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Variation in the active compounds among natural populations of *Swertia cordata*

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Swertia cordata (Wall. ex G. Don) C.B. Clarke is an important medicinal plant of the family Gentianaceae and is found distributed throughout temperate regions of the Himalaya. The species is used in various ethno-medicinal systems and as an adulterant of *Swertia chirayita*. Plants collected during the flowering stage from four different populations were air dried and crushed to make extract. The extract was analyzed using HPLC for the presence of bioactive molecules. Quantitative variations exist in the bioactive compounds among different populations. Variations among studied populations are due to long term adaptation in particular ecological niche. As *S. chirayita* has been banned for collection due to rarity in natural populations, *S. cordata* may be used as an alternate source. Presence of amarogentin, amaroswerin, and mangiferin increases the medicinal importance along with further research on chemistry, pharmacology, domestication, and crop improvement aspects of *S. cordata*.

Keyword: Bioactive molecules, Chemotype, Ethno-medicine, Gentianaceae, Secoiridoid glucosides, TSM.
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Introduction

In recent times, much attention has been paid on herbal products for the treatments of various ailments that increased demand of natural products in the market. Higher demand and lower supply increased adulteration in herbal products in the market samples. Amarogentin, amaroswerin, and mangiferin are such important bioactive molecules of *Swertia chirayita* (family Gentianaceae), but their supply is very limited from natural source. Medicinal properties of genus *Swertia* is well documented in British pharmacopoeia, American pharmacopoeia and different traditional systems of medicines (TSM) such as the Ayurveda, Unani, Siddha, and ethno-medicinal system. *Swertia* has an established international market, which is increasing 10 % annually[1] and India needs about 711 Metric Tons (Mt) annual raw drug supply to fulfill domestic and export requirements[2]. However, plants available in the market many a times is adulterated and substituted by close relatives of chirayita[2,3]. To fulfill market demand, *S. chirayita* was extracted from natural populations, which ultimately decreased its population and species became critically endangered (CR). Being a CR species, attention has increased on adulterant of *S. chirayita*. Therefore, a strong need was felt to screen different species of this genus from different phyto-geographical locations[1] to fulfill market demand.

Swertia cordata (Wall. ex G. Don) C.B. Clarke is one of the important medicinal plant species distributed between 2400-3600 m asl. The plants are annual, erect, solitary or tufted branched herb. Leaves are usually sessile, opposite, ovate to chordate in shape. Plant produce flowers during the month of August and many, minute seeds inside capsule during the months of September-October. The species was found useful in the treatment of malarial fever[4] along with various other medicinal uses in TSM. Antioxidant, antibacterial, and anti-diabetic properties of this species had also been reported recently[5]. Mangiferin reported from *S. cordata*[6] is the most active compound till date along with swertianolin, ursolic acid, etc. Investigations revealed that major bioactive constituents in the genus *Swertia* are mangiferin, amaroswerin and amarogentin[1,7]. Therefore, phytochemical investigation was carried out on under examined species of the genus *Swertia* from North-West Himalaya to find out an alternate source of these bioactive molecules from a wide

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geographical region. Present study revealed wide range of variation in active compounds among natural populations of *S. cordata*.

Material and Methods

Plant material

Highest concentration of amaroswerin and amarogentin was recorded from aerial parts of *S. chirayita*[3], whereby harvesting during the flowering stage is believed to give the highest yield of active constituents[8]. In view of this, 20 plants of *S. cordata* were collected in the flowering stage (during the last week of August) from four different populations (covering three Himalayan states of India) i.e. Dugalbittha (2580 m asl) in Uttarakhand, Soja (2500 m asl) and Gulaba (2700 m asl) in Himachal Pradesh and Baltal (3200 m asl) in Jammu and Kashmir. The voucher specimen were authenticated by Dr. P. Prasad of Botanical Survey of India, Dehradun and deposited in same herbarium (BSD 005-008).

