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A HPTLC method for estimation of solasodine and diosgenin in *Solanum xanthocarpum* Schrad. and Wendl.

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ABSTRACT

The present paper deals with development and standardization of HPTLC method used for quantification of Solasodine and Diosgenin in Solanum xanthocarpum. *Solanum xanthocarpum* Schrad. and Wendl. (Kantakari) Solanaceae is known in Ayurveda for its activity in bronchial asthma, fungal infections, heart diseases, diabetes and a general low vitality of the system. It contains many phyto-constituents such as Diosgenin, Solasodine and other steroidal alkaloids. As there is no single chromatographic method for estimation of Solasosodine and Diosgenin, which can be used for standardization of extract of *Solanum xanthocarpum*, an attempt has been made to quantify Solasodine and Diosgenin in *Solanum xanthocarpum* dried crude fruit and extracts by single HPTLC method. The lowest detectable limit of Solasodine and Diosgenin was found upto 10 ng and 20 ng respectively. This method also provides good resolution and separation of Solasodine and Diosgenin from other constituents of *Solanum xanthocarpum* Schrad. and Wendl. Further, recovery values of Solasodine and Diosgenin were found to be about 92% and 93% respectively, which shows the reliability and suitability of the method. This HPTLC method was found to be reproducible, accurate and precise. The structure of isolated Solasodine and Diosgenin was characterized and confirmed by various advanced spectroscopic methods.

KEYWORDS

HPTLC;
Solanum xanthocarpum Schrad. and Wendl.;
Kankatari;
Solasodine;
Diosgenin.

INTRODUCTION

Kantkari (Solanum xanthocarpum) is one of the members of the dasamula (ten root) of the Ayurveda[1]. It is a very spiny diffuse herb up to 1.2 m tall, commonly found throughout India. *Solanum xanthocarpum* Schrad. and Wendl. Solanaceae, possesses a range of medicinal activities in bronchial asthma, fungal infections, heart diseases, diabetes and a general low vitality of the system[2-5]. *Solanum xanthocarpum* Schrad. and Wendl. is a potential herbal alternative as anti-asthma or anti-inflammatory agent and one of the active principles reported to be responsible for this action is Diosgenin and Solasodine[6-11]. It is therefore important to standardize the extract of *Solanum xanthocarpum*.

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There is no single chromatographic method available for estimation of Solasodine and Diosgenin, which are active constituents of *S.xanthocarpum* and hence, an attempt has been made to develop a HPTLC method, for estimation of both compounds, which is fast, precise, sensitive and reproducible with good recoveries for standardization of crude or/and extract of *Solanum xanthocarpum* Schrad. and Wendl.

EXPERIMENTAL

The fruits of *Solanum xanthocarpum* Schrad. and Wendl. Solanaceae were collected from three different location near Mumbai, Maharashtra, India and used as samples for the quantification of Solasodine and Diosgenin.

Preparation of the extract

The 100 gms of Solanum xanthocarpum fruit dried powder was extracted with 500 ml solvent (chloroform, methanol and water) by stirring at 50^0C for 1 hr. The filtered extract was concentrated under reduced pressure to remove the solvent. The extraction carried out for two times with the above-mentioned protocol. The extract was obtained by drying the concentrated pooled extract under vacuum. These extracts were used for estimation and comparison of Solasodine and Diosgenin content.

Pure Solasodine and Diosgenin were isolated from the dried fruits of *Solanum xanthocarpum.* Purity and structure of isolated compounds (Solasodine and Diosgenin) were confirmed by HPTLC and spectral analysis like NMR and MS. This isolated Solasodine and Diosgenin were used as working standards for quantification of content in dried crude herbs and their extracts.

Sample preparation

Accurately weighed 100 mg of aqueous extract, methanol extract and chloroform extract of dried Kantakari fruit powder and 1 gm of crude dried powder of S. xanthocarpum were separately extracted with methanol (10 ml×3) by vortexing and allowed to stand for 5 min. at room temperature. The methanol extract was then filtered through Whatmann no.42 filter paper; extracts were pooled and concentrated to dryness under vacuum. Final volume was made to 10 ml with methanol in volumetric flask. Solasodine and Diosgenin content were then analyzed after subjecting to HPTLC.

