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ORIGINAL ARTICLE

Reverse Phase-ultra Flow Liquid Chromatography-diode Array Detector Quantification of Anticancerous and Antidiabetic Drug Mangiferin from 11 Species of *Swertia* from India

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ABSTRACT

Background: 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. Mangiferin one of xanthoids is referred with enormous pharmacological potentials. **Objective:** The aim of the study was to quantify and compare the anticancerous and antidiabetic drug mangiferin from 11 *Swertia* species from India. The study also evaluates hierarchical relationships between the species based on mangiferin content using multivariate analysis. **Materials and Methods:** The reverse phase-ultra flow liquid chromatography-diode array detector analyses was performed and chromatographic separation was achieved on a Lichrospher 100, C18e (5 μm) column (250–4.6 mm). Mobile phase consisting of 0.2% triethylamine (pH-4 with O-phosphoric acid) and acetonitrile (85:15) was used for separation with injection volume 20 μL and detection wave length at 257 nm. **Results:** Results indicated that concentration of mangiferin has been found to vary largely between *Swertia* species collected from different regions. Content of mangiferin was found to be highest in *Swertia minor* compared to other *Swertia* species studied herein from the Western Ghats and Himalayan region also. The same was also evident in the multivariate analysis, wherein *S. chirayita*, *S. minor* and *Swertia paniculata* made a separate clade. **Conclusion:** Conclusively, the work herein provides insights of mangiferin content from 11 *Swertia* species of India and also presents their hierarchical relationships. To best of the knowledge this is the first report of higher content of mangiferin from any *Swertia* species.

Key words: Dendrogram, mangiferin, multivariate analysis, reverse phase-ultra flow liquid chromatography, *Swertia*

SUMMARY

- The present study quantifies and compares mangiferin in 11 species of *Swertia* from India. The study also evaluates hierarchical relationships between the species based on mangiferin content using multivariate analysis. The mangiferin content was highest in *S. minor* compared to the studied *Swertia* species. To the best of our knowledge this is the first report of higher content of mangiferin from *Swertia* species.

Abbreviations used: LOD: Limit of detection, LOQ: Limit of quantification, RP-UFLC-DAD: Reverse phase-ultra flow liquid chromatography-diode array detector, RSD: Relative standard deviation, SAN: *Swertia angustifolia*, SAP: *Swertia angustifolia* var. *pulchella*, SBI: *S. bimaculata*, SCH: *S. chirayita*, SCO: *S. corymbosa*, SDE: *S. densifolia*, SDI: *S. dialatata*, SLA: *S. lawii*, SMI: *S. minor*, SNE: *S. nervosa*, and SPA: *S. paniculata*.

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INTRODUCTION

Genus *Swertia* (family Gentianaceae), comprises of ~170 species in world.[1] Nearly, 40 are endowed to India out of which 32 occur in the Himalayan regions[2] and remaining eight are confi ed endemic to the Western Ghats of India. The genus is valued for its medicinal potential, most importantly *Swertia chirayita* (Roxb. ex Fleming) H. Karst which is known for its use in traditional medicine in range of ailments including anthelmintic, hypoglycemic, and antipyretic.[3]

The pharmacological actions of any plants depend upon its chemical diversity, *Swertia* being no different have been reported for a wide range of such phytochemicals. The plant is reported for its marker compounds swertiamarin, swerchirin, amaroswerin, and amarogentin.[3] Studies such as antioxidant, hypoglycemic, and antiglycation activities of some *Swertia* species from India have been well documented.[4,5] Triterpenoids such as betulinic acid, oleanolic acid, and ursolic acid; singly or collectively are been reported from different medicinal plants including *Swertia* species.[6-12] Similarly, mangiferin a widely distributed xanthonoid found in *Mangifera indica* and members of Anacardeaceae is also reported from *Swertia*.[13] Mangiferin exhibits diverse pharmacological activities such as antidiabetic,[14]

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anti-HIV,[15] anticancer,[16] anti-inflammatory,[17] antiproliferative, diuretic and antioxidant,[18-20] antifungal,[21] antitubercular,[22] chemopreventive,[23] hepatoprotective,[24] immunomodulatory,[25] and many other activities. The interest in this multipotential molecule is mainly due to its anticancerous and antidiabetic properties.

