



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading

doi:

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Screening of different East Himalayan species and populations of *Swertia* L. based on exomorphology and mangiferin content

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ARTICLE INFO

Article history:

Received 30 September 2012

Received in revised form 3 October 2012

Accepted 1 December 2012

Available online 28 December 2012

Keywords:

Swertia chirata

Exomorphology

Mangiferin

HPTLC

Adulteration

ABSTRACT

Objective: The present report embodies detailed diagnostic features of five important Red listed *Swertia* species for easy and authentic identification along with HPTLC fingerprinting using the c–glucoxanthone mangiferin as a biomarker. The key objectives of this study were to reduce indiscriminate harvesting of different *Swertia* species from its natural habitat and the development of a reliable kit based on HPTLC fingerprinting to reduce adulteration in commercial trade. **Methods:** Chromatography was performed on Silica gel 60 F254 TLC plates with ethyl acetate–glacial acetic acid–formic acid– water– 100:11.0:11.0:26 as mobile phase. Densitometric measurement was performed at $\lambda = 254$ nm. The method was found to be simple, reliable, precise and convenient for routine analysis. **Results:** Although mangiferin was detected in substantial amount from all populations of *Swertia chirata* irrespective of their geographical locations and to a lesser degree in *Swertia nervosa*, the biomarker was totally absent in *Swertia bimaculata*, *Swertia dilatata* and *Swertia paniculata*. **Conclusions:** Floral morphology can be effectively used for the construction of an easy identification tool that can be utilized at the field level to screen out *Swertia chirata* from other closely related species that flower at the same time along with *Swertia chirata*. High Performance TLC using mangiferin as a biomarker can be utilized for quality screening and checking adulteration among different species and populations of *Swertia*.

1. Introduction

Swertia chirata (Roxb. ex Fleming) H. Karst known commonly as Chirata [Chiretta (trade name), Chirayita and Chirato (in Nepal)] is a critically endangered temperate Himalayan medicinal herb (occurring between 1,200–3,000m and above) that has been mentioned as a potent herbal drug in Indian traditional systems of medicine (Ayurvedic, Unani and Siddha) and also in American and British pharmacopeias. *Chirata* is one the most reputed herbal drugs used extensively for the treatment of various health ailments including liver disorders, malaria, gastrointestinal infection and diabetes [1]. Reputed herbal medicines like Ayush–64, Diabecon, Menstrual syrup, Sudarshan churna and Melicon V ointment contain *S. chirata* extracts in different amounts [2]. The important constituents of this plant are iridoids, xanthenes, xanthone glycosides, flavonoids and triterpenoids [3]. Xanthenes comprises a large group of secondary metabolites and studies reveal that out of

the available xanthenes, the c–glucoxanthone mangiferin exhibits diverse pharmacological activities like anti–diabetic [4], anti–HIV [5], anti–cancer [6], immunomodulatory [7] and anti–inflammatory properties [8] as well as antiproliferative, diuretic and antioxidant properties [9,10,11]. Ever increasing demand in the herbal drug market coupled with poor seed germination and rapid overexploitation from its natural habitat has led the Medicinal Plant Board of India to declare *S. chirata* as an endangered and priority plant for conservation. *Swertia* is a diverse genus of the family Gentianaceae. Different species belonging to the genus exhibit few overlapping characters as well as a wide range of morphological variations within and among the populations resulting in considerable uncertainty about the delimitation of different species. Due to lack of correct information, lack of proper authentication process and accidental substitution or intentional adulteration with other inferior species, a lot of confusion still prevails in the Indian herbal drug market regarding the true identity of Chirata. It has been reported that *Swertia dilatata*, *Swertia bimaculata*, *Swertia paniculata*, *Swertia alata*, *Swertia minor*, *Swertia nervosa* and *Swertia angustifolia* are common less bitter adulterants found with true chirata [2]. Taking into account the widespread use and pharmacological importance of *S.*

