International Journal of Bioassays
ISSN: 2278-778X
CODEN: IJBNHY
OPEN ACCESS

Original Research Article

MANGIFERIN QUANTIFICATION IN SOME *SWERTIA* SPECIES COLLECTED FROM SOUTH-WEST ZONE OF MAHARASHTRA

Aruna Nangare* and VD Mendhulkar
Department of Botany, Institute of Science, Mumbai-32, India

Received for publication: September 16, 2014; **Accepted:** September 22, 2014

Abstract: The present work deals with the analytical aspect of three species of *Swertia* viz. *Swertia densifolia* Griseb, *Swertia minor* Knobl., and *Swertia lawii* Syn. collected from southwest-zone of Maharashtra for quantification of mangiferin content. A xanthone derivative, mangiferin exhibits diverse pharmacological activities like anti-diabetic, anti-HIV, anti-cancer and antioxidant properties. Quantification of xanthone compound mangiferin was done by High Performance Liquid Chromatography method using methanol: water solvent system as the mobile phase. Injection volume for species as well as standard Samples was 10µl and flow rate of 1ml/min at room temperature. Standard calibration curve was generated for quantification of Mangiferin in studied samples. Leaf extract samples were used for the analysis. The highest quantity of Mangiferin, 6.02mg/g was estimated in *Swertia densifolia* while *Swertia minor* indicated the estimate of 4.21mg/g whereas, *Swertia lawii* contains 0.24mg/g of mangiferin, indicating the lowest content among the three species.

Key words: High performance liquid chromatography, Mangiferin, *Swertia* species.

INTRODUCTION

Nutrients from various food components have a vital role to play in maintaining normal function of the human body. With recent advances in plant sciences, natural products and health promoting foods have received extensive attention from both health professionals and the common population. New concepts have appeared with this trend, such as nutraceuticals, nutritional therapy, phytonutrients and phytotherapy [1, 2 and 3]. These functional or medicinal foods and phytonutrients or phytomedicines play positive roles in maintaining well-being, enhancing health and modulating immune function to prevent specific diseases. They also hold great promise in clinical therapy due to their potential to reduce side effects associated with chemotherapy or radiotherapy and significant advantages in reducing the health care cost [4]. Therefore, the research area in plant sciences has been extensively widened with high significance.

Swertia, commonly Known as Chiretta 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 of the most reputed herbal drugs used extensively for the treatment of various health ailments including liver disorders, malaria, gastrointestinal infection and diabetes [5]. The important constituents of this plant are iridoids, xanthones, xanthone glycosides, flavonoids and triterpenoids [6]. Xanthone comprises a large group of secondary metabolites. Studies reveal that out of the available xanthones, the c-glucoxanthone mangiferin exhibits diverse pharmacological activities like anti-diabetic [7], anti-HIV [8], anti-cancer [9], immunomodulatory [10], and anti-inflammatory properties [11], as well as antiproliferative, diuretic and antioxidant properties [12,13 and 14]. The presence of C-glucoxanthone Mangiferin was initially reported from the leaves and bark of *Mangifera indica* –Anacardiaceae [15]. But at present the occurrence of Mangiferin has been reported from many phylogenetically distant families like Hippocrateaceae [16], Rubiaceae [17] and Gentianaceae the family of *Swertia* [18]. HPLC method was also authentically used by other workers in quantification of secondary metabolites [19].

The objective of the present study was to quantify the Mangiferin in three species viz. *Swertia densifolia* Griseb, *Swertia minor* Knobl. and *Swertia lawii* Burkill *collected* from south-west zone of Maharashtra in order to exploit Mangiferin rich wild flora as alternate source.

MATERIALS AND METHODS

Plant material: The fresh matured leaves of *Swertia lawii* was collected randomly from Panhala (Dist. Kolhapur) during November, *Swertia minor* from Sinhagad (Dist. Pune) during July and *Swertia densifolia* from Kas (Dist. Satara) during December. The plant species were identified by standard morphological characters (keys) according to the Flora of Kolhapur District (20).

Chemicals: Standard Mangiferin was purchased from sigma Aldrich (U. S.).All HPLC grade chemicals were obtained from Merk Co.

Preparation of standard Mangiferin and calibration curve: 10 mg of standard Mangiferin was weighed accurately and dissolved in 10 ml methanol. This was diluted to give a series of concentrations. Three injections were performed for each dilution. A calibration curve was plotted between 20µg -100µg/ml (Fig. 1).