Solvents and chemicals

All the chemicals including solvents were of HPLC grade. Both HPLC-grade methanol and distilled ethyl alcohol were purchased from Ranbaxy chemicals (Mohali, Punjab, India) and water was purified using a Milli-Q water purification system (Millipore, Billerica, MA). Marker compounds (i.e. mangiferin, amaroswerin, and amarogentin) were isolated previously from *S. chirayita* according to the procedure described earlier[9] with slight modifications.

Preparation of analytical samples

Plants were air dried for two weeks at room temperature and crushed to make fine powder. For isolation of mangiferin, amaroswerin, and amarogentin from different samples, powdered material (5 g) was percolated in 300 mL (100x3 times) ethanol : water (1:1) for 3 h at 30±2 °C (with sonication; 3x30 min). The aqueous ethanolic extracts were filtered and dried in Rotacool system by evaporation at reduced pressure and temperature (60±2 °C). For the final removal of the moisture, 10 mL of acetone was added and extract was stored in vacuum till further analysis. The dried filtrate (20 mg) was dissolved in 1 mL methanol to yield mangiferin, amaroswerin, and amarogentin residue and 10 µL of each sample was analyzed by HPLC.

Preparation of the stock solutions and calibration curve

Stock solutions of the pure reference compounds (1000 mg/mL) were prepared in HPLC grade water and stored in a refrigerator at 4 ^{o}C. From the stock solutions, working solutions for each reference compounds were prepared by dilution with HPLC grade water. These working solutions of all the reference compounds (5 µL of each) were mixed together in equal volume for further analysis. 10 µL aliquot of this solution was used for the preparation of the calibration curve.

HPLC analysis

The marker compounds were separated and quantified by a Waters HPLC system consisting of two pumps (Waters 515) with Waters pump control module II, an automatic sampling unit (auto sampler) Waters 717^{+}, photodiode array detector (Waters 2996) and temperature control module II with Waters Empower software for data analysis and data processing was used. Extracts were separated on a Merck RP-18 column (250 mmx 4 mmx 5 µm particles, I.D.). The mobile phase was a gradient prepared from 2 % aqueous acetic acid (A) and methanol (B) in the proportion of 40:60. The flow rate was 0.7 mL/min, the run time was 30 min, and the detection wavelength was 271 nm. All the analyses were performed at 30±2 ^{o}C and the injection volume was 10 µL. Marker compounds were quantified by using an external standard method.

Results and Discussion

A standard calibration curve was generated for mangiferin, amaroswerin, and amarogentin using the linear least square regression equation derived from peak area. r^2 value for the standard plot was 0.9986 for mangiferin, 0.9997 for amaroswerin, and 0.9998 for amarogentin. Auto scale chromatogram of standard marker compounds (mangiferin, amaroswerin, and amarogentin) is presented in Fig. 1. Samples collected from four different populations' revealed presence of amaroswerin and mangiferin in different concentration, whereas amarogentin was present only in Gulaba population (Table 1). A typical HPLC chromatogram showing presence of mangiferin and amaroswerin in *S. cordata* (Fig. 2) and chromatogram from Gulaba population showing presence of mangiferin, amaroswerin, and amarogentin (Fig. 3) are also presented. Chemical structures of the isolated bioactive compounds are presented in Fig. 4.

Mangiferin in present study had earlier been reported from *S. cordata*[6] and medicinal properties are well described in literature. Mangiferin is

found to be anti-tubercular[10,11], hypoglycemic[12,13], anti-inflammatory[14,15], hepatoprotective[13], anti-oxidative[16], and anti-fungal[17] along with other pharmacological properties[18,19].