HPTLC method

Silica gel 60 F_{254} precoated plates (10×10cm) were used with Petroleum ether : Acetone (80:20) as solvent system. 10µl of sample was spotted on pre-coated TLC plates. Ascending mode was used for development of thin layer chromatography. TLC plates were developed upto 8 cms. The TLC plates were scanned at 535 nm after spraying with 10% Sulphuric acid methanolic reagent (10 ml concentrated sulphuric acid added in 90 ml of methanol with cooling and the reagent must be prepared freshly), heated at 110^0C for 2-3 min and brought to room temperature.

Procedure: -1 (Calibration curve of standard Diosgenin)

One milligram of each working standard Solasodine and Diosgenin were dissolved in 10 ml of methanol to yield stock solution of 100µg/ml concentration each. Calibration curve from 400 ng to 5 µg was prepared and checked for reproducibility, linearity and validating the proposed method. The correlation coefficient, coefficient of variance and the linearity of results were calculated.

Procedure: -2 (Calibration curve using extract spiked with Diosgenin)

The content of Diosgenin and Solasodine in dried crude and extracts was determined by comparing with the calibration curve of the working standard of Diosgenin. The extract, which showed lowest content of Diosgenin and Solasodine were then used as blank. This blank was then used to spike with the working standard of Diosgenin and Solasodine. Different samples with varying amount of standard Diosgenin and Solasodine were spiked separately in 100 mg of blank extract in which the content of both working standards had already been estimated. Procedure for sample preparation was followed as mentioned above. In each sample preparation, 10 µl of spiked solution were then subjected to HPTLC with 10 µl of blank solution for comparison. The percent recoveries of both working standards were calculated. Reproducibility, precision and validation of the method were achieved by analyz-

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ing six replicate of spike sample solutions. Correlation coefficient, coefficient of variance was calculated.

RESULTS AND DISCUSSION

Standard Diosgenin and Solasodine showed single peak in HPTLC chromatogram individually and by using the proposed method, the Rf of Diosgenin and Solasodine were found to be 0.40 and 0.28 respectively in mixture (1:1) (Figure 1). Comparative HPTLC fingerprinting profile of Diosgenin, Solasodine and mixture of both compounds shows that both the compounds were very well separated from each other as well as other constituents of Solanum xanthocarpum. The calibration curve of Diosgenin and Solasodine were obtained by spotting both the standards on HPTLC plate after scanning at 535 nm after derivatization. Dried fruits from various sources and various extracts of Solanum xanthocarpum were analysed by the proposed method and the data are recorded in TABLE 1.

Chromatographic precision and recoveries from spike sample solution

Specificity

It was observed that the other phytoconstituents present in the extract or herb did not interfere with the band of Diosgenin and Solasodine. Therefore the method was specific and helps in separation of Disogenin and Solasodine from other constituents of herb and hence, help to get the exact content of Diosgenin and Solasodine in single HPTLC method.

Limit of detection

By applying the proposed method, the minimum detectable limit of Diosgenin was found to be 20 ng/spot at 535 nm after derivatization with spraying reagent. The lowest detectable limit of Solasodine was found upto 10 ng after spraying with 10 % sulphuric acid reagent. The lowest detectable limits of Diosgenin and Solasodine were comparable to the sensitivity, which can be offered by HPLC methods, which is another advantage of this HPTLC method.

Linearity

The linearity of the method was checked with standard Diosgenin with the calibration curve in the concentration range of 400 ng-1000ng based on a 10µl sample volume. The regression equations (Y = 1442.028+8.791*X r) and correlation coefficient were obtained with 6-8 replicate analysis for each concentration. Correlation coefficients were obtained in the range of 0.97851-0.97884 indicated fair linearity of the procedure for standard Diosgenin analyzed. Calibration curve of standard Diosgenin is shown in figure 3.

The linearity of the method was checked with standard Solasodine with the calibration curve in the concentration range of 800ng-5000ng based on a 10µl

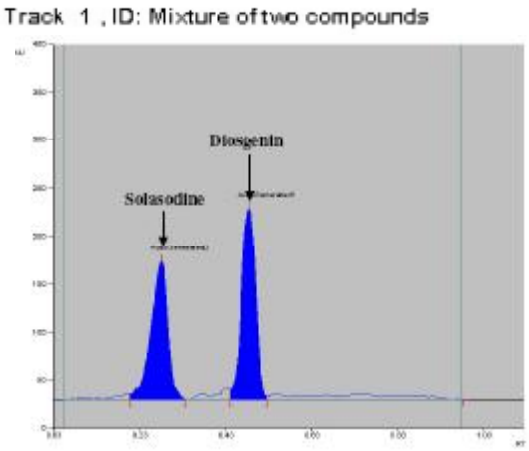

Figure 1: TLC Chromatogram of standard Diosgenin and Solasodine in mixture (1:1)

