailments, including nervous disorders of the digestive tract such as colic, diarrhoea and heartburn (Khosh, 2000), for menstrual disorders, and as a sedative and a nervine tonic. It is popular in western herbal medicine for anxiety and related disorders; such as nervous exhaustion, muscular tension, hysteria, hypertension, tremors, convulsions, neuralgia, insomnia and headache. It is also used for tranquiliser and barbiturate withdrawal (Joshee et al., 2002).

Preparations of *S. lateriflora* are made from the aerial parts and are sold in the form of tinctures, teas and tablets; and capsules containing powders, liquids or freeze-dried material (Wills and Stuart, 2004).

Due to the risk of contamination with potentially toxic botanicals and other hazardous substances, including pesticide residues, bacteria, fungi, toxic metals and radioactive chemicals, the importance of strict quality control of all herbal preparations cannot be underestimated (de Smet, 1999). The large number of *Scutellaria* species, many of which are similar in appearance, and consequent problems of substitution or adulteration of *S. lateriflora* with other skullcaps or potentially harmful herbs such as germander (*Teucrium*) species, make its correct identification prior to commercial use particularly essential. In the 1980s and 1990s, although reports of liver damage, including hepatitis and fibrosis, were linked to preparations containing *S. lateriflora*, there was no evidence that the hepatotoxicity resulted from intake of *S. lateriflora* (Upton and Dayu, 2012). The cause of the hepatotoxicity was subsequently attributed to germander species, such as American germander (*T. canadense*) and Wall germander (*T. Chamaedrys*), which contain hepatotoxic furan neoclerodane diterpenoids such as teucrin, being used in some European commercial preparations in place of *S. lateriflora* (de Smet, 1999; Bedir et al., 2003; Lin et al., 2009; Upton and Dayu, 2012).

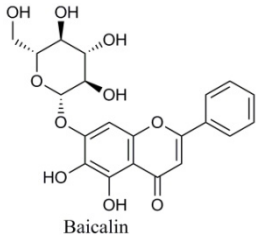
Figure 1 *Scutellaria lateriflora*

S. lateriflora is possibly the most substituted species in western materia medica. The phytochemical profile of other skullcaps is different from that of *S. lateriflora.* They are not considered to be medicinally interchangeable (Wolfson and Hoffmann, 2003).

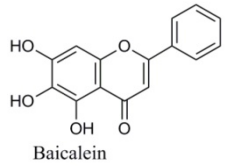
The aim of this paper is twofold. Firstly, it will inform herbal medicine practitioners, researchers and manufacturers of the extent of adulteration and substitution of commercially available *S. lateriflora* products (and consequent safety issues). Secondly, it will demonstrate a simple and effective method for authentication of herbal medicines; using high performance liquid chromatography (HPLC), which commercial herb supply companies could use routinely alongside pharmacognosy methods, without the necessity of employing more sophisticated methods of analysis such as random amplified polymorphic DNA (RAPD) markers, estimation of genome size or mass spectrometry.

The purpose of conducting this present HPLC analysis was to ensure the quality, identity and safety of freeze-dried whole aerial parts of *S. lateriflora* to be used in a study to test its efficacy in healthy volunteers. A freeze-dried preparation was selected for optimum preservation of the whole plant phytochemistry.

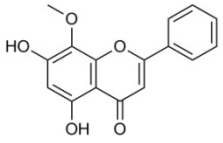
The specific objectives were to: Develop a characteristic chromatogram or 'fingerprint' from authenticated *S. lateriflora*; compare the chromatographic profile of the commercial product with the fingerprint of authenticated herb; qualify and quantify major flavonoids (Figure 2) baicalin (baicalein 7-*O*-glucuronide) baicalein (5,6,7-trihydroxyflavone) and wogonin (5,7-dihydroxy-8-methoxyflavone) found in the commercial product; verify the absence of *Teucrium* from the commercial product by verifying the absence verbascoside (a phenylethanoid glycoside found in *Teucrium* but not *Scutellaria* species).



Baicalin



Baicalein



Wogonin

Figure 2 Three important biomarker flavonoids found in *S. lateriflora*

Issues surrounding authenticity of *S. lateriflora* in the market have probably been a problem for over a hundred years. Wohlmuth (2001) pointed out that King's American Dispensatory, published in 1898, describes *S. versicolor* and *S. canescens* as being two substitute species that constituted the majority of the commercial drug. Botanical descriptions in herbal text books are general and tend to be gathered from secondary sources. It is also apparent that authors tend to cite from one another when providing information on the chemical constituents of the herb. This no doubt adds to identity issues surrounding the herb. A notable example is that, according to Roy Upton, executive director of the American Herbal Pharmacopoeia®, even the description of *S. lateriflora* in the 1983 British Herbal Pharmacopoeia® is believed to relate to *S. incana* (Foster, 2012).

Misidentification may occur with small scale herb farming and the possibility of those with an untrained eye gathering skullcap herb from the wild (the relative safety of most herbs has

perhaps led to a cavalier attitude to plant identification). Accidental substitution of *S. lateriflora* in the market may occur and perpetuate when the wrong species is cultivated by even large-scale commercial growers.

Substitution or adulteration of *S. lateriflora* with other skullcaps, particularly *S. galericulata*, *S. cordifolia* (syn. *S. ovata*) and *S. canescens* (Gorman, 2008; Wolfson and Hoffmann, 2003), or potentially harmful herbs such as morphologically similar germander (*Teucrium*) species may be accidental or it may be deliberate when *S. lateriflora* crops fail. *Teucrium*, for example, has a heavier dry weight than *S. lateriflora* (Gorman, 2008). *S. lateriflora* crop damage may occur due to its susceptibility to a number of diseases such as leaf spot, stem rot and powdery mildew (Greenfield and Davis, 2004); also, adult species of the leaf beetle (*Phyllobrotica* genus) are known to attack the aerial parts and their larvae attack the roots (Farrell and Mitter, 1990).

Evidence that *S. lateriflora* is plagued by substitution problems has been demonstrated in various studies. For example, when German plant extraction company, Extract Chemie, tested 12 samples of material from different sources claimed to be *S. lateriflora* all were in fact other *Scutellaria* species (Gorman, 2008).

Zhang et al. (2009) analysed 10 different commercial preparations claimed to be *S. lateriflora,* by HPLC, and found a wide variation in individual and total phenolic content and chromatographic profiles (the relative proportions of flavonoids differed). Baicalin content, for example, varied from 0.48% to 10.10% while total phenolic content varied from 1.11% to 20.55% respectively (Zhang et al., 2009).

Gao et al. (2008), also found wide variations in individual and total flavonoid content of seven commercial so-called *S. lateriflora* preparations (obtained from five companies), in an HPLC analysis comparing baicalin, baicalein and wogonin content. One sample contained no baicalin, baicalein or wogonin. Of all three flavonoids, µg/ml ranged from 180, 280 and 97.5 to 12,700, 629 and 152 respectively (Gao et al., 2008). In another HPLC analysis (Wills and Stuart, 2004) a liquid commercial product, which was also claimed to be *S. lateriflora,* had the chromatographic profile of *S. incana*. Of five combination commercial preparations claimed to contain *S. lateriflora*, when tested, none had the typical profile of the herb and may therefore either have contained different *Scutellaria* species or may have lost a considerable amount of flavonoids during processing (Wills and Stuart, 2004).

More than one species within a genus may possess exactly the same flavonoids, although in different proportions. HPLC methods can quickly and easily distinguish between species and genera by qualitative and quantitative analysis of the flavonoids and other phytochemicals contained in extracts of the plants (de Oliveira et al., 2001). For example, it can detect the presence of verbascoside (acteoside; kusaginin), a phenylethanoid glycoside found in *Teucrium* but not in *Scutellaria* species, which may indicate contamination of a commercial *S. lateriflora* product with *Teucrium* (Upton et al., 2009). Although the skullcap species most commonly substituted for *S. lateriflora* is thought to be *S. galericulata*, HPLC analysis can distinguish between the two species by the use of the compound 2'-methoxychrysin, which is found in *S. galericulata* but not *S. lateriflora* (Gafner et al., 2003).

In addition to correctly identifying *S. lateriflora* it is also important to ascertain its quality. Evidence suggests that *S. lateriflora*'s flavonoids provide it with its therapeutic actions (Gorman, 2008). Therefore, the concentration of flavonoids in any commercial preparation of the herb is important in defining its quality and efficacy. However, flavonoids in extracts from dried *S. lateriflora* have demonstrated extreme instability over time (Wills and Stuart, 2004). There may also be significant qualitative variations in phytochemical profile within a plant species according to geographic region, biodiversity, ecological variations, cultivation, seasonality,

harvesting and storage time (affecting stability), processing method, marc to menstrum ratio and alcohol concentration (Ciddi, 2006; Gao et al., 2008). Freeze-dried herb, on the other hand, is believed to be superior in its preservation of phytochemicals (Luthria, 2006) and has superior storage time (Gafner and Bergeron, 2005).

As *S. lateriflora's* flavonoids are unstable in both dried herb and extracts and because of the risk of its substitution and adulteration, some bodies of work have been devoted to the development of reliable HPLC methods for verifying its purity and quality. A characteristic profile of the HPLC chromatogram or 'fingerprint', which is altered by adulteration, can be used for accurate identification of the herb. The pattern's relative percentage of flavonoids is the key point to ascertain the quality and identity of *S. lateriflora* (Wills and Stuart, 2004).

2. Experimental

The following protocol is modified from Wills and Stuart (2004), who developed a reliable HPLC method for the purpose of aiding the production of high quality *S. lateriflora* to be grown commercially in Australia.

2.1 Chemicals

HPLC grade baicalin (baicalein 7-*O*-glucuronide), wogonin (5,7-dihydroxy-8-methoxyflavone) and verbascoside were purchased from Extrasynthese, Genay, France. Baicalein (5,6,7-trihydroxyflavone) (98%) and HPLC grade methanol and phosphoric acid were purchased from Sigma-Aldrich, Dorset, UK. HPLC water was obtained from an Elga de-ioniser set at 18.2 MΩ.

2.2 Plant materials

Dried, expertly morphologically authenticated *S. lateriflora* reference material (aerial parts) was donated by the American Herbal Pharmacopoeia® (AHP), Scott's Valley, California. The reference *S. lateriflora* was grown in Colorado, USA and harvested in 2008. A voucher specimen for the reference sample is deposited at the herbarium of the AHP. Freeze-dried herb was purchased from the Eclectic Institute Inc., Sandy, Oregon. The herb used in the commercial product had been botanically identified morphologically and organoleptically at source and was grown on the supplier's own farm in Oregon, USA.

2.3 Marker compounds and verbascoside

Retention times (RTs) and calibration curves of the flavonoid reference standards were developed in order to determine their presence and concentration in the known (identified) *S. lateriflora* samples and for comparison with the freeze-dried sample (Eclectic Institute Inc.). Retention time of verbascoside was also determined to ensure there was no adulteration of the commercial product with germander species.

From stock solutions of 100 µg/ ml concentration, serial dilutions of (5, 10, 20, 50, 60, 80, 100) µg/ml were made of the marker flavonoids and (40, 60, 100) µg/ml for verbascoside.

Each sample was injected into the column three times and an average peak area (PA) was taken. The average retention time was noted for each reference flavonoid and for verbascoside in order to identify the major relevant peaks of *S. lateriflora* and to determine potential adulteration with germander respectively. The calibration curves also enabled dry weight (mg/g) calculation of the concentration of major flavonoids for each of the identified *S. lateriflora* samples analysed by HPLC.

2.4 General

Aerial parts of *Scutellaria lateriflora* reference material, ground to a fine powder, and a freeze-dried, powdered commercial sample were extracted with methanol: water and their UV spectra were compared. Qualitative and quantitative analyses of flavonoids were based on RTs and PAs respectively of flavonoid biomarkers; baicalin, baicalein and wogonin. RT of verbascoside was also established.

2.5 Extraction of *S. lateriflora*

Using a coffee grinder (Braun™) the dried herb (AHP) was ground to a fine powder. The powdered plant material was extracted with methanol: water (80: 20 v/v) at a solvent/solute ratio of 100: 1 (1g plant material in 100 ml methanol/water). Maceration was augmented by placing in a sonicator twice for 15 minutes. The extract was filtered through Whatman™ filter paper. The residue on the filter paper was then washed 3 times through the filter paper with 80% methanol until the extract reached 100 ml. The extract was then filtered through a Spartan® membrane filter (Sigma-Aldrich), pore size 0.45µ and then centrifuged for 60 minutes at 6000 r.p.m. The procedure was repeated with the commercial product (Eclectic Institute) with the omission of grinding to a fine powder.

2.6 HPLC analysis of plant materials

The supernatant of the MeOH/H_2O extracted *S. lateriflora* reference material (AHP) was used for obtaining a 'fingerprint' of the herb for comparison with the commercial product. Quantification of flavonoids was based on peak areas of the flavonoid glycoside baicalin and the aglycones baicalein and wogonin, all considered to be important flavonoid biomarkers in *S. lateriflora* (Gao et al., 2008), which were used as working flavonoid reference standards.

Mobile phase = linear gradient of 30% - 90% methanol/water (v/v). A continuous gradient elution was used. Methanol was acidified with 0.007M phosphoric acid, final pH 3.2; water was acidified with 1% 0.001M phosphoric acid, final pH 4.5. Injection volume = 20 µl. Stationary phase = silica C18 column (Polaris® 5 µ C18-A, 250 x 4.6 mm, Varian Ltd) fitted with a pre-column and a 2 µ pre-column filter (Metasaver, Varian/Agilent Technologies). Flow rate = 1 ml/minute. UV wavelengths were set at 330, 300, 280 and 254 nm. Optimum flavonoid peak detection was at 280 nm. For verbascoside optimum peak detection was 330 nm but reported at 280 nm. Total run time = 30 minutes. The column temperature was set at 25^{o}C. Reference and test samples were prepared fresh daily and injected in triplicate. The use of phosphoric acid to control pH was for its enhancement of flavonoid separation (Wills and Stuart, 2004).

3. Results

3.1 Comparison of chromatograms

HPLC analysis of the commercial sample showed reproducible RTs[1] of baicalin (RT= 14.8 min; mean ± SD = 11.71 ± 1.16 mg/g); baicalein (RT= 20.4 min; 7.67 ± 0.89 mg/g); wogonin (RT= 23.7 min; 0.65 ± 0.06 mg/g). The commercial sample appeared to be free from adulteration with germander (verbascoside was not detected; RT= 9.1 minutes) and its phytochemical profile was consistent with that of the *S. lateriflora* reference standard (Figure 3: A & B).

[1]Retention time (RT) is the time in minutes taken from when a sample is injected into the column until a particular compound contained in the sample is displayed as a maximum peak height.

A. **B.**

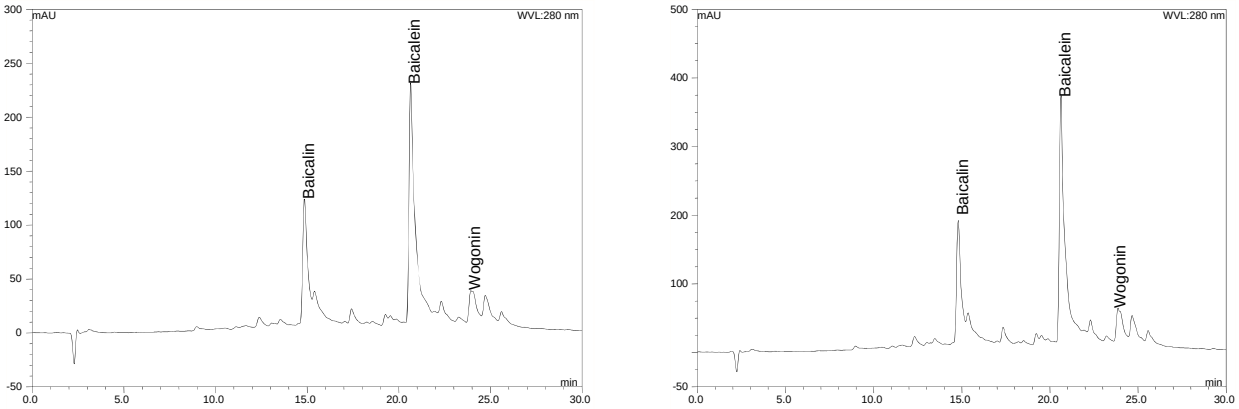


Figure 3: HPLC chromatograms demonstrating consistency between the flavonoid profiles of a commercial product of *S. lateriflora* (A) and *S. lateriflora* reference material (B).

3.2 Estimation of baicalin concentration in *S. lateriflora*

At 11.71 ± 1.16 mg/g concentration of baicalin in methanol extracts of the commercial sample of *S. lateriflora* freeze-dried aerial parts, our results compare favourably with those of other workers (Table 1).

Table 1 Comparison of baicalin concentration found in *S. lateriflora* in previous studies

***S. lateriflora* source material**	**Solvent/solute ratio**	**Concentration**	**Extraction**	**Reference**
Fresh, freeze dried	1:100	11.71 ± 1.16 mg/g	80% MeOH	Current study (Brock et al.)
Dried, powdered	1:10	21.3 mg/g*	95% EtOH	Awad et al. (2003)
Dried, powdered	1:50	40.7 mg/g*	50% EtOH	Awad et al. (2003)
Fresh, rapid heat dried	1:100	14-18 mg/g [a]	80% MeOH	Wills and Stuart (2004)
Various	1:50	1.79 ± 0.55 mg/g [b]	70% EtOH	Wohlmuth et al. (2009)
Commercial tincture	Not known	12.66 mg/ml [c] *	45% EtOH	Gao et al. (2008)

Abbreviations: MeOh = methanol; EtOH = ethanol

[a] Calculated as 40-50% of a mean concentration of 36mg/g total flavonoids from whole *S. lateriflora* at 4 different stages of growth. Of total flavonoid content of *S. lateriflora*, baicalin constituted 40 -50% (Wills and Stuart, 2004).

[b] Mean in a total of 27 authenticated samples extracted in 70% ethanol (Wohlmuth, 2011, personal communication, unreferenced) including genuine commercial *S. lateriflora* and herbarium specimens (Wohlmuth et al., 2009).

[c] The highest amount of baicalin in one of seven different products analysed.

*It is unknown whether samples were linked to voucher specimens so identity as *S. lateriflora* cannot be verified.

4. Discussion

4.1 Factors affecting flavonoid concentration

Differences between the baicalin content reported in the various studies may be due in part to differences in solvents used and water: solvent ratios. Extraction methods have an important

impact on flavonoid content and therefore quality of a commercial product. When Gao et al. (2008) compared extraction in 25% and 45% ethanol from the same batch of *S. lateriflora* plant material they found that 45% ethanol yielded around five times more flavonoids than 25% ethanol (Gao et al., 2008). Similarly, Wills and Stuart (2004) found 40 - 60% ethanol extractions from dried powder preparations yielded the maximum flavonoid content of around 70%.