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chirata and the issue of adulteration in commercial trade a strong need was felt to develop a reliable and affordable screening system based on comparative morphology and chromatographic fingerprinting that can accurately screen elite populations of *Swertia chirata* and reduce adulteration in commercial trade. The presence of C- glucoxanthone mangiferin was initially reported from the leaves and bark of *Mangifera indica* (Anacardiaceae) [12]. But at present the occurrence of mangiferin has been reported from many phylogenetically distant families like Hippocrateaceae [13], Rubiaceae [14] and Gentianaceae [15], the family of *Swertia*. Mangiferin thus can be used as an effective biomarker for distinguishing different species/varieties/populations of *Swertia*. Mangiferin has been detected and estimated previously in *Swertia davidi* and *Swertia chirata* using HPLC and LC-MS [15, 16]. HPTLC based detection of amarogentin and *swertiamarin* from West Himalayan populations of *Swertia chirata* has also been reported previously [17]. However no systematic screening with any biomarker has been carried out involving different *Swertia* species/populations growing in the temperate regions of Eastern Himalaya. Thus, considering the continuous loss of valuable wild germplasms, preexisting morphotaxonomic confusion regarding the identity of closely related *Swertia* species, increasing adulteration in commercial trade and non availability of affordable screening methods, the primary objectives of this study was to separate *S. chirata* from other less bitter adulterants based on morphology and fingerprinting pattern and secondarily to screen and identify elite East Himalayan populations of *S. chirata* in terms of mangiferin content.

2. Materials and methods

2.1. Sample collection & Morphological studies

Different species and populations of *Swertia* were collected from three states of India viz, Darjeeling District of West Bengal, Sikkim and Arunachal Pradesh (Tables: 1 & 5). Majority of the samples were collected in vegetative state as well in the post flowering stage. The specimens were identified and authenticated on the basis of morphological characters and by direct comparison of herbarium specimens available at the Central National Herbarium (Botanical Survey of India, Howrah). Voucher specimens are available at the Department of Botany, Presidency University, Kolkata, India. Comparative morphological studies of the different species and populations were carried out from live materials and also from herbarium specimens available at the Central National Herbarium

2.2. Reagents and standard solution

Standard mangiferin was obtained from Sigma Aldrich (USA). All chemicals used in the experiments were of HPLC grade [E. Merck (Mumbai, India)].

2.3. Sample preparation

Leaf samples were carefully washed, oven dried (60 ± 2) °C and then powdered in a grinder separately. Powdered leaves

(1 g) for all four species and populations were separately extracted with methanol (2×20 ml, thrice for each sample) for 24 hrs under constant agitation (150 rpm). The extracts were pooled and filtered through Whatman No: 1 filter paper (separately for each species/population) and finally evaporated under vacuum using a rotary evaporator (Eyela, N-1100, China) to furnish a solid mass of extract. For each sample the dried methanolic extracts were dissolved in 10 ml methanol and transferred in 10 ml graduated test tubes.

2.4. Preparation of standard solution

The standard solution was prepared by dissolving 3 mg of the pure mangiferin in 30 ml of methanol (0.1 mg ml^{-1}).

2.5. Quantitative estimation of mangiferin by HPTLC

2.5.1. Chromatography

The HPTLC system consisted of a CAMAG (Muttentz, Switzerland) Linomat-5 automatic sample applicator and CAMAG TLC scanner-3 equipped with CATS software (version:4.03). The stationary phase consisted of pre-coated silica gel 60 F254 TLC plates (20 cm \times 10 cm; with 0.25 mm layer thickness: Merck KGaA; 1.05554. 0007). Known amounts of samples and standard were applied to the layers as bands 6 mm wide, 10 mm from the bottom of the plate, by use of a CAMAG (Muttentz, Switzerland) Linomat 5 automated TLC applicator with nitrogen flow. Delivery from the syringe was always $100 \mu\text{L s}^{-1}$. Plates were developed to a distance of 8 cm, at room temperature (22 ± 2) °C and 50% relative humidity with ethyl acetate-glacial acetic acid-formic acid water-100:11.0:11.0:26 (v/v) as mobile phase, in a CAMAG twin-trough glass chamber previously saturated with mobile phase vapor for 15 min. After development the plates were dried by using hair drier at 65°C for 15 min. Peak areas for the sample and standards were recorded by densitometry in absorbance-reflectance mode at 254 nm, slit width 5 mm \times 0.45 mm, scanning speed 20 mm/s and data resolution 100 $\mu\text{m/step}$ by help of a CAMAG Model-3 TLC scanner. Data acquisition and processing were performed with WINCATS 4 software.