Keeping this in view, the present work was aimed to determine mangiferin content from 11 *Swertia* species from India. It also enumerates superiority among the species based upon the higher mangiferin content as determined by reversed phase-ultra fl w liquid chromatography-diode array detector (RP-UFLC-DAD) analysis and evaluates similarity between the species using multivariate analysis.

MATERIALS AND METHODS

Collection of plant materials and extract preparation

Plant material of all the species were obtained from different localities of the Western Ghats and Eastern Himalayan region. The whole plant material was air-dried at the room temperature and ground to fi e powder in laboratory grinder. Extracts were prepared by dissolving 500 mg plants powder in 25 ml methanol for 24 h. The filtrates were re-volumized and subjected to RP-UFLC analysis after passing through 0.2 µm nylon filters.

Chemicals and standard

All solvents and chemicals used during the study were of high-performance liquid chromatography (HPLC) grade. A HPLC grade mangiferin was procured from Sigma-Aldrich, India. An accurately weighed standard mangiferin was dissolved in known amount of methanol to obtain mg/ml concentration stock. The stock solution was then diluted to obtain desired working concentrations (1, 10, 25, 50, 100, and 250 µg/ml).

Reverse phase-ultra flow liquid chromatographic analysis

The RP-UFLC analysis was performed on Shimadzu Chromatographic System consisting of a quaternary pump, manual injector, and dual λ ultraviolet absorbance DAD. The built in LC-solution software system was used for the data processing. Chromatographic separation was achieved on a lichrospher 100, C18e (5 µm) column (250–4.6 mm). Mobile phase consisting of 0.2% triethylamine (pH-4 with O-phosphoric acid) and acetonitrile (85:15) was used for separation with injection volume 20 µL. The fl w rate was 1 ml/min and the detection wavelength of the dual λ absorbance detector beam was set at 257 nm. The analysis time was 8 min for both standard and samples. The system suitability test was assessed by three replicate injections of the standard solutions at a particular concentration.

Statistical and multivariate analysis

Statistical analysis was performed using the statistical software Graph Pad Prism Evaluation version (GraphPad Software, USA). The data were reported as means and ± standard deviation (SD). The chromatographic profiles of all extracts were analyzed using built in Shimadzu LC-solution software version 1.25 (Shimadzu corporation, Japan). Multivariate analysis for correlations was analyzed using Biodiversity Pro, version 2 (N Mc Aleece, PJD Lambshead, GLJ Paterson and JD Gage, The Natural History Museum & The Scottish Association for Marine Science.)eco-statistical software to understand the possible natural groupings and correlation in and among the samples collected. The hierarchical clustering analysis performed was based on the relative peak area of the mangiferin from all the samples.

RESULTS AND DISCUSSION

In this study, 11 *Swertia* species, five from the Western Ghats, and six from Himalayan region of India were considered for the study. Quantitative determination of mangiferin in the various species was achieved using RP-UFLC-DAD method and results were expressed as mg/g on dry weight basis. The analysis yielded clear, sharp peaks for standard and sample runs [Figure 1a-c]. Calibration curve was constructed from six different concentrations of standard mangiferin against their respective area under curve with coeffici t of determination (R^2) not < 0.990 [Figure 1d]. A lowest calibrator concentration of 1 µg/ml was used during the study with 0.158 µg/ml limit of detection and 0.479 µg/ml limit of quantifi ation values. The relative SD values were <2% indicating precision and reproducibility of the used method. Validation test was performed by injecting equal volume spiking of standard with *S. chirayita* sample extract to attain recovery at 95–100% range.

Mangiferin was retained and detected at 5.220 ± 0.053 min in standards and samples. Out of the 11 *Swertia* species collected, *S. minor* (63.84 ± 3.19 mg/g) and *S. chirayita* (47.78 ± 2.39 mg/g) had a higher content of mangiferin, followed by *S. paniculata* with 13.62 ± 0.69 mg/g and *Swertia bimaculata* (2.18 ± 0.11 mg/g) [Table 1]. *Swertia angustifolia* var. *pulchella, Swertia densifolia* and *Swertia nervosa* were among the other species with mangiferin

