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Development and validation of high-performance thin layer chromatography method for the quantification of solasodine in *Solanum trilobatum* L.K. Vijayakaran\*<sup>◆</sup>, V. Ranganathan\*\*<sup>◆</sup>, P. Senthil Kumar\*\*<sup>◆</sup>, S. Balakrishnan\*\*\*<sup>◆</sup>, R. Velusamy\*\*\*\*<sup>◆</sup>, K. Kannan\*\*<sup>◆</sup> and A. Elamaram\*\*<sup>◆</sup>

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## Article Info

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## Abstract

The current study aims in validating a high-performance thin layer chromatography (HPTLC) method for examining solasodine in *Solanum trilobatum* L. as per the International Conference on Harmonization (ICH) Harmonized Tripartite guideline. In Siddha medicine, *S. trilobatum* has long been used to treat cough, bronchitis, and tuberculosis. This plant contains a bioactive phytochemical called solasodine, which has been shown to have immunomodulatory, diuretic, antifungal, anticancer, cardiotoxic, and antispermatic and cures neurogenetic diseases. Nevertheless, there have never been HPTLC methods in quantifying solasodine in *S. trilobatum*. A Soxhlet device was used to prepare a plant extract after fresh leaves were gathered from Thanjavur, Tamil Nadu. Using ethanol, the yield percentage was determined to be 2.29%. Initial phytochemical screening revealed the presence of flavonoids, terpenoids, alkaloids, phenols, and saponins. This research additionally analyzed total alkaloids, flavonoids, and phenols. Linearity of solasodine ranged within 200-800 ng/spot concentration, with coefficient of variation of 2.3% and a correlation coefficient of 99.65%. Solasodine content has been identified to be 0.5168% on a dry weight basis, with quantification limit being 334.5 ng/spot and detection limit being 110.4 ng/spot. Developed HPTLC method is accurate, precise, robust, and specific, therefore being suitable for quality monitoring and standardization of drugs containing solasodine in *Solanum* species. This provides potential for further research and development of pharmacological applications.

## 1. Introduction

Plants of the genus *Solanum* (Solanaceae) are abundant in steroidal glycoalkaloids, a significant class of secondary compounds, and are well known for their wide range of therapeutic applications and have been recognized for various bioactivities (Ranjitha and Shobha, 2022; Karthikeyan *et al.*, 2024). Researchers have focused considerable attention on solasodine, glycoalkaloid, given its numerous medicinal properties, that include immunomodulatory (Bahr and Hansel, 1982), anti-schistosomicidal (Miranda *et al.*, 2012), antifertility (Gupta and Dixit, 2002), anti-amnesic (Desai *et al.*, 2011), anti-inflammatory (Malik *et al.*, 2018), hepatoprotective (Lin *et al.*, 1988), antioxidant (Sharma *et al.*, 2014), antinociceptive (Pandurangan *et al.*, 2010), anticonvulsant (Chauhan *et al.*, 2011), and other neurogenetic disorders (Lecanu *et al.*, 2011). Given that it

is a precursor for the semi-synthesis of contraceptives and the anabolic steroidal hormones progesterone and cortisone, it has also garnered a lot of attention considering its great pharmacological and industrial significance (Kumar *et al.*, 2019). Solasodine is an aglycone component of glycoalkaloids, a nitrogen counterpart of sapogenins, found in the majority of solanaceous plants. Solasodine is a glycoalkaloid with a C<sub>27</sub> cholestane structure that is composed of six sugar molecules and a basic nitrogen bonded to a steroidal skeleton (Figure 2). A critical stage in the synthesis of steroidal drugs, 16-dehydropregnenolone, could be readily converted from it (Ghisalberti, 2006; Pawar Pankaj *et al.*, 2008).