2.5.2. Preparation of standard curve

For preparation of calibration curve and to assess the linearity, different concentrations of the marker stock solution [5 μL (0.5 μg), 10 μL (1.0 μg), 15 μL (1.5 μg), 20 μL (2.0 μg), 25 μL (2.5 μg)] were applied in different tracks as a bands by Linomat v applicator to furnish amounts in the range 0.5 – 2.5 $\mu\text{g/ band}$. Peak areas were plotted against the corresponding concentrations and regression analysis was performed to generate the calibration equation.

2.5.3. Scanning

The plates were kept in the above mentioned solvent system and allowed to run up to a distance of 8 cm (in ascending mode). After drying, they were scanned densitometrically at 254 nm.

2.6. Method development and validation

Repeatability: The plant material was extracted by the above mentioned procedure and analyzed in triplicate.

Reproducibility: In order to access the reproducibility of the method identical volumes of the standard solution (1 μ g in 1 μ L) were applied five times on HPTLC plates and analyzed by densitometry.

Linearity range: Calibration graphs for HPTLC were recorded with sample amounts ranging from 0.5–2.5 μ g.

2.7. Method validation: The HPTLC method developed was validated for the following parameters.

2.7.1. Sensitivity

The sensitivity of the method was determined with respect to Limit of Detection (LOD) and Limit of Quantification (LOQ). The standard solutions were spotted in the range from 0.5–2.5 μ g. The LOD and LOQ were calculated on the basis of signal to noise ratio.

2.7.2. Specificity

Specificity of the method was ascertained by analyzing the standard and sample solutions. The bands of mangiferin in the samples were confirmed by comparing their R_f values and overlaid spectra of the spotted bands with standard.

2.7.3. Accuracy

The accuracy of the analytical procedure was evaluated using the recovery test. This involved the addition of known quantities of the reference standard compound taken from stock solution to one of the pre-analyzed sample (SC-4). Three concentration levels were tested at three levels (low, middle and high). At each level, samples were prepared in triplicate and analyzed according to the procedure described previously. Accuracy was expressed as percentage (observed concentration \times 100 / theoretical concentration).

2.7.4. Precision

Repeatability: Repeatability expresses the precision of the method under the same operating conditions over a short interval of time. It is also termed intra day precision.

Intermediate precision: Intermediate precision express the precision variation within laboratory in different days, different analysts or different equipments and is expresses as % R.S.D.

3. Results

3.1. Diagnostic characters based on morphology

Comparative morphological characters of the different species and populations collected across three Indian states are summarized in Table 1. Considerable heterogeneity was observed in vegetative as well as floral morphology among different species and populations of *Swertia*. It appears that the flowers of the five collected species can be categorized into either pentamerous or strongly tetramerous groups. The pentamerous group consisted of *S. bimaculata*, *S. dilatata* and *S. paniculata* while the tetramerous group comprised of *S. nervosa* and *S. chirata* (Figure 1 A–H; Table 1). Among all the collected species, *Swertia bimaculata* was the most abundant one. This species was characterized by the presence of large pentamerous (often heptamerous) flowers (30–35 mm long) with slightly connate calyx and a rotate