An earlier study (Awad et al., 2003) using HPLC analysis also found higher ethanol concentrations extracted *S. lateriflora* flavonoids well. Baicalin content was greater at 50% than at 95% ethanol extraction (40.7 mg/g and 21.3 mg/g respectively) whereas 95% ethanol was better at extracting baicalein than was 50% ethanol (32.7 mg/g and 23.5 mg/g respectively). The analysis also indicated that an aqueous extract contained no baicalein and minimal baicalin (Awad et al., 2003).

Plant parts may also affect flavonoid content. According to Wills and Stuart (2004), when averaged over different stages of plant growth, the leaves of *S. lateriflora* have the highest concentration of flavonoids (50.8 mg/g) and the stems (21.3 mg/g) contain a lower flavonoid concentration than the roots (35.8 mg/g). At full maturity the highest baicalin content is in the leaves (54.5%), followed by the stems (45.2%) and roots (38.1%). Differences in individual flavonoid concentrations between the leaves, stems and roots result in differences in their chromatographic profiles, which can be used to assist in identification of the plant part used for medicinal purposes (Wills and Stuart, 2004).

Stability of an herbal product is important with regard to efficacy and safety and may be affected by various factors, such as pH, light, enzymatic degradation (for example due to harvesting stress, heat or insects) and temperature (Gafner and Bergeron, 2005). While heat treatment destabilises the enzymes responsible for degradation of some flavonoids, for example hydrolysis of baicalin to baicalein by endogenous beta-glucoronidase, not all *S. lateriflora* flavonoid degradation is prevented in this way. Some, such as degradation of dihydrobaicalin, can only be prevented by addition of antioxidants, indicating oxidation is also involved in flavonoid instability (Russell et al., 2003). Wills and Stuart (2004) found total flavonoid loss from dried herb extracted in 40 - 60% ethanol to be considerable, at 0.17% per day at room temperature (this would amount to over 30% loss of flavonoids in 6 months) and about 50% greater than with dried herb under the same storage conditions. We did not compare the flavonoid biomass of the reference sample with that of the freeze-dried product as we have previously observed a minimal amount of the flavonoids baicalin, baicalein and wogonin in a number of authenticated samples (linked to AHP herbarium vouchers) harvested two years or more prior to analysis, most likely indicating a significant loss of flavonoids over time. Our sample was harvested two years prior to analysis.

Although dry heat is believed to negatively affect the activity of herbal medicines, little research has been done to this affect. However, the flavonoid profile of freeze-dried extracts of *S. lateriflora* was not affected by exposure to dry heat for 8 hours at 77^{o}C (Gafner and Bergeron, 2005).

There appears to be no data available on flavonoid loss from fresh *S. lateriflora* extracted in ethanol although Russell et al. (2003) assert that fresh are more unstable than dried extractions. Future studies on this aspect are important as fresh material is believed to be most efficacious (Felter and Lloyd, 1898; Kuhn and Winston, 2001; Yarnell and Abascal, 2001).

In summary, strict quality control must be ensured before the commencement of any *in vitro* or clinical studies. This should include botanical identification to rule out adulteration with different *Scutellaria* species or with *Teucrium* spp. as has occurred in the past. Storage, harvesting, extraction and processing methods, and marc to menstrum strength should be at their

optimum for avoidance of flavonoid loss. Many companies extract using 25% ethanol in water. However, it is clear from HPLC studies (Gao et al., 2008; Wills and Stuart, 2004; Wohlmuth et al., 2009) that *S. lateriflora* flavonoid extraction and stability is poor at this strength. Gao et al. (2008) suggest that more research into the anxiolytic properties of *S. lateriflora* is needed in order to convince manufacturing companies to extract the herb at higher alcohol strength.