Table 1: Content (mg/g) of mangiferin reported in different *Swertia* species

Speceies	Content (mg/g)	Method	References
S. franchetiana	2.00-6.00	HPLC	[26]
S. mussotti	15.10-44.21	HPLC	[9]
S. chirayita	NM	LC-MS	[27]
S. franchetiana	0.06	HPLC	[28]
S. punicea	0.42-8.86	HPLC	[29]
S. kouichensis	1.32		
S. bifolia	1.58		
S. cincta	0.69		
S. macrosperma	1.66		
S. diluta	Trace		
S. erythrosticta	Trace		
S. franchetiana	NQ	HPLC	[30]
S. chirayita	0.10-0.70	HPLC	[31]
S. bimaculata	ND	HPTLC	[32]
S. nervosa	7.89-11.20		
S. chirayita	12.36-43.70		
S. dilata	ND		
S. paniculata	ND		
*S. chirayita**	0.69-3.03	HPLC	[33]
S. densifolia	6.02	HPLC	[34]
S. minor	4.21		
S. lawii	0.24		
S. angustifolia	ND	UFLC	Present study
S. angustifolia var. *pulchella*	0.14±0.01		
S. bimaculata	2.18±0.11		
S.chirayita	47.78±2.39		
S. corymbosa	ND		
S. densifolia	0.76±0.04		
S. dialatata	Trace		
S. lawii	Trace		
S. minor	63.84±3.19		
S. nervosa	0.19±0.01		
S. paniculata	13.62±0.69		

*Tissue culture grown sample. HPLC: High performance liquid chromatography; HPTLC: High performance thin layer chromatography; CE: Capillary electrophoresis; UFLC: Ultra fl w liquid chromatography; Trace: Content lower than limit of quantifi ation; ND: Not detected; NM: Not mentioned; NQ: Not quantifi d; LC-MS: Liquid chromatography-mass spectrometry