corolla with characteristic black spots and 2 distinct green spots on each lobe (Figure 1A–C). Interestingly, in all the populations collected from Arunachal Pradesh the two spots were merged (Figure 1C). Flowers of *Swertia dilatata* was characterized by the presence of ovate to triangular calyx lobes and rotate corolla lobes with a characteristic dark purple band encircling 2 green dots (Figure 1D). Considerable variation was also noticed among the different populations collected. The populations collected from Lava exhibited serullate corolla and puberulous stigmatic lobes (Figure 1E) not reported earlier. The characteristic dark purple band was not noticed in Lava populations. Flowers of *Swertia paniculata* were strongly pentamerous with campanulate calyx lobes and a rotate pale green corolla with a characteristic dark purple band and one horse-shoe shaped nectary per lobe (Figure 1F). *S. nervosa* was characterized by the presence of strongly tetramerous flowers with calyx lobes larger than corolla lobes and the presence of a single pocket like gland in each corolla lobe (Figure 1G). Flowers of *Swertia chirata* however differed considerably from all the related species which are used commonly as adulterants. *Swertia chirata* can be characterized by the presence of much smaller tetramerous flowers (6–10 mm long) with nearly equal, slightly connate calyx and rotate 4 lobed dark brown to red corolla. Presence of 2 distinct oblong glands was also noticed in each corolla lobe (Figure 1H). The exomorphological variations observed among the different species and populations of *Swertia* can be used effectively for construction of an easy and reliable key for authentic botanical identification.

3.2. Sensitivity

Under the proposed experimental conditions, the lowest amount of compounds which could be detected (LOD) was 30 ng/spot and the lowest amount of compound which could be quantified (LOQ) was found to be 90 ng/spot. The calibration curve was found to be linear in the range of 500–2500 ng/spot with good correlation coefficient (0.99) (Table 2; Figure 2).

3.3. Accuracy

The mean recovery values for mangiferin was (98.64%, 98.36%, 99.72% as mentioned in Table:3) from lowest to highest level spiked i.e 50%, 100%, 150% respectively (Table 3).

3.4. Specificity

The bands of mangiferin in the samples were confirmed by comparing the R_f values and overlaid spectra of the spotted bands with that of the standard. The peak purity of mangiferin was confirmed by comparing the UV spectra at three different levels viz. peak(s) apex and peak end position with the aid of WINCATS 4 software (Figure 3).

3.5. Precision

3.5.1. Repeatability (intra day precision)

For repeatability, three samples of same concentration were prepared as per method and analyzed by proposed method to determine the variations arising from the method and

Table 1Comparative exomorphology of some East Himalayan *Swertia* species for easy and authentic in-situ identification.