Bitter secoiridoid glucosides amaroswerin and amarogentin are identified for the first time in *S. cordata*. Amaroswerin and amarogentin were biosynthesized from 3 units of acetates and

Table 1 — Variations in the amount (mg/g, DW basis) of biologically active compounds in the aerial parts of *S. cordata* from different populations

Active principal	Populations			
	Soja (2500 m asl)	Dugalbittha (2580 m asl)	Gulaba (2700 m asl)	Baltal (3200 m asl)
Mangiferin	0.963	0.932	1.054	0.621
Amaroswerin	0.225	0.156	0.009	0.006
Amarogentin	ND	ND	0.007	ND

ND- Not detected

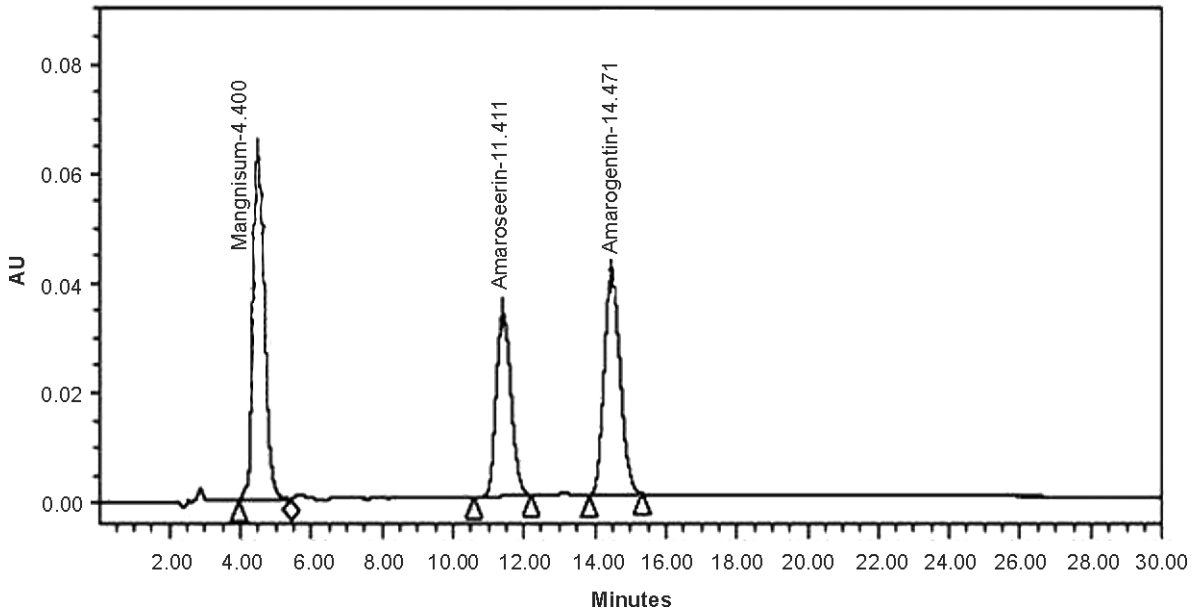

Fig. 1 — HPLC chromatogram of mangiferin, amaroswerin and amarogentin obtained from standard