A perennial herb in Solanaceae family, *Solanum trilobatum* L. (Figure 1) is utilized for treating respiratory ailments that include chronic bronchitis and cough (Nee, 1999). This noteworthy medicinal plant is found in southern India and is used in Ayurvedic and Siddha medicine systems (Nataraj and Ramachandramurthy, 2014). It is frequently employed in traditional medicine for treating rheumatism, asthma, tuberculosis, respiratory disorders, and rheumatism. It additionally assists in lower blood glucose levels and has antibacterial, antifungal, antioxidant, and anticancer qualities (Govindan *et al.*, 2004; Sugnanam and Rajanala, 2015). Among phytochemical

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components of this plant that contribute to its numerous medicinal properties: glycoalkaloid, diosgenin, sobatum, solamarine, solanine, solasodine, and tomatidine (Giulietti *et al.*, 1991). This species has been found to contain the glycoalkaloid solasodine (Krishnamurthy *et al.*, 1996). Therefore, estimation of solasodine content is essential to isolate this glycoalkaloid for commercial use from this plant.

Several analytical methods, including HPLC and HPTLC, have been employed for assessing the amount of solasodine from several *Solanum* species, including *Solanum xanthocarpum*, *S. khasianum*, *S. nigrum*, *S. gracile*, and *S. laciniatum* (Trivedi and Pundarikakshudu, 2007; Chaudhary *et al.*, 2021). Since plant-based traditional remedies are affordable and free from side effects, their pharmacological evaluation and active principle estimation using analytical methods are typically limited mostly to extracts and are critical (Nagaiah, 2022). According to the review of the literature, there is currently no HPTLC method available for determining solasodine in *S. trilobatum*. Given this, the current research intends to develop a quick and accurate analytical method for employing HPTLC to quantify solasodine from *S. trilobatum*.



Figure 1: *Solanum trilobatum* L.

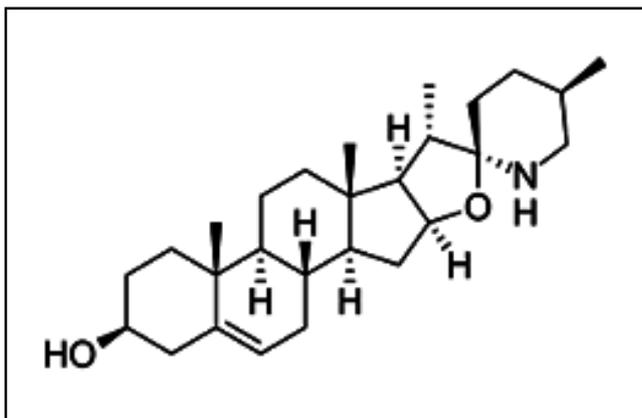


Figure 2: Chemical structure of solasodine.

## 2. Materials and Methods

### 2.1 Collection of plant materials

Ethno Veterinary Herbal Product Research and Development Centre's Herbal Garden at Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Orathanadu, Thanjavur District, Tamil Nadu, India, is where fresh *S. trilobatum*

leaves have been collected. Dr. K.N Sunil Kumar, Department of Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai-600106, Tamil Nadu, India verified the authenticity of the plant where the Voucher Specimen Number (S22042323T) had been preserved in the same Institute in the form of herbarium for future use. A 40-mesh pulverizer was used to grind the leaves after they had been manually cleaned and shade-dried at  $25 \pm 3^\circ\text{C}$ . For additional research, the leaves were pulverized then preserved in an airtight container.

### 2.2 Preparation of plant extract

For 72 h, precisely weighed crude powdered sample of *S. trilobatum* had been extracted utilizing Soxhlet with HPLC-grade ethanol (Shunmugadevi *et al.*, 2022). Extracted filtrate concentrated at a lower temperature ( $25 \pm 2^\circ\text{C}$ ) and pressure (40 m bar) in rotatory evaporator (Buchi Rotavapor R-300, Switzerland). Concentrated extract then gathered, weighed, and kept for additional examination at  $4^\circ\text{C}$ . Petroleum ether was used to defatten the samples before extraction to eliminate any fatty substances or contaminants.

### 2.3 Estimation of yield percentage

The extraction yield percentage of the plants used in the present study has been calculated applying following formula:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of the extract (g)}}{\text{Weight of the powdered plant material (g)}} \times 100$$

### 2.4 Phytochemical analysis

#### 2.4.1 Qualitative phytochemical screening

To determine presence of various phytochemicals, freshly prepared plant extracts subjected through an initial phytochemical screening (Trease and Evans, 1989; Sahu *et al.*, 2014).