Species	Places of collection	No: of populations collected/ surveyed	Brief Exomorphology	
			Vegetative morphology	Floral morphology
<i>S. bimaculata</i>	Senchal, Lava (Darjeeling) Ramalingam (Arunachal) & Rabangla (Sikkim)	04	Erect dwarf shrub, 0.3 – 1.5 m high with light yellow fibrous roots. Stems 3 – 5 mm in diam., terete, subquadrangular to winged	Inflorescence panicles of cymes, lax, spreading, each raceme 30 – 40 mm long, 5 – 6-flowered, glabrous, winged, 2-bracteate. Flowers pentamerous to heptamerous (30–35 mm long) [Figure 1 A–C]. Calyx lobes 5 – 6 mm long, lobes 5, slightly connate at base, subequal, linear-elliptic, persistent in fruit. Corolla rotate, 18 – 22 mm across, greenish white with black spots in their upper half of each lobe, lobes 5, elliptic to oblong-obovate. Flowering: peak in late September to early October. Fruiting: late October – November.
<i>S. dilatata</i>	Lava (Darjeeling) & Bomdila (Arunachal)	03	Erect dwarf shrub up to 80 cm high with light yellow fibrous roots. Stems 2 – 3.5 mm in diam., terete with ridges, branched, glabrous.	Inflorescence panicles of cymes, each raceme 30 – 100 mm long, 6 – 15-flowered, glabrous, terete, 2-bracteate. Flowers strongly pentamerous (12–18 mm long). Calyx lobes campanulate, c. 12 mm long, lobes 5, basally connate, unequal, ovate-triangular. Corolla rotate, 12 – 19 mm across, pale yellow-green to light green with dark purple band encircling 2 green dots and one horseshoe-shaped naked nectary gland per lobe. [Figure 1 D&E]. Flowering: September – October. Fruiting: October – November
<i>S. paniculata</i>	Tiger Hill (Darjeeling)	01	Erect dwarf shrub up to 50 cm high. With light yellow fibrous roots. Stem 1–1.5mm in diameter, 4-lineolate to 4 winged.	Inflorescence panicles of cymes, each raceme 25 – 60 mm long, 5 – 10-flowered, sparsely pubescent, quadrangular, 2-bracteate. Flowers strongly pentamerous (8 – 14 mm long). Calyx lobes campanulate, c. 12 mm long, lobes 5, basally connate, unequal, ovate-triangular. Corolla rotate, 12 – 19 mm across, pale yellow-green to light green with dark purple band encircling 2 green dots and one horseshoe-shaped naked nectary gland per lobe. [Figure 1F]. Flowering: peak in mid October to November. Fruiting: October – November
<i>S. nervosa</i>	Lava (Darjeeling)	01	Erect dwarf shrub up to 80 cm high with light yellow fibrous roots. Stems 3 – 5 mm in diam., 4-winged, often narrowly winged on angles, branched.	Inflorescence panicles of cymes, each raceme 22 – 42 mm long, 8 – 15-flowered, glabrous, 4-winged, 2-bracteate. Flowers strongly tetramerous, 12 – 23 mm long incl. pedicels. Calyx lobes longer than corolla lobes (c. 16 mm) slightly connate at base, subequal, oblong-linear, persistent in fruit. Corolla rotate, 14 – 16 mm across, light green with purple markings, lobes 4, shortly acuminate at apex, glabrous with 1 pocket-like gland near the base of each lobe. [Figure 1 G]. Flowering: peak in mid September to early October. Fruiting: late October – November
<i>S. chirata</i>	Lava & Ghum (Darjeeling); Hilley (Sikkim); Lama Camp, Eagle Nest, Ramalingam & Bomdila (Arunachal)	07	Erect dwarf shrub up to 90 cm high. Roots light yellow, fibrous. Stems 2 – 2.5 mm in diam., terete to winged, twigs rarely quadrangular.	Inflorescence panicles of cymes, each raceme 12 – 40 mm long, 12 – 25-flowered, glabrous, winged, 2-bracteate. Flowers strongly tetramerous (6 – 10 mm long). Calyx lobes c. 4 mm long, lobes 4, slightly connate at base, equal, oblong-linear, persistent in fruit, shortly acuminate at apex, glabrous. Corolla rotate, 10 – 11 mm across, 4 lobed, ovate to ovate-elliptic, persistent in fruit [Figure 1 H]. Flowering: peak in mid September to early October. Fruiting: late October – November

expressed as % RSD. Percentage R.S.D. of method precision was in the range of 1.34–1.65%. (Table 4)

Table 2

Method validation parameters for the quantification of mangiferin.

Parameters	Value
R_f	0.5
Linearity range (ng/spot)	500–2500
Regression Equation (area)	$Y = 2135 + 7.484X$
LOD	30
LOQ	90
SD	5.36
Linearity R^2 (Correlation Coefficient)	0.99

3.5.2. Repeatability (intermediate precision)

Inter day precision or intermediate precision express within laboratory variations in different days. % RSD was found to vary from 1.23–1.56 % (Table 4).

3.6. Detection of mangiferin in different species and populations of *Swertia*

HPTLC studies revealed that the solvent system ethyl acetate–glacial acetic acid–formic acid water–100:11.0:11.0:26 (v/v) was ideal and gave a single compact spot with a R_f 0.5 for the marker and well resolved spots for the test samples. The spots of the chromatogram were visualized both in UV at 254 nm and after derivatization with 5%

Table 3
Recovery study of mangiferin by proposed HPTLC method.

Marker compound	amount present in the sample Sc-4($\mu\text{g mg}^{-1}$)	amount added(μg)	amount founda (μg)	recovery%	average recovery%
mangiferin	43.7	20	62.83 \pm 0.34	98.64	98.9
	43.7	40	82.32 \pm 0.45	98.36	
	43.7	60	103.40 \pm 0.32	99.72	

a mean \pm standard deviation (SD, n=3)