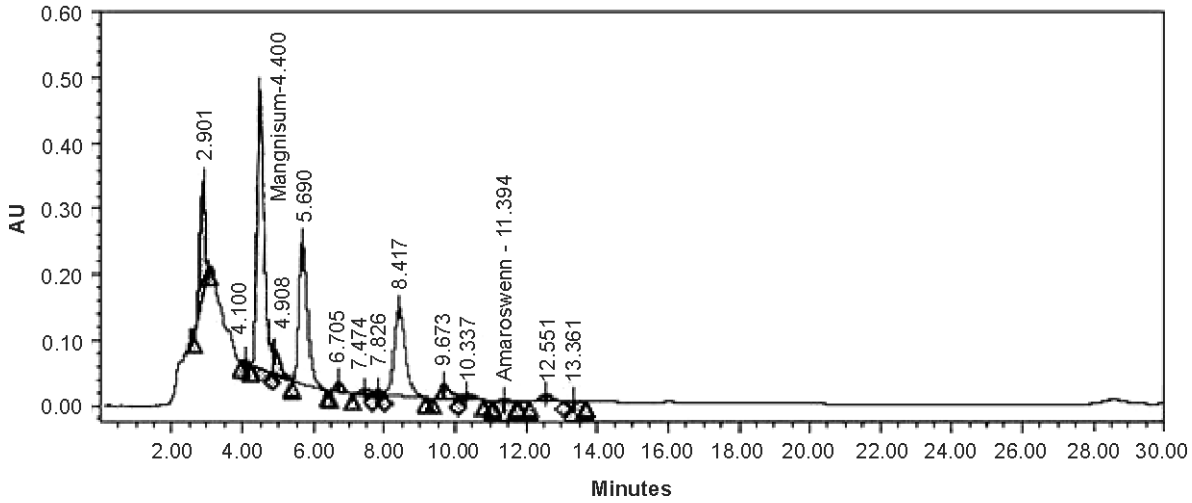

Fig. 2 — A typical HPLC chromatogram showing presence of mangiferin and amaroswerin in *S. cordata*

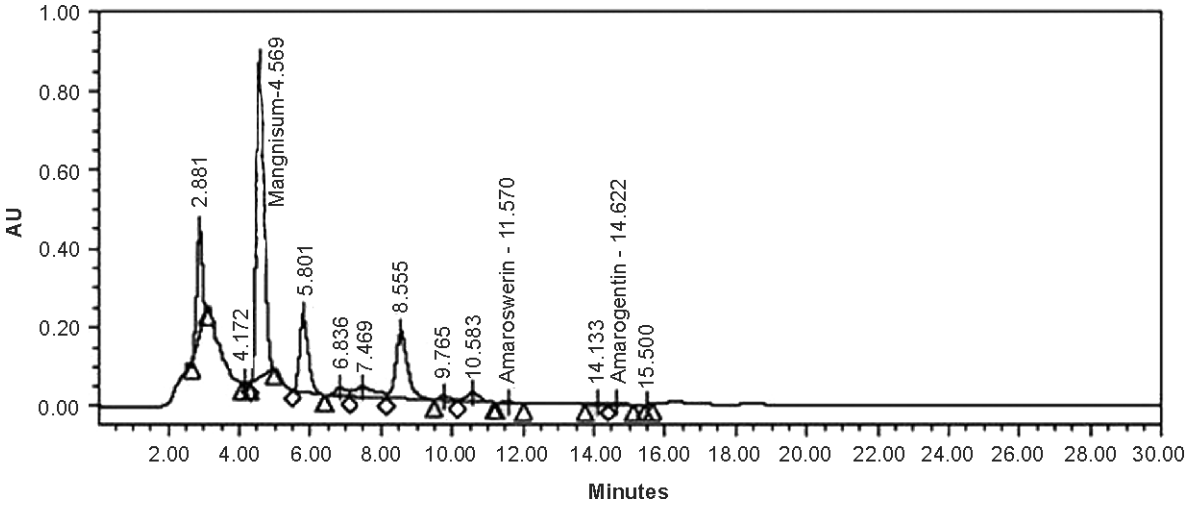

Fig. 3 —HPLC chromatogram from Gulaba population showing presence of mangiferin, amaroswerin and amarogentin in *S. cordata*