***Corresponding Author:**
Aruna Shahaji Nangare,
Department of Botany,
Institute of Science, Mumbai-32, India.

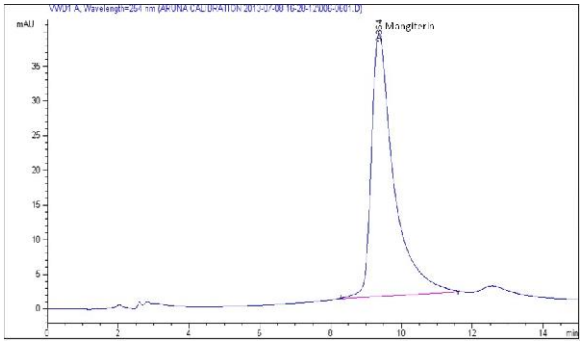

Figure 1: HPLC separation of Standard mangiferin.

Extraction and preparation of test samples: The leaves of each species were dried and powdered. Appropriate weight of each sample was accurately weighed and then extracted twice with 10mL Methanol (70%) in an ultrasonic bath at 45°C for 30 min. The sample solution was filtered through 0.45 µm syringe membrane filter prior to HPLC.

Chromatographic analysis: All extracts were analyzed using an HPLC chromatograph equipped with an ultraviolet detector and a Zorbax C18, 5µm, 250 ×4.60 column. The HPLC protocol developed for analyzing Mangiferin and xanthones by Demizu *et al.,* [21] was adopted using methanol: water as the mobile phase in 32:68 proportions. All concentrations were determined by injecting 10µl of the standard solutions and sample extracts at a flow rate of 1ml/min at room temperature. The peak wavelength was 254nm for Mangiferin. Standard calibration curve was generated and it is used for quantification of Mangiferin in species samples. Chromatographic peaks of the samples were identified by comparing retention time with that of the standard compounds and were subsequently quantified using the standard method.

The chromatographic method for analyte separation involves specificity, which is the ability of the method to accurately measure the analyte response in presence of all the interferences. Therefore, the extraction mixtures obtained from the sample preparation were analyzed and the analyte Mangiferin peak was evaluated for the peak purity and resolution from the nearest peak. The reported peaks were completely separated from the other interfering compounds. Due to the verification of the normal distribution of results, linearity was evaluated through the relationship between the concentration of Mangiferin and the absorbance obtained from the UV-HPLC detector. The linear relationship between the detector response and different concentrations of Mangiferin was confirmed in the range of 20-100µg/ml. A linear relationship was obtained with a correlation of coefficient, r=0.92968. The limit of detection (LOD) and Limit of quantization (LOQ) was 0.4978 and 1.5087 respectively.

RESULTS AND DISCUSSION

The studied species of *Swertia* were collected from three different localities; *Swertia densifolia* from Kas- Satara Dist. (Latitude- 17° 45' N, longitude- 73° 56' E, Elevation 1200m, *Swertia minor* from Sinhagad - Pune Dist.(Latitude18°21'56.39"N, longitude- 73°45'18.97" E, Elevation-1, 312m) *Swertia lawii* were collected from Panhala- Kolhapur Dist. (Latitude 16.82°N, longitude- 74.12°E, Elevation- 1, 312m).

Quantity of Mangiferin found in *Swertia* species was 5mg/g, in *Swertia densifolia;* 2.5mg/g in *Swertia minor* and 1mg/g in *Swertia lawii.* The highest quantity of Mangiferin was found in *Swertia densifolia* (6.02mg/g). *Swertia minor* (4.21mg/g) has moderate percent of Mangiferin, whereas *Swertia lawii* (0.24mg/g) contains lowest amount of Mangiferin than in other two studied species. The compound mangiferin appeared at retention time 9.28±0.06m. in standard and studied samples. Fig. 2-4 indicates the separated peak of the mangiferin compound in *Swertia densifolia, Swertia lawii* and *Swertia minor leaf samples.* The compound however exhibits variations in its estimates in studied *Swertia* species.