Figure 1. Floral morphology of different species and populations of *Swertia* L.
 A: Pentamerous flower of *Swertia bimaculata* with characteristic black spots and two distinct green spots (indicated by arrow) [bar: 30mm].
 B: Heptamerous flower of *Swertia bimaculata* with characteristic black spots and two distinct green spots [bar: 30mm].
 C: *Swertia bimaculata* flower (Arunachal population) with two green spots merged together (indicated by arrow) [bar: 30mm].
 D: Pentamerous flower of *Swertia dilatata* (Arunachal population) showing a characteristic dark purple band encircling 2 green dots [bar: 20mm].
 E: Pentamerous flower of *Swertia dilatata* (Lava population) with–out the characteristic dark purple band [bar: 20mm].
 F: Pentamerous flower of *Swertia paniculata* (Tiger Hill population) with a characteristic dark purple band and one horse–shoe shaped nectary per lobe [bar: 20mm].
 G: Strongly tetramerous flower of *Swertia nervosa* (Lava population) showing the presence of a single pocket like gland in each corolla lobe (indicated by arrow) [bar: 20mm].
 H: Tetramerous flower of *Swertia chirata* showing lobed dark brown to red corolla and the presence of 2 distinct oblong glands (indicated by arrow) [bar: 20mm].

Table 4
Intra– and Inter– day precision study (intermediate precision) the quantification of mangiferin.

Marker compound	Concentration($\mu\text{g/spot}$)	Intra–day	Inter– day
Mangiferin	20	1.34	1.45
	40	1.65	1.23
	60	1.39	1.56

a Relative standard deviation (% RSD , n=3)

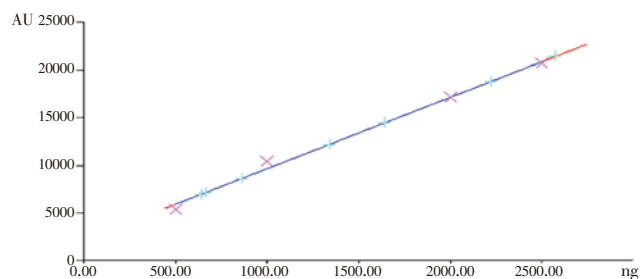


Figure 2. Calibration plot for Mangiferin in the 0.5–2.5 μg (500–2500 ng) per spot range.

