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

Parthraj R. Kshirsagar¹,²,³, Nikol B. Gaikwad¹, Subhasis Panda⁴, Harsha V. Hegde²,³, Sandeep R. Pai³

¹Department of Botany, Shivaji University, Kolhapur, Maharashtra, ²Herbal Medicine, ³Plant Biotechnology and Tissue Culture Division, Regional Medical Research Centre, Indian Council of Medical Research, Nehru Nagar, Belagavi, Karnataka, ⁴Department of Botany, Darjeeling Government College, Darjeeling, West Bengal, India

**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. Mangiferone one of xanthonoids 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.

**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 regions² and remaining eight are confined 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.¹ Studies such as antioxidant, hypoglycemic, and antglycation activities of some *Swertia* species from India have been well documented.⁴ Triterpenoids such as betulonic acid, oleanolic acid, and ursolic acid; singly or collectively are been reported from different medicinal plants including *Swertia* species.⁵–¹² 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

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*.

**Correspondence:**
Dr. Sandeep R. Pai, Plant Biotechnology and Tissue Culture Division, Regional Medical Research Centre, Indian Council of Medical Research, Nehru Nagar, Belagavi, Karnataka, India.
E-mail: dpaisr@gmail.com

**DOI:** 10.4103/0973-1296.176105

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Kshirsagar PR, Gaikwad NB, Panda S, Hegde HV, Pai SR. Reverse phase-ultra flow liquid chromatography-diode array detector quantification of anticancerous and antidiabetic drug mangiferin from 11 species of *Swertia* from India. Phcog Mag 2016;12:S32-S6.
### 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 coefficient of determination (R²) 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 quantitation 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.

### 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 fine 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 flow 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 Lambshad, GL) Paterson and JD Gage, The Natural History Museum & The Scottish Association for Marine Science).jeco-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.

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

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

*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 quantification value; ND: Not detected; NM: Not mentioned; NQ: Not quantified; LC-MS: Liquid chromatography-mass spectrometry
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* spp.

**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.
11 Swertia species from India. Mangiferin content (mg/g) has earlier been determined in Swertia species by using various chromatographic techniques [14] 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. angustifolia 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. angustifolia 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.[15] in different banana varieties and Wohlmuth et al.,[16] 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.

Acknowledgment

Authors are indebted to offic -in-charge, RMRC, ICMR, Belagavi and Head, Department of Botany, Shivaji University, Kolhapur for their kind support. Authors also thank Dr. Manoj Lekhak, Department of Botany, Shivaji University, Kolhapur for his help in collection of Swertia species. SRP is also indebted to SERB, DST, and New Delhi for providing financial support during the work (SB/YS/LS-71/2013).

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Brahmacari G, Mondal S, Gangopadhyaya A, Gora D, Mukhopadhyaya B, Saha S, et al. Swertia jGenianaceaet. Chemical and pharmacological aspects. Chem Biodivers 2004;1:1627-61.

2. Kumar N, Singh B, Kaul VK, Ahuja PS. Chemical and biological aspects of indigenous bearing plants of temperate region. Vol. 32, Part L, Attar-Rahman, editor. Studies in Natural Products Chemistry Pages Bioactive Natural Products; 2005. P. 247-302.

3. Joshi P, Dhawan V, Swertia chirayita: Overview. Curr Sci 2005;89:635-40.

4. Kshirsagar P, Chavan J, Nimbalkar M, Yadav S, Dixit G, Gaikwad N. Phytochemical composition, antioxidant activity and HPLC profiles of Swertia species from Western Ghats. Nat Prod Res 2015;29:780-4.

5. Kshirsagar P, More T, Arvindakar A, Gaikwad N. Antioxidant, antihyperglycemic and antiglycation properties of some Swertia species from Western Ghats. Int J Pharm Pharm Sci 2014;6:300-6.

6. Kshirsagar PR, Pai SR, Nimbalkar MS, Gaikwad NB. Quantitative determination of three pentacyclic triterpenes from five Swertia L. endemic species to Western Ghats, India, using RPHPLC analysis. Nat Prod Res 2015;29:1783-8.