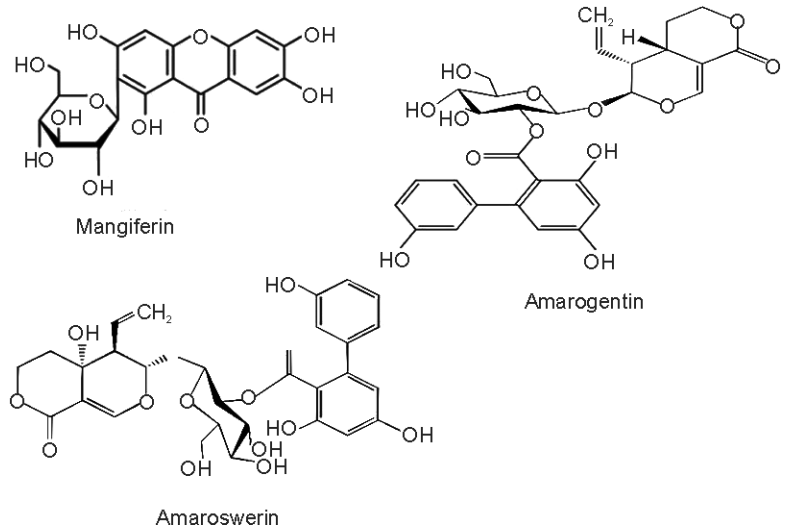

Fig. 4— Chemical structures of isolated bioactive compounds from *S. cordata*

3-hydroxybenzoic acid formed from phenylealanine via benzoic acid[20]. The non-mevalonate pathway is operative during the biosynthesis of the bitter flavour additive amarogentin[21]. These compounds are biologically active and found useful in the treatment of many diseases. Amarogentin is responsible for the bitterness, anthelmintic, hypoglycemic, and antipyretic properties of *Swertia*[3]. These bioactive compounds are also reported from *S. chirayita*[22] and *S. ciliata*[23]. The antimalarial properties of *S. cordata* described in ethno-medicinal systems[4] may be attributed due to presence of amaroswerin and amarogentin. Present study revealed that mangiferin does not show any correlation with altitude as reported for *S. mussotii*[24].

However, presence of amaroswerin and amarogentin from *S. cordata* is contradictory to earlier report[3]. He reported that amaroswerin and amarogentin were completely lacking in *S. cordata* collected from Shimla (Himachal Predesh)[3]. Presence of amaroswerin in all the four studied population and amarogentin in Gulaba population may be due to existence of chemotypes in *S. cordata.* However, further studies are needed for authentication of chemotypes by growing them in similar environmental regime. Therefore, it can be concluded that the presence or absence of these compounds in the samples of a particular region may be used as taxonomic marker for infrageneric classification of the genus. Chemical polymorphism or chemotypes have also been reported for a number of medicinal plant species on the basis of variation in chemical composition like *Lychnophora ericoides*[25], *Leptospermum scoparium*[26], *Delphinium occidentale*[27], and *Artemisia dracunculus*[28].

Difference in the concentration of amaroswerin and mangiferin among different populations of *S. cordata* may be due to difference in habitats of collection i.e. ecological, whereas, occurrence of chemotype might be subject to ecological as well as genetic control. Geographical variations in the levels of phytochemicals are also reported in the genus *Swertia* like *S. frnchetiana*[29], *S. mussotii*[24], and *S. alternifolia*[30]. Ecological factors like soil type, temperature, precipitation, etc. might affect the synthesis and turnover of secondary compounds[31]. Genetic differentiation generally has stronger effects on the contents of the secondary metabolites than ecological factors[32] and the mutation of a single gene might affect the production of certain compounds[33]. Mutation and long term adaptation of *S. cordata* in specific habitats may be responsible for the development of such variations. However, more studies are needed to clarify the relationships between synthesis of such compounds, genetic control and ecological factors as suggested for *S. frnchetiana*[29].

Conclusion

Presence of mangiferin and amaroswerin in all the four studied populations and amarogentin in one population provides base for further studies on existence of variations in the bioactive compounds among the natural populations of *S. cordata.* The antimalarial properties of this species may be attributed to the presence of amaroswerin and amarogentin. As biologically active amaroswerin, amarogentin, and mangiferin are present in *S. cordata*, species will be useful for the synthesis of medicines as an alternate source of *S. chirayita*. Occurrence of these compounds increase usefulness of the species and open new avenues for investigations on chemistry, pharmacology, domestication, and crop improvement aspects.

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