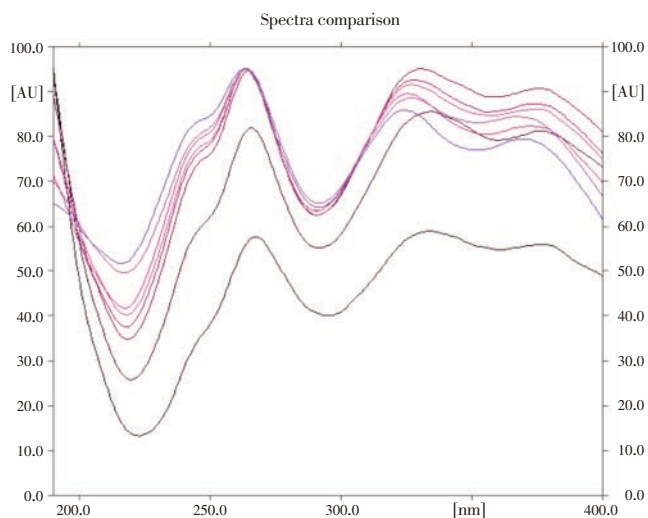


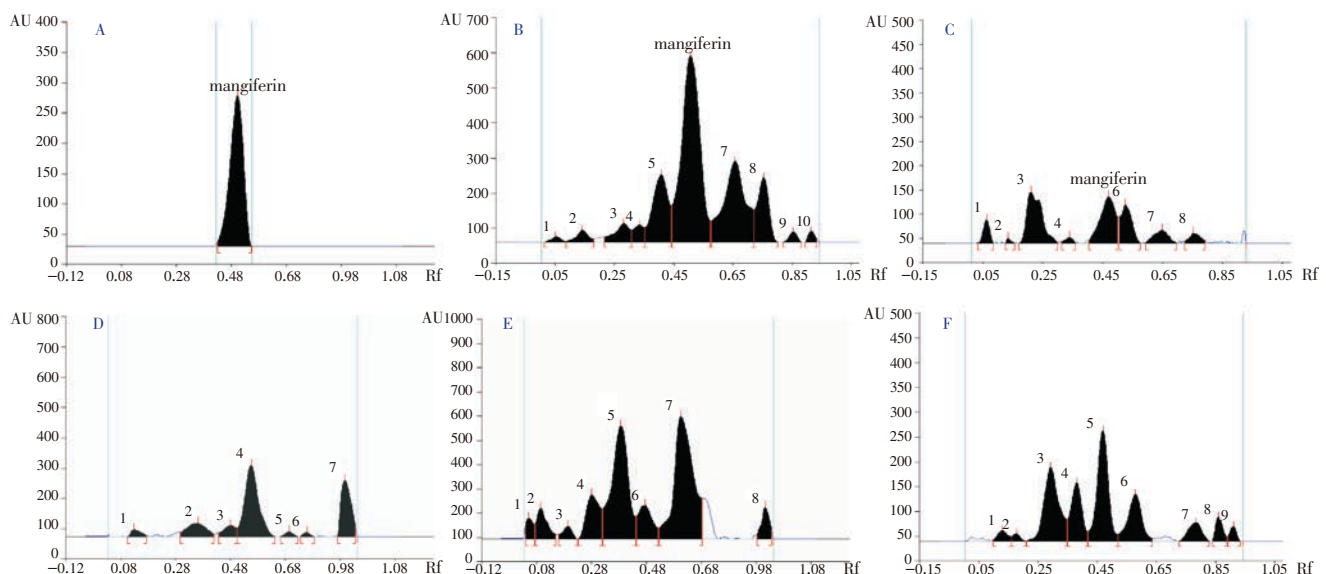
Figure 3. Overlay of spectra of standard mangiferin (pink lines) and mangiferin in *Swertia* samples (violet and dark brown lines)

Table 5

Mangiferin content in leaf parts of different populations of *Swertia chirata*, *Swertia bimaculata*, *Swertia nervosa*, *Swertia paniculata* and *Swertia dilatata* collected from West Bengal, Sikkim and Arunachal Pradesh.

Species	Population No:	Place of collection	Altitude & Co-ordinates	% of Mangiferin in leaves collected from different growth stages	
				Vegetative	Flowering
<i>Swertia bimaculata</i>	SB1	Senchal (Darjeeling)	2490 m 27.3 ° N 88.160E	N.D	N.D
<i>Swertia bimaculata</i>	SB2	Lava (Darjeeling)	2200 m 27.60N 88.70	N.D	N.D
<i>Swertia bimaculata</i>	SB3	Ramalingam(Arunachal Pradesh)	1800 m 27.110N 92.280E	N.D	N.D
<i>Swertia bimaculata</i>	SB4	Rabangla (Sikkim)	2133 m 27.17 0N 88.210E	N.D	N.D
<i>Swertia dilatata</i>	SD1	Lava (Darjeeling)	2200 m 27.60N 88.70E	N.D	N.D
<i>Swertia dilatata</i>	SD2	Bomdila (Arunachal)	2530 m 27.150N 92.240E	N.D	N.D
<i>Swertia dilatata</i>	SD3	Eagle nest Arunachal)	2850 m 27.70N 92.280E	N.D	N.D
<i>Swertia nervosa</i>	SN1	Lava (Darjeeling)	2200 m 27.60N 88.70E	0.789	1.12
<i>Swertia paniculata</i>	SP1	Tiger Hill Darjeeling)	2555m 27.00N 88.280E	N.D	N.D
<i>Swertia chirata</i>	SC1	Lava (Darjeeling)	2200 m 27.60N 88.70E	1.786	2.61
<i>Swertia chirata</i>	SC2	Chum (Darjeeling)	2438 m 27.420N 88.150E	2.679	3.124
<i>Swertia chirata</i>	SC3	Hilley (Sikkim)	3200 m 27 ° 11'6"N 88 ° 7'21"E	1.236	2.7
<i>Swertia chirata</i>	SC4	Lama camp (Arunachal)	2500 m 27.10N 92.280E	3.46	4.37
<i>Swertia chirata</i>	SC5	Ramalingam (Arunachal)	1800 m 27.110N 92.280E	2.19	3.318
<i>Swertia chirata</i>	SC6	Eagle nest Arunachal)	2850 m 27.70N 92.280E	N.C	3.591
<i>Swertia chirata</i>	SC7	Bomdila (Arunachal)	2530 m 27.150N 92.240E	N.C	3.325

N.D: Not Detected; N.C: Not Collected

**Figure 4.**

A: Chromatogram obtained from Standard mangiferin.