7. Li G, Zhang X, You J, Song C, Sun Z, Xia L, et al. Highly sensitive and selective pre-column derivatization high-performance liquid chromatography approach for rapid determination of triterpenes oleanolic and ursolic acids and application to Swertia species: Optimization of triterpenes acids extraction and pre-column derivatization using response surface methodology. Anal Chim Acta 2011;688:208-18.

8. Gupta M, Bish D, Khatoon S, Srivastava S, Rawat AK. Determination of ursolic acid a biomarker in different Swertia species through high performance thin layer chromatography. Chin Med 2011;2:121-4.

9. Yang H, Ding C, Duan Y, Liu J. Variation of active constituents of an important Tibet folk medicine Swertia mussotii Franch. Genianaceaet between artificially cultivated and naturally distributed. J Ethnopharmacol 2000;88:316.

10. Pai SR, Nimbalkar MS, Pawar NV, Dixit GB. Optimization of extraction techniques and quantification of betulinic acid (BA) by RP-HPLC method from Ancistrocladus heyneanus Wall. Ex Grav. Ind Crops Prod 2011;34:1458-64.

11. Upadhye V, Anikad GM, Pai SR, Hegde HV, Khokute SD. Accumulation and trends in distribution of three triterpenoids in various parts of Achyranthes coyneri determined using RP-UFLC analysis. Pharmacogn Mag 2014;10:398-401.

12. Upadhye V, Hegde HV, Joshi RK, Khokute SD. New report of triterpenoid betulinic acid (BAI) along with oleanolic acid (OA) from Achyranthes aspera by RP-UFLC analysis and confirmation using HPTLC and FTR techniques. J Planar Chromatogr Modif TLC 2014;27:38-41.

13. Chavan JJ, Ghadge DM, Kshirsagar PR, Kudele SS. Optimization of extraction techniques and RP-HPLC analysis of antidiabetic and anticancer drug mangiferin of roots of ‘Saptarangi’ (Salacia chinensis L.). J Liq Chromatogr Relat Technol 2015;38:963-9.

14. Miura T, Ichiki H, Iwamoto N, Kato M, Kubo M, Sasaki H, et al. Antidiabetic activity of the rhizoma of Anemarrhena asphodeloides and active components, mangiferin and its glucoside. Biol Pharm Bull 2001;24:1009-11.

15. Guha S, Ghosal S, Chattopadhyay U, Antitumor, immunomodulatory and anti-HIV effect of mangiferin, a naturally occurring glucosylxanthone. Chemotherapy 1996;42:443-61.

16. Nakai Y, Kengo M, Masaki K, Yasuhiro Y, Toshiya K, Zheng Q, et al. The inhibitory effects of mangiferin, a naturally occurring glucosyl xanthone, in bowel carcinogenesis of male F344 rats. Cancer Lett 2001;163:163-70.

17. Garindo G, González D, Lemus Y, García D, Lodeiro L, Quintero G, et al. In vivo and in vitro anti-inflammatory activity of Mangifera indica L. extract (VIMANG). Pharmocol Res 2004;50:143-9.

18. Hsu MF, Raung SL, Tsao LT, Lin CN, Wang JP. Examination of the inhibitory effect of norathyl in formylmethionyl-leucyl-phenylalanine-induced respiratory burst in rat neutrophils. Free Radic Biol Med 1997;23:1035-45.

19. Sánchez GM, Re L, Giuliani A, Núñez-Selles AJ, Davison GP, León-Fernández OS. Protective effects of Mangifera indica L. extract, mangiferin and selected antioxidants.
PARTHRAJ R. KSHIRSAGAR, et al.: RP-UFLC-DAD Analysis of Mangiferin from 11 *Swertia* spp.

against TPA-induced biomolecules oxidation and peritoneal macrophage activation in mice. Pharmaco Res 2000;42:565-73.