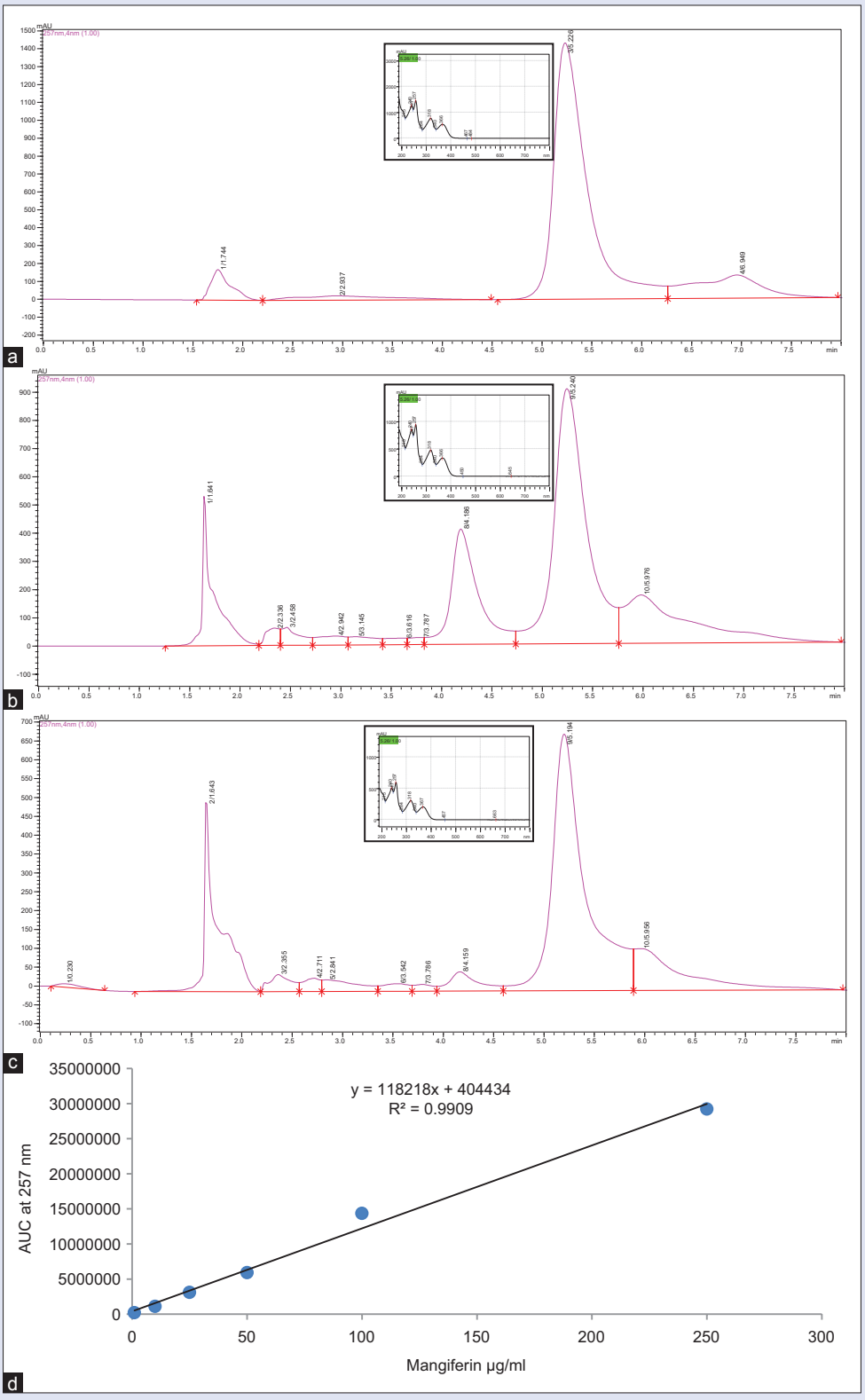

Figure 1: Reverse phase-ultra flow liquid chromatography profiles of (a) standard mangiferin (250 µg/ml); (b) *Swertia minor* extract; (c) *Swertia chirayta* extract; (d) six point calibration curve (1, 10, 25, 50, 100, and 250 µg/ml); figures in inset shows spectrum maximum wavelength for peak at 5.220 ± 0.053 min

content below 1 mg/g [Table 1]. Among the rest, *Swertia dialatata* and *Swertia lawii* showed mangiferin content less than LOQ hence termed as trace. Whereas, it was not detected in *S. angustifolia* and *Swertia corymbosa*. Thus, the paper provides a data on mangiferin content of

11 *Swertia* species from India. Mangiferin content (mg/g) has earlier been determined in *Swertia* species by using various chromatographic techniques [Table 1][8,31] and it was observed that the mangiferin content determined in the present study for *S. chirayita* and *S. minor* was the highest among all.

The multivariate analysis was performed using area under curve for mangiferin from all the samples. A percent similarity dendrogram was obtained based on area of mangiferin run of RP-UFLC-DAD analysis. The *Swertia* species were arranged in ascending order of mangiferin content from top to bottom [Figure 2]. *S. chirayita*, *S. minor* and *S. paniculata* made a separate clade at bottom with a higher content and a similarity of 44.45%. Among this *S. minor* showed higher similarity with *S. chirayita* (85.67%). This followed by *S. densifolia*, *S. bimaculata*, *S. aungustifolia* var. *pulchella* and *S. nervosa* with medium content and a percent similarity of 55.55%. *S. lawii* and *S. dialatata* with trace amount of mangiferin showed 46.20% similarity. *S. corymbosa* and *S. aungustifolia* with no content of mangiferin remained separated from all others with a lower percent similarity (4.40%). Similar analysis has been also performed by Deshmukh *et al.*[35] in different banana varieties and Wohlmuth *et al.*,[36] in developing method to improve detection of adulteration in *Ginkgo biloba*, hence justifying use of such tools in understanding hierarchical relations.

CONCLUSION

In conclusion, the work herein provides insights of mangiferin content from 11 *Swertia* species of India and also presents their hierarchical relationships. Besides, we also stress upon *S. minor* from the Western Ghats of India, to have a higher content of mangiferin than any other species reported hereto. To best of the knowledge this is the first report of a higher content of mangiferin from any *Swertia* species.

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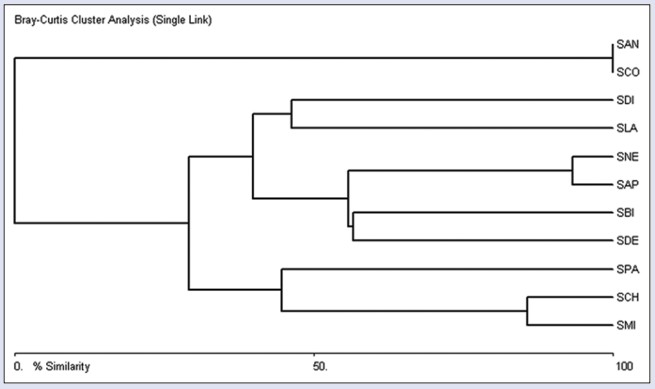

Figure 2: Dendrogram generated using area under curve obtained from reverse phase-ultra flow liquid chromatography-diode array detector analysis of mangiferin from various *Swertia* species

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Conflicts of interest

There are no confli ts of interest.

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