B: Chromatogram obtained from methanolic leaf extract of Arunachal Pradesh sample (SC-4; *Swertia chirata*).

C: Chromatogram obtained from methanolic leaf extract of Darjeeling sample (SN-1; *Swertia nervosa*).

D: Chromatogram obtained from methanolic leaf extract of *Swertia dilatata* showing the absence of mangiferin (SD- 1; Lava sample).

E: Chromatogram obtained from methanolic leaf extract of *Swertia paniculata* showing the absence of mangiferin (SP-1; Tiger Hill sample).

F: Chromatogram obtained from methanolic leaf extract of *Swertia bimaculata* showing the absence of mangiferin (SB-3; Arunachal Pradesh sample).

methanolic H₂SO₄. Initially HPTLC finger printing was done on the marker compound and parameters were optimized. Under identical parameters, the finger printing pattern of the test samples was recorded. The marker compound was found to be present at Rf 0.5. The finger printing pattern of the leaf samples of different species and populations of *Swertia* are depicted in Figure 4 (a-f) and quantitative data on mangiferin content of leaf samples are summarized in Table 5.

4. Discussion

Results clearly reveal that floral morphology can be effectively used for the construction of an easy identification tool that can be utilized at the field level to screen out *Swertia chirata* from other closely related, less bitter adulterant species that flower at the same time along with *Swertia chirata*. The results also emphasizes the fact that High Performance TLC using mangiferin as a biomarker can be utilized for quality screening among different species and populations of *Swertia*. The results summarized in Table: 5 are indicative of the fact that diversity in mangiferin content exists within and between the species. Considerable

heterogeneity has also been observed among the different populations of *S. chirata*. The bioactive marker mangiferin was detected in leaf samples of *S. chirata* and *S. nervosa* only. While it was totally absent in all the populations of *Swertia bimaculata*, *Swertia dilatata* and *Swertia paniculata* irrespective of their geographical location Figure 4 (Figs: a–f). Also it is noteworthy to mention that leaf samples collected in the post flowering stage contained more mangiferin than the samples collected in the vegetative state. Harvesting of crude drugs with higher concentration of active principle is a prerequisite in preparation of efficacious drugs. Maximum production of metabolites depends on age and growth phase of the plant. Studies conducted by previous workers indicate that the reproductive stage of a plant is characterized by high increment in levels of secondary metabolites [18–21]. Thus, it can be concluded that the flowering stage (characterized by the highest content of mangiferin) can be considered as the best stage for harvesting. The bioactive marker mangiferin has been detected and quantified previously from aerial parts of two Chinese *Swertia* species (viz, *Swertia davidi* and *Swertia mussotti*) [15, 22] but profiling of different East Himalayan *Swertia* species/populations at different developmental stages has not been attempted previously. From the data it is significant to note that *Swertia chirata* populations collected from different locations of Arunachal Pradesh contains higher mangiferin in comparison to the populations collected from West Bengal (Darjeeling district) and Sikkim. Among all the populations collected from Arunachal Pradesh SC– 4 was found to contain maximum amount of mangiferin (4.37 %) followed by SC–6, SC– 7 and SC–5 respectively. These elite populations need attention for in–vivo as well as in– vitro conservation. *Swertia* is an important medicinal plant of Gentianaceae which not only contains mangiferin but a plethora of important bioactives (like amarogentin *swertiamarin*, amaroswerin, swerchirin etc). Although out of nearly 32 available Indian species *Swertia chirata* is considered to be superior in medicinal quality, unexplored species like *S. nervosa* may also emerge as important source of valuable bioactives in future, if exploited properly.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are grateful to the Council of Scientific & Industrial Research (CSIR), New Delhi, India, for financial support [Project No: 38(1211)/09/EMR–II, 2009].

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