20. Martínez G, Delgado R, Pérez G, Garrido G, Núñez Sellés AJ, León OS. Evaluation of the *in vitro* antioxidant activity of *Mangifera indica* L. extract (Vimang). Phytother Res 2000;14:424-7.

21. Rodríguez S, Wolfender JL, Hakizamungu E, Hostettmann K. An antifungal naphthoquinone, xanthones and secoiridoids from *Swertia* calycina. Planta Med 1995;61:362-4.

22. Bian QY, Luo NC, Xiao PG. Four xanthone glycosides from *Swertia* calycina Franch. Pharm Pharmacol Commun 1998;41:597-8.

23. Yoshimi N, Matsunaga K, Katayama M, Yamada Y, Kuno T, Qiao Z, et al. The inhibitory effects of mangiferin, a naturally occurring glucosylxanthone, in bowel carcinogenesis of male F344 rats. Cancer Lett 2001;163:163-70.

24. Komatsu K, Purusotam B, Kadota S, Namba T. A comparative study on swertia herbs from Japan, Nepal and China and their hypoglycemic activities in streptozotocin (STZ)-induced diabetic rats. Nat Med 1997;51:265-8.

25. Leiro J, Arranz JA, Yáñez M, Ubeira FM, Sanmartín ML, Orallo F. Expression profiles of genes involved in the mouse nuclear factor-kappa B signal transduction pathway are modulated by mangiferin. Int Immunopharmacol 2004;4:763-78.

26. Yang H, Duan Y, Hu F, Liu J. Lack of altitudinal trends in phytochemical constituents of *Swertia franchetiana* (Gentianaceae). Biochem Syst Ecol 2004;32:961-6.

27. Suryawanshi S, Mehrotra N, Asthana RK, Gupta RC. Liquid chromatography/tandem mass spectrometric study and analysis of xanthone and secoiridoid glycoside composition of *Swertia chirata*, a potent antigastic. Rapid Commun Mass Spectrom 2006;20:3761-8.

28. Li Y, Wang L, Wang X. Separation and determination of the major active components in Tibetan folk medicinal species *Swertia franchetiana* by HPLC with DAD. J Liq Chromatogr Relat Technol 2007;30:1687-96.

29. Tan L, Chen J, Huang F, Fang J. Simultaneous determination of four active components in *Swertia* by RP-HPLC. Chin J Nat Med 2008;6:444-9.

30. Sun Y, Zhang X, Xue X, Zhang Y, Xiao H, Liang X. Rapid identification of polyphenol C-glycosides from *Swertia franchetiana* by HPLC-ESI-MS-MS. J Chromatogr Sci 2009;47:190-6.

31. Phoboo S, Pinto MD, Bhownik PC, Jha PK, Shetty K. Quantification of major phytochemicals of *Swertia chirayita*, a medicinal plant from Nepal. Ecoprint 2010;17:59-68.

32. Pandey DK, Basu S, Jha TB. Screening of different East Himalayan species and populations of *Swertia* L. based on exomorphology and mangiferin content. Asian Pac J Trop Biomed 2012;2:S1450-6.

33. Kumar V, Chauhan RS, Sood H. *In vitro* production and efficient quantification of major phytopharmaceuticals in an endangered medicinal herb, *Swertia chirata*. Int J Biotechnol Bioeng Res 2013;4:495-506.

34. Nangare A, Mendhukar V. Mangiferin quantification in some *Swertia* species collected from south-west zone of Maharashtra. Int J Biosci 2014;3:3488-91.

35. Deshmukh NH, Pai SR, Nimbalkar MS, Patil RP. Biochemical characterization of banana cultivars from Southern India. Int J Fruit Sci 2009;9:305-22.

36. Wohlforth H, Savage K, Dowell A, Misutt P. Adulteration of *Ginkgo biloba* products and a simple method to improve its detection. Phytomedicine 2014;21:912-8.

ABOUT AUTHOR

Dr. Sandeep R. Pai, Scientist (DST-YS), research interests in plant based compounds, extraction and analytical methods, presently working on elicitation of secondary metabolites using biotechnological tools and prospecting of medically important compounds from plants with over 10 years research experience.