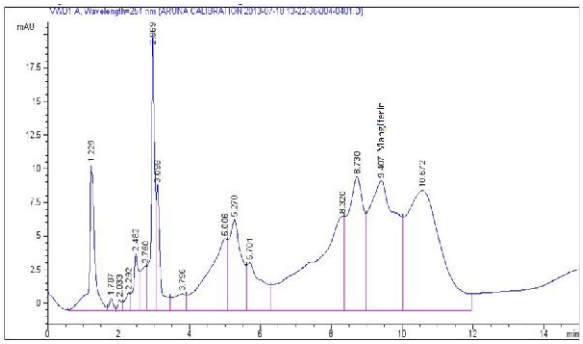

Figure 2: HPLC separation of Mangiferin in *Swertia densifolia.*

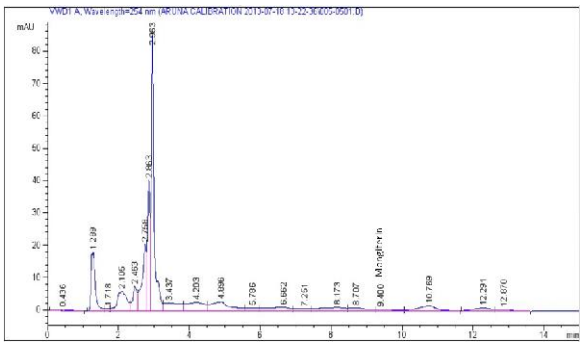

Figure 3: HPLC separation of Mangiferin in *Swertia lawii.*

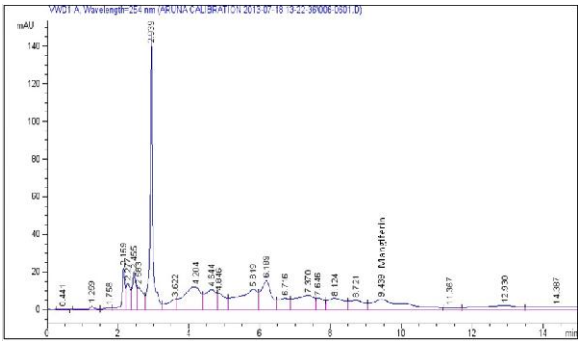

Figure 4: HPLC separation of Mangiferin in *Swertia minor*.

Many widely distributed species shows physiological trends along altitudinal gradients [22]. For example contents of carotenoids, flavonoids, sucrose, fructose, glucose and total soluble sugar [~~10~~ 23], Geographical variations in levels of phytochemicals can occur in some species (e.g. Volatile terpenoids in Juniperous [24, 25].

Three possible factors might account for the variations in the mangiferin content in *S. densifolia, S. lawii* and *S. minor*. First altitude, ecological factors e.g. Soil type, temperature, and precipitation might affect the synthesis and turnover of secondary compounds [26]. Genetic differentiation generally has stronger effect on the contents of the secondary compounds than ecological factors [27]. More studies are needed to clarify the relationship between synthesis of these compounds, genetic controls and ecological factors.

The presence of C-glucoxanthone mangiferin was initially reported from the leaves and bark of *Mangifera indica* (Anacardiaceae), [15]; but at present the occurrence of mangiferin has been reported from many phylogenetically distant families like Hippocrateaceae [16], Rubiaceae [17] and Gentiaceae [18] the family of *Swertia*. Mangiferin has been detected and estimated in *Swertia davidi* and *Swertia chirata* using HPLC and LC-MS [18,28]. The bioactive marker mangiferin has been detected and quantified previously from aerial parts of two Chinese species viz, *Swertia davidi* and *Swertia mussotti* [15, 18]. In different East Himalayan *Swertia* species, the bioactive marker mangiferin was detected in leaf samples of *S. chirata* and *S. nervosa* only [29].

CONCLUSION

Mangiferin is a bioactive xanthone compound and the quantification of this compound provides an idea about the potentials of utilizing *Swertia* species as the compound, mangiferin has recommendable utilities for different pharmacological preparations and health applications that includes anti-diabetic, anti-inflammatory and diuretic properties. Due to importance of the xanthone in this plant, it is used by the pharmacologist for the preparation of medicinal product like Ayush- 64, Diabecon, and Mensturyl syrup and also for veterinary purposes like Melicon V ointment. Present work has provided an insight regarding quantitative status of Mangiferin in studied experimental system *Swertia* species. It is a need of hours to exploit the availability of such potent target compound for the benefit to society. To this context, the studies on this aspects deserve high significance.

ACKNOWLEDGEMENT

Authors are thankful to Director, Institute of Science, Mumbai, for availing the laboratory facilities and encouragement.

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Source of support: Nil
Conflict of interest: None Declared