TABLE 1 : Percentage of Diosgenin and Solasodine in different samples of Solanum xanthocarpum fruits and extract by measuring area in HPTLC method

Sr.	Sample name	Region of collection	Solvent used for extraction	Diosgenin content (in mg)	Solasodine content (in mg)
1	Dried fruits	Keshavshrishti	-	36	39
2	Dried fruits	Sanjay Gandhi National Park, Mumbai	-	42	46
3	Dried fruits	Virar	-	39	41
4	Dried fruit extract-1	Sanjay Gandhi National Park, Mumbai	Chloroform	630	680
5	Dried fruit extract-3	Sanjay Gandhi National Park, Mumbai	Methanol	450	490
6	Dried fruit extract-3	Sanjay Gandhi National Park, Mumbai	Water	90	50

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TABLE 2 : Chromatographic precision and recoveries of spiked working standard 1 (Diosgenin)

Standard spiked concentration (ng/ml)	Precision (C.V.)	Recovery (%)
550	6.59	93.31
650	6.89	93.12
750	6.71	93.17
850	6.23	92.37
950	5.79	92.66

TABLE 3 : Chromatographic precision and recoveries of spiked working standard 2 (Solasodine)

Standard spiked concentration (ng/ml)	Precision (C.V.)	Recovery (%)
1500	6.74	93.01
2500	6.62	93.34
3500	6.48	93.08
4500	5.61	92.66
5500	5.07	92.34

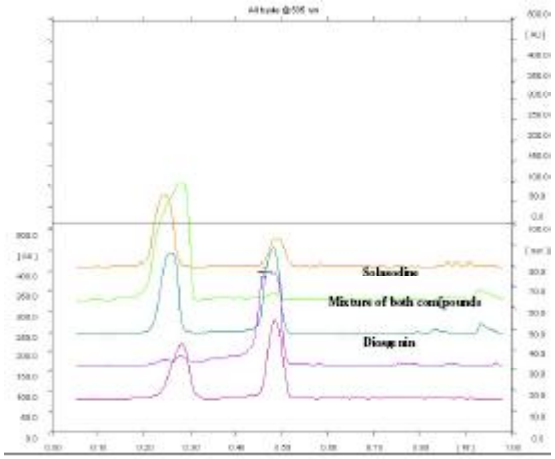


Figure 2: Comparative HPTLC fingerprinting profile of Diosgenin, Solasodine and mixture of both compounds

sample volume. The regression equations (Y = 304.736 + 1.052* X r) and correlation coefficient were obtained with 6-8 replicate analysis for each concentration. Correlation coefficients were obtained in the range of 0.98901-0.99030 indicated excellent linearity of the procedure for standard Solasodine analyzed. Calibration curve of standard Solasodine is shown in figure 4.

Precision

Six replicate HPTLC analysis of spiked Diosgenin and Solasodine in sample performed as per the procedures were carried out to check chromatographic precision. The results are recorded in TABLES 2 and 3.

Accuracy and precision

The method was applied to determine concentration of spiked Diosgenin samples in the range of 550-950 ng for assessing the accuracy and precision of the

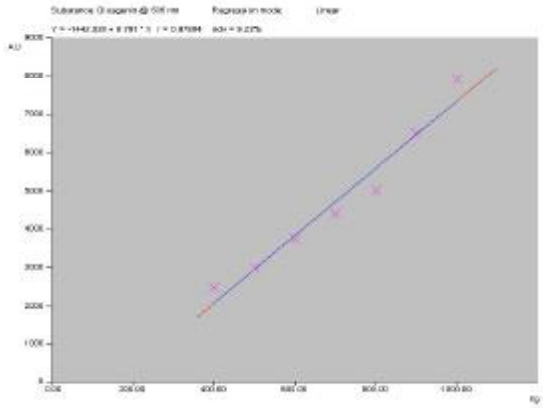


Figure 3 : Calibration curve of standard Diosgenin with respect to the area under curve at various concentration