#### 2.4.2 Estimation of total phenolic, flavonoid, and alkaloid content

Total phenolic contents of ethanolic extracts have been determined utilizing Folin-Ciocalteu assay, that adapted from Supriya and Radha's (2018) description. Gallic acid equivalent (GAE)  $\text{mg g}^{-1}$  served as result's unit. Rutin equivalent (RE)  $\text{mg g}^{-1}$  was the end result of estimating the total flavonoid content using rutin as the standard (Bedraj and Meena, 2014). The method described by Fazel *et al.* (2010) had been slightly adjusted for determining total alkaloid content employing atropine as a reference. The results were reported in  $\text{mg g}^{-1}$ .

### 2.5 Analytical studies: Development of HPTLC protocol for the identification and estimation of solasodine from *S. trilobatum*.

#### 2.5.1 Chemicals and reference compounds

Analytical or HPLC grade reagents and solvents had been used (M/s Merck Specialities Ltd., Worli, Mumbai, India). Prior to use, 0.45 mm membrane (Millipore, Billerica, MA, USA) had been employed for filtering solvents. Merck (M/s Merck Specialities Ltd., Worli, Mumbai, India) supplied pre-coated TLC silica gel 60F254 aluminum plates (Product number: 1.05554). Solasodine (Product number: PHL80004), the standard reference substance, has been purchased from M/s Sigma Aldrich Chemicals Pvt. Ltd., USA.

### 2.5.2 Preparation of stock and working solution

Before HPTLC analysis, marker solasodine 1 mg had been dissolved in HPLC-grade methanol (1 ml) to make stock solution, that was then kept at 4°C. To produce working solution of 100 µg ml<sup>-1</sup>, aliquots of stock solution (standards) have been diluted 10 times with methanol in volumetric flasks. Working standard dilutions have been filtrated with 0.45 µm membrane filter (Millipore, Burlington, MA, USA) before HPTLC analysis.

### 2.5.3 HPTLC instrumentation

Silica gel 60F254 had been precoated on 20 x 10 cm, 0.2 mm thick HPTLC plates. Densitometer: TLC Scanner 4 with vision CATS software (Camag, Muttenz, Switzerland); syringe: 100 µl (Hamilton Bonaduz, Switzerland); TLC chamber: 20 × 10 × 4 cm Camag glass twin trough chamber.

### 2.5.4 Chromatographic conditions

For chemical profiling and process optimization, 20 × 10 cm TLC aluminum pre-coated plates with 0.2 mm silica gel 60F254 (M/s Merck Specialities Ltd., Worli, Mumbai, India) have been employed for detecting solasodine. Standard and sample tracks have been applied independently at 100 nl s<sup>-1</sup> delivery speed as 8 mm wide bands, 10 mm from plate's bottom, and 15 mm from its edge employing an automated nitrogen-flowing TLC applicator. The mobile phase used was n-butanol: ethyl acetate: 10% acetic acid (10:7:3). For enhanced resolution, twin-trough glass chamber had been saturated with mobile phase vapors for 20 min at 25 ± 2°C and 55 ± 2% relative humidity. This procedure is conducted through linear ascending development. About 80 mm of height was reached by plate's development from application site (total length ran by mobile phase). Developed chromatographic plates have been derivatized employing an automatic derivatizer with anisaldehyde-sulfuric acid, then dried in an air current for 3 min with hair drier set to normal mode for visualization of bands. A wavelength of 530 nm has been employed for scanning solasodine in absorbance-reflectance mode. Area versus standard marker concentration was used to quantify solasodine.

### 2.5.5 Method validation

ICH Harmonized Tripartite guidelines (ICHHT, Q2 (R1) 2005) have been followed in verification of HPTLC method for measuring solasodine in *S. trilobatum*. The performance of recommended strategy has been verified by applying a variety of metrics, that includes recovery as accuracy, robustness and precision, linearity, limit of detection (LOD), specificity, and limit of quantification (LOQ).

#### 2.5.5.1 Calibration curve for solasodine

An absolute amount of 100, 200, 300, 400, 500, 600, 700, and 800 ng/band was obtained by applying a solasodine standard solution with concentration of 100 µg ml<sup>-1</sup> in triplicate at eight different volumes. The plