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Quantification of Swertiamarin from Whole Plant Powder of *Swertia densiflora* (Griscb.) Kashyap. Collected in Different Seasons

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In this paper, the comparative quantification of Swertiamarin from *Swertia densiflora* (Griscb.) Kashyap in different periods, in order to identify the best period for harvest is investigated. The plant powder was first extracted with methanol and then in chloroform. The final residue was reconstituted in methanol and used for quantitation. Chromatography was performed on silica gel 60 F_{254} HPTLC plate, with ethyl acetate:methanol:water, 7.5:1.5:1.2 (v/v), as mobile phase. Quantitation was achieved by densitometric scanning at 244 nm (λ_{max}) in reflectance-absorbance mode. The response to swertiamarin was linear function of concentration over the range 30 to 100 µL mL^{-1} in the extract of *Swertia densiflora* (Griscb.) Kashyap. The amount of swertiamarin in *Swertia densiflora* (Griscb.) Kashyap. whole plant powder was found to be 2.94 mg g^{-1} in the month of March, 1.59 mg g^{-1} in October and 1.18 mg g^{-1} in December.

Key Words: Swertiamarin, *Swertia densiflora* (Griscb.) Kashyap., Seasonal variation, Quantitation, HPTLC.

INTRODUCTION

Swertia chirata (Wall) Clarke is listed in the British and United States Pharmacopoeias. Several species of *Swertia* are used as substitutes of *Swertia chirata* (Wall) Clarke. Most of these species are found[1] wild in the upper Himalaya at altitudes of 1200-3000 cm from Kashmir to Bhutan and in the Khasi hills in Meghalaya at 1200-1500 cm.

Swertia densiflora (Griscb.) Kashyap. which is used as a substitute of *Swertia chirata* (Wall) Clarke is a native of Northern Deccan and Mahabaleshwar region of peninsular India. The present study comparatively evaluates the swertiamarin content in *Swertia densiflora* (Griscb.) Kashyap. collected in different months (October, December and March) during the flowering season. Swertiamarin, 2′- 3″, 5″ |, 3″-trihydroxydiphenyl-2″-carboxylic acid ester, is a secoiridoid glucoside found in members of Gentianaceae family like *Swertia chirata* (Wall) Clarke, Swertia ciliata, *Swertia japonica* Makino, *Swertia angustifolia* Buch. - Ham.ex D. Don and *Swertia densiflora* (Griscb.) Kashyap. Swertiamarin has antidepressant and anticholinergic activity and thus can be used as a bioactive marker[2,3].

Swertia densiflora (Griscb.) Kashyap. syn. *Swertia decussata* Nimmo.ex Grah.[4] belonging to family Gentianaceae is an annual herb found on Western Ghats at altitude 1500-2000 cm. It grows on flat lands of Panchgani near Mahabaleshwar, India. *Swertia densiflora* (Griscb.) Kashyap. is considered an excellent substitute for *Swertia chirata* (Wall) Clarke and Gentian. The plant drug lacks odour but is extremely bitter in taste. Like *Swertia chirata* (Wall) Clarke, it is a bitter stomachic tonic, febrifuge and laxative[1,5]. The whole plant is medicinal but the root is reported to be most valuable part. It is also prescribed as a blood purifier in skin diseases. According to the Pharmacopoeia of India the drug should contain not less than 1.3 % of the bitter principles[1]. Literature survey shows that no reports are available on the comparative quantification of Swertiamarin from *Swertia densiflora* (Griscb.) Kashyap in different periods, so as to identify the best period for harvest.

The quantitation of Swertiamarin from *Swertia chirata* (Wall) Clarke has already been reported[6]. The reported method, however, did not provide adequate resolution at R_f of standard Swertiamarin when applied to quantitate Swertiamarin from *Swertia densiflora* (Griscb.) Kashyap. The reported method was therefore, modified with cold extraction in methanol and suitable changes in the chromatographic conditions.

EXPERIMENTAL

Whole plant of *Swertia densiflora* (Griscb.) Kashyap. was collected from Mahabaleshwar and Panchgani region of India in different months during the flowering season. It was authenticated from Blatter Herbarium, St. Xavier's College, Mumbai, India. After collection, the whole plant was washed with water thoroughly to remove soil particles, dust and extraneous matter. The plant material was drained to remove excess of water by spreading over filter paper for 6 h in shade away from sunlight. The plant material was then placed in an oven at 45 ± 5 ºC and allowed to dry for 4 d. Immediately after drying, it was powdered using an electrical mixer-grinder and sieved through a BSS mesh No. 80 sieve and stored in airtight Pearlpet® containers at 25 ºC. The containers were labeled with details such as date of collection, weight of powder, time of collection and the season of collection.

