SHORT COMMUNICATION

RP-HPLC analysis of seco-iridoid glycoside swertiamarin from different Swertia species

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ABSTRACT
Genus Swertia is valued for its great medicinal potential; mainly Swertia chirayita (Roxb. ex Fleming) H. Karst. is used in traditional medicine for a wide range of diseases. Seco-iridoid glycosides like swertiamarin is referred with enormous pharmacological potentials. The aim of the study was to identify a suitable substitute to S. chirayita by quantifying seco-iridoid swertiamarin from five different Swertia species endemic to the Western Ghats. The reverse-phase high-performance liquid chromatography diode array detector analyses were performed and chromatographic separation was achieved on a Lichrospher 100, C18e (5 µm) column (250–4.6 mm). A mobile phase consisting of acetonitrile and water (25:75) was used for separation. Results indicated that the concentration of the marker compound has been found to vary largely between and within the species from different localities. The content of swertiamarin was the highest in S. chirayita compared to the other species studied herein, advocating the use of Swertia minor as an alternate source to S. chirayita.

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1. Introduction

The genus *Swertia* (Family: Gentianaceae) comprises ~970 species in the world. Nearly 40 are endemic to India out of which 32 occur in the north-west Himalayas (Kumar et al. 2005), and the remaining eight are confined to the Western Ghats of India. The genus is valued for its great medicinal potential, mainly *Swertia chirayita* (Roxb. ex Fleming) H. Karst. is used in traditional medicine for a range of medicinal properties including anthelmintic, hypoglycemic and antipyretic (Joshi & Dhawan 2005). Recently, the species under study have been evaluated for triterpenoids viz. betulinic acid, oleanolic acid and ursolic acid (Kshirsagar et al. 2015).

Among the different seco-iridoids present in the genus *Swertia*, swertiamarin is the marker compound used for an array of therapeutic applications. Swertiamarin has been reported as antioxidant, hepatoprotective (Jaishree & Badami 2010), gastroprotective, antiulcerogenic, anticholinergic and CNS depressant (Soni & Gupta 2009). Keeping this in view, the present work was aimed to determine the possibility of the other *Swertia* species present in the Western Ghats having antioxidant, antihyperglycemic and antiglycation activities (Kshirsagar, Chavan, et al. 2014; Kshirsagar, More, et al. 2014) as a source of this seco-iridoid glycoside and to find a superior substitute for *S. chirayita*.

2. Results and discussion

Seven different concentrations (10, 20, 40, 80, 100, 250 and 500 ppm) of standard swertiamarin were detected at 238-nm wavelength using the RP-HPLC technique. The analysis yielded profiles with a retention time of 3.611 ± 0.004 min (Figure S1). The linearity and sensitivity of the method were analysed using the set conditions, three independent calibration curves for the compound were plotted correlating the detector signals with concentrations of swertiamarin (Table S1). The regression equation was obtained using the least squares method and the standard deviation did not exceed 2% level. Linear calibration curves were obtained with the coefficient of determination ($R^2$) not less than 0.999 for standard swertiamarin (Figure S1, Table S1).

The limit of detection and limit of quantification were calculated based on the signal:noise ratio (Table S1). Five injections, each of swertiamarin with concentration 1, 5 and 10 ppm, showed excellent inter-day precision. Similarly, the intra-day precision was determined using triplicate readings at same concentration ranges of analytes. A lower RSD (<0.79%) of retention time here indicated acceptable reproducibility of the method.