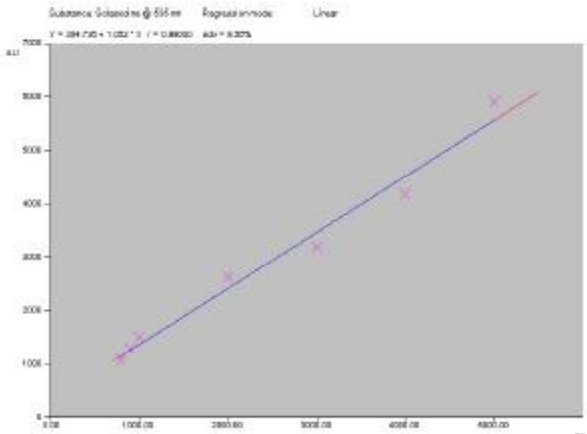
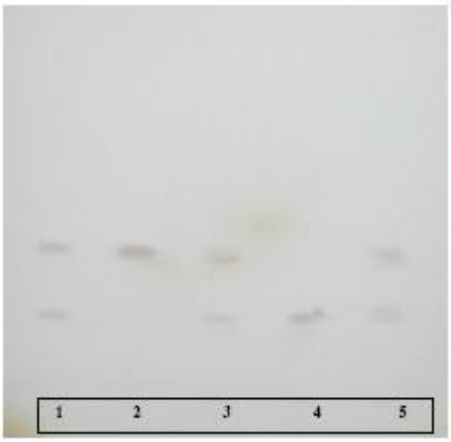


Figure 4 : Calibration curve of standard Solasodine with respect to the area under curve at various concentration



1: Disogenin and Solasodine in mixture, 2: Diosgenin, 3: Disogenin and Solasodine in mixture 4: Solasodine,5: Disogenin and Solasodine in mixture

Figure 5 : HPTLC photograph of Diosgenin and Solasodine in mixture in white light after spraying with 10% sulphuric acid-Alcoholic reagent

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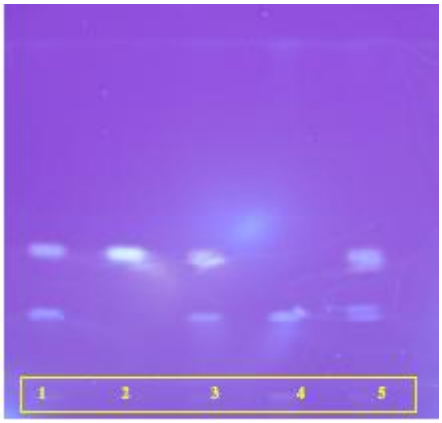

1: Disogenin and Solasodine in mixture, 2: Diosgenin, 3: Disogenin and Solasodine in mixture,4: Solasodine,5: Disogenin and Solasodine in mixture

Figure 6 : HPTLC photograph of Diosgenin and Solasodine in mixture at 366 nm after spraying with 10% sulphuric acid-alcoholic reagent

TABEL 4 : Precision and accuracy of the method applied to spiked Diosgenin samples

Amount added (ng/ml)	Amount found(ng/ml) (Mean±S.D., n=6)	Precision / Reproducibility (C.V.)	Mean Recovery (%)
550	508.1 ± 8.87	5.48	92.38
750	698.52 ± 7.60	5.43	93.13
950	881.31 ± 7.59	5.70	92.77

TABLE 5 : Precision and accuracy of the method applied to spiked Solasodine samples

Amount added (µg/ml)	Amount found (µg /ml) (Mean ± S.D., n=6)	Precision / Reproducibility (C.V.)	Mean Recovery (%)
1.5	1.408 ± 8.71	6.71	93.92
3.5	3.262 ± 8.02	6.02	93.22
5.5	5.107 ± 8.19	5.88	92.87

procedure. TABLE 4 represents the mean values and coefficient variance (C.V.) results indicate the levels in the above range can be estimated with accuracy and precision.

The method was applied to determine concentration of spiked Solasodine samples in the range of 1.5-5.5 µg for assessing the accuracy and precision of the procedure. TABLE 5 represents the mean values and coefficient variance (C.V.) results indicate the levels in the above range can be estimated with accuracy and precision.

CONCLUSION

The structure of Diosgenin and Solasodine were confirmed by NMR, Mass and IR spectroscopy. The lowest detectable limit of Diosgenin and Solasodine were found upto 20 ng and 10 ng respectively and provides good resolution and separation of Diosgenin and Solasodine from other constituents of *Solanum xanthocarpum* Schrad. and Wendl. in single developed HPTLC method. Further, recovery values of Diosgenin were found to be about 92-93%, which shows the reliability and suitability of the method. The proposed HPTLC method is rapid, simple and accurate for quantitative monitoring of Diosgenin and Solasodine in *Solanum xanthocarpum* Schrad. and Wendl. dried fruits and extracts.

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