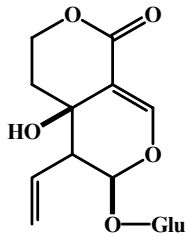

Fig. 1. Structure of Swertiamarin

Standard Swertiamarin (98 % purity) was received as a generous gift from B.V. Patel Pharmaceutical Education and Research Development Center (PERD), Thaltej, Ahmadabad, India. The solvents, chloroform, ethyl acetate and methanol were of

analytical grade and were purchased from Qualigens Fine Chemicals, Mumbai, India. Distilled water was used in the analysis.

A TLC scanner with a computer system and Cats 3 Version Software were used (Camag Muttenz, Switzerland). The source of radiation was deuterium lamp. Camag Linomat IV was used as applicator. Separation was done on silica gel 60 F_{254} HPTLC pre-coated plate procured from Merck (Darmstadt, Germany).

Standard and sample preparation: A stock solution of swertiamarin (100 µg mL^{-1}) was prepared by dissolving 1 mg of accurately weighed swertiamarin in methanol and diluting to 10 mL with methanol. Aliquots (0.3 mL to 1.0 mL) of this stock solution were transferred to 10 mL standard volumetric flasks and the volume of each was adjusted to 10 mL with methanol to obtain working standard solutions containing 30 to 100 µg mL^{-1}.

0.5 g of the dried plant powder was accurately weighed and placed in a stoppered tube and 25 mL of methanol was added, the sample was vortexed for 1-2 min and left to stand 2 h at room temperature (28 ± 2 ºC). The contents of the tube were filtered through Whatmann No. 41 paper (E. Merck, Mumbai, India) and filtrate obtained was evaporated to dryness in a water bath. The residue was reconstituted in 25 mL of chloroform and vortexed. The chloroform extract was filtered through Whatmann No. 41 paper and the filtrate was evaporated to dryness in a water bath. The final residue was again reconstituted in 10 mL of methanol. The methanolic extract was used for further quantification and validation.

Chromatography was performed on aluminium HPTLC plates coated with silica gel 60 F_{254} (Merck # 5554)[7]. Before use, plates were pre washed with methanol and dried in an oven at 105 ºC for 2 h. Samples (10 µL) were spotted as 7 mm, starting 14 mm from edge of the plates, by means of a Camag Linomat IV sample applicator. The plates were developed up to a distance of 85 mm above the position of sample application in Camag twin-trough chamber previously equilibrated with mobile phase for 0.5 h. The mobile phase consisted of ethyl acetate:methanol:water, 7.5: 1.5:1.2 (v/v). The chromatographic conditions had been previously optimized to achieve the best resolution and peak shape.

After development, plates were dried under current of air at room temperature and densitometric evaluation of the plates was performed at 244 nm (λ_{max}) in reflectance-absorbance mode using deuterium lamp with a Camag Scanner II in conjunction with Cats 3 Version Software. The wavelength used for densitometry was selected after acquiring spectra of the standard. The identity of the band of Swertiamarin in the sample was confirmed by overlaying the chromatogram of sample with that of the swertiamarin and by comparing their R_f (0.31). The chromatographic plate of standard swertiamarin and *Swertia densiflora* (Griscb.) Kashyap. samples is shown in Fig. 2.

The linearity of detector response was evaluated in triplicate using seven different concentrations (20, 30, 40, 50, 60, 70, 80, 100 µg mL^{-1}) of swertiamarin prepared in methanol. The plot was linear in the range 30 to 100 µg mL^{-1} (Table-1).