Samples prepared were injected and chromatograms were obtained under similar conditions as those of standards mentioned above. The RP-HPLC analysis of swertiamarin found in methanolic extract of the *Swertia* species is shown in Table 1 and Figure S1. A comparison of the content of swertiamarin from samples showed variations from species to species and also in different populations within the species. Among the species studied, swertiamarin was found to be in abundance in *Swertia minor* followed by *S. chirayita, Swertia lawii, Swertia corymbosa, Swertia densifolia* and *Swertia angustifolia* var. *pulchella*. Among the localities studied for *S. minor*, the highest amount of swertiamarin (145.368 ± 72.68) was observed in the sample collected from Walmiki, while the Panchgani sample reported the lowest content of swertiamarin (2.562 ± 1.28 mg/g). Similarly in *S. densifolia*, the highest amount of swertiamarin (93.744 ± 46.87 mg/g) was reported in the sample collected from locality Chalkewadi, while the Kas sample showed the lowest swertiamarin (20.201 ± 10.10 mg/g) content (Table 1). At the same time, localities were studied for *S. lawii* in which the Pushpagiri locality
showed the highest amount of swertiamarin and locality Panhala showed the lowest amount of swertiamarin content. The presence of swertiamarin in the Swertia species is in accordance with the earlier reports of Takei et al. (2001) of Swertia herbs, Yang et al. (2004) in Swertia franchetiana, Cao et al. (2005) in Swertia mussotii, Bhandari, Kumar, et al. (2006) and Bhandari, Gupta, et al. (2006) in Swertia cordata and S. chirayita, Tian et al. (2008) from different Swertia species, Shailajan and Abhishhek (2009) in Swertia densiflora and Phobo et al. (2010) from S. chirayita. The large variation in the content of swertiamarin observed by different workers in various species of Swertia, may be due to factors involved in procedures followed for time of collection, extraction, detection and determination of the compound by various research groups (Ankad et al. 2014). Thus, there is a need to revise all the species phytochemically for their marker compound considering such parameters. Even, it is important to address the population-level variation, which may be done by including 10 individuals per population. A similar study for the determination of camptothecin from Nothapodytes nimmoniana from the Western Ghats has been reported by Ankad et al. (2014).

### 3. Conclusion

Results obtained from this analysis observed that the Swertia species from the Western Ghats have a good amount of swertiamarin content. The highest Swertiamarin content was observed in S. minor in comparison to the other Swertia species collected from the Western Ghats. Thus, S. minor can be used as a substitute to S. chirayita, this may in due course release pressure of exploitation of S. chirayita from natural resources and may help to bring it out from endangered category from the conservation point of view. Yet there is further need to evaluate other compounds and biological activities also. Further, it is important to

| Sr. No | Name of the species | Locality | Latitude (N) | Longitude (E) | Elevation (m) | Swertiamarin* mg/g ± SD | Voucher No. |
|--------|---------------------|----------|--------------|--------------|--------------|-------------------------|-------------|
| 1      | Swertia minor       | Panchgani| 17° 55.475′  | 73° 46.472′  | 1288.0       | 2.562 ± 0.128           | PRK-1       |
| 2      | Swertia minor       | Kas      | 17° 55.425′  | 73° 41.606′  | 1002.2       | 31.066 ± 1.553          | PRK-2       |
| 3      | Swertia minor       | Chakewadi| 17° 35.356′  | 73° 50.294′  | 1104.3       | 113.546 ± 5.677         | PRK-3       |
| 4      | Swertia minor       | Walniki  | 17° 14.954′  | 73° 52.451′  | 925.98       | 145.368 ± 7.268         | PRK-4       |
| 5      | Swertia minor       | Panhala  | 16° 49.073′  | 74° 06.425′  | 829.67       | 119.221 ± 5.961         | PRK-5       |
| 6      | Swertia densifolia  | Panchgani| 17° 55.185′  | 73° 47.176′  | 1215.8       | 56.391 ± 2.820          | PRK-6       |
| 7      | Swertia densifolia  | Kas      | 17° 42.579′  | 73° 54.220′  | 1109.8       | 20.201 ± 1.010          | PRK-7       |
| 8      | Swertia densifolia  | Chakewadi| 17° 35.060′  | 73° 49.800′  | 1148.2       | 93.744 ± 4.687          | PRK-8       |
| 9      | Swertia densifolia  | Walniki  | 17° 14.585′  | 73° 48.807′  | 1005.5       | 31.647 ± 1.582          | PRK-9       |
| 10     | Swertia densifolia  | Torana   | 18° 16.897′  | 73° 37.400′  | 1074.7       | 67.208 ± 3.360          | PRK-10      |
| 11     | Swertia lawii       | Jamboti  | 15° 44.530′  | 74° 24.191′  | 828.75       | 22.237 ± 1.112          | PRK-11      |
| 12     | Swertia lawii       | Pushpagiri| 12° 39.944′  | 75° 38.775′  | 771.14       | 127.443 ± 6.372         | PRK-12      |
| 13     | Swertia corymbosa   | Bababudangi| 13° 25.408′  | 75° 45.944′  | 548.94       | 119.336 ± 5.967         | PRK-13      |
| 14     | Swertia angustifolia| Palakkad | 10° 33.312′  | 76° 42.527′  | 896.11       | 83.212 ± 4.161          | PRK-14      |

*Figures in the tables are calculated as mean content of three injection and represented as mg/g ± SD.
phytochemically revise all the species of India using a similar methodology so as to get a comprehensive idea about the elite species wealth.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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