Additionally, empirical evidence and research have both indicated that fresh freeze-dried or fresh herb extract is likely to be more efficacious than the dried herb (Gafner and Bergeron, 2005; Kuhn and Winston, 2001; Yarnell and Abascal, 2001), due to instability of the latter and hence fewer flavonoids (Wills and Stuart, 2004). Unfortunately, freeze dried material is not widely available as it needs to be processed at harvest by immediately freezing freshly harvested plants, which have been washed in spring water, at low temperature e.g. -18^{o}C. They then undergo sublimation, a process whereby the frozen water is vaporised under vacuum. The vaporised water is passed through condenser plates, a process which converts the water vapour back to a solid and removes it from the vacuum chamber. Only the dried plant material remains and the separation process is complete. Any remaining moisture is removed (around 5%) by gentle heat. Active constituents remain, including flavonoids, enzymes, oils and fatty acids. The colour, smell and taste of the plant material are not affected and it has a long shelf life (Eclectic Institute Inc., 2003; Luthria, 2006)

4.2 The importance of using voucher specimens in laboratory studies of *S. lateriflora*

It is important when carrying out a phytochemical analysis study that samples are compared with authenticated reference material. Problems of substitution may have been exacerbated by authentication problems in the scientific literature, some relating to analyses of the chemical composition of *S. lateriflora* and the generation of non-reproducible data. For example, according to Yaghmai (1988) *S. lateriflora* grows on riverbanks and marshes in northern Iran. However, the species that is known to grow in this region is *S. pinnatifida* (Barceloux, 2008). When Wohlmuth et al. (2011) compared the composition of essential oil from morphologically and LC-MS authenticated *S. lateriflora* specimens grown in North America and Australia with that reported from the Iranian samples the results differed regarding which were the dominant oils (Wohlmuth et al., 2011).

Another study (Zhang et al., 2009), in which the chemical ingredients of *S. lateriflora* were characterised, used 10 samples of plant material purchased from herbal product stores and companies. There is therefore no guarantee that any of this material, including a whole extract against which other samples were compared, was authentic *S. lateriflora*. The authors even used one of the samples to test for anti-convulsant activity in a rat seizure model (Zhang et al., 2009) without first verifying the material against a voucher specimen. In agreement with Rader et al. (2007), the results of a study may be invalid if the botanical material tested has not been authenticated as it may have been misidentified or be adulterated with other species.

5. Conclusions

S. lateriflora has been beset by problems of substitution and adulteration for many years and must therefore be rigorously authenticated morphologically by a trained botanist (macroscopic and microscopic) and, to confirm quality and identity, by HPLC or other advanced laboratory methods (such as gas chromatography-mass spectrometry (GC-MS), or DNA analysis) against a voucher specimen before entry onto the market, whether for commercial or scientific use. Although herbal practitioners do not have access to HPLC technology they can ensure that, when purchasing herbal supplies, they receive a positive statement regarding appropriate identification from growers or manufacturers. Likewise, instead of relying on text-book descriptions, small-

scale herb farmers should be able to verify their herbs or seeds have been botanically identified by a trained botanist at source. Furthermore, plant material or products should not be subject to deterioration.

Our study represents a simple and effective method for assessing the authenticity of a sample of the herb and demonstrates the importance, when carrying out a phytochemical analysis study, of comparing samples with authenticated reference material. The HPLC method is rapid, simple and robust, would enable the efficient screening of large numbers of commercial products and is not resource intensive. The identity and safety of a commercial product of *S. lateriflora* to be used in a clinical study have been verified through the matched patterns of investigated flavonoid biomarkers in the reference material and freeze-dried sample. Additionally, HPLC chromatograms enabled the quantification of flavonoids (baicalin; baicalein and wogonin) in the Eclectic Institute product, which proved to be free from adulterants (germander) and/or other skullcap species. The results justify the potential use of the freeze-dried product in future clinical efficacy studies.

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