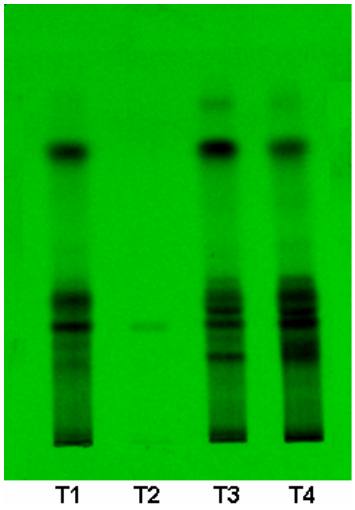

Fig. 2. Chromatographic plate of Swertiamarin standard and *Swertia densiflora* (Griscb.) Kashyap. samples; T1 = *Swertia densiflora* (March); T2 = Swertiamarin standard; T3 = *Swertia densiflora* (October); T4 = *Swertia densiflora* (December)

TABLE-1
LINEARITY DATA

Linearity range (µg mL^{-1})	30 to 100
Slope (m)*	3.0886
Intercept (c)*	37.625
Correlation coefficient (R)	0.9865
LOD (µg mL^{-1})	5
LOQ (µg mL^{-1})	15
Instrument precision (n = 5 RSD)	0.28
Intra-day precision (n = 3 RSD)	0.22
Inter-day precision (n = 3 RSD)	0.22

*The equation y = mx + c, where y is peak area, m is the slope, x is the concentration and c is the intercept.

The accuracy of the method was evaluated by performing recovery experiments by the standard addition method. The per cent recovery of swertiamarin was found to be 96.8 % calculated. The results are given in Table-2. The robustness of the method was studied, during method development, by determining the effects of small variations of mobile phase composition (± 2 %), chamber saturation period, development distance and scanning time (10 % variation of each). No significant change of R_f or response to Swertiamarin was observed, indicating the robustness of the method.

Assay procedure: Standard solution of swertiamarin and *Swertia densiflora* (Griscb.) Kashyap. were prepared as explained earlier. 10 µL of their solutions were spotted on a HPTLC plate. The amount of swertiamarin present was calculated

TABLE-2
RESULTS OF ACCURACY ANALYSIS

Amount of swertiamarin in pre analyzed sample (mg)	Amount of swertiamarin standard added to pre analyzed sample (µL)	Total amount of swertiamarin (mg)	SD	RSD (%) n = 7	Recovery (%)
0.12	0.0	0.12	0.020	0.048	96.8
	20	0.125			

and the assay was repeated 7 times. The mean assay value of swertiamarin was found to be 1.59 ± 0.011 mg g^{-1} in October, 1.18 ± 0.010 mg g^{-1} in December and 2.94 ± 0.014 mg g^{-1} in March. The corresponding per cent content of swertiamarin was found to be 0.16 ± 0.0017 in October, 0.12 ± 0.0083 in December and 0.30 ± 0.0081 in March, respectively. The results of assay are given in Table-3.

TABLE-3
RESULTS OF ASSAY

Sample	Month	Weight of sample in mg	Amount of swertiamarin present in sample in mg g^{-1}	Average % content of swertiamarin
Whole plant powder of *Swertia densiflora* (Griscb.) Kashyap.	October	500	1.59 ± 0.011	0.16 ± 0.0017
	December	500	1.18 ± 0.010	0.12 ± 0.0083
	March	500	2.94 ± 0.014	0.30 ± 0.0081

RESULTS AND DISCUSSION

An earlier reported method for swertiamarin estimation has been modified and has been found to be sensitive, precise, accurate and robust. The recovery of extraction is 96.8 %. The swertiamarin content in a marketed raw material of *Swertia chirata* (Wall) Clarke has been already reported[6] as 0.36 ± 0.006 %. In the present study, swertiamarin content in *Swertia densiflora* (Griscb.) Kashyap. varied in different months during the flowering season. The maximum concentration of swertiamarin was obtained in month of March while the least was obtained in the month of October. This is interesting since, maximum flowers are seen in the month of March but the plants start drying. Thus, it is best to harvest the plant material in the month of March rather than the earlier months which is normally the case.

The results confirm the importance of comparative evaluation of active markers in herbal drugs to decide the time and mode of their harvest. In the case of *Swertia densiflora* (Griscb.) Kashyap. harvest in the month of March could also assist in sustaining the species survival since the harvest falls in later half of flowering season. The modified TLC method can also be applied in quality control of the plant raw material.

Conclusion

Swertia densiflora (Griscb.) Kashyap. which is used as a substitute for *Swertia chirata* (Wall) Clarke shows significant variations in swertiamarin content in different months during the flowering season. On the basis of swertiamarin content the month of March is the best month for its harvest. The modified method for swertiamarin quantitation using a cold extraction technique is sensitive, accurate and robust. The method can be used as a quality control method for the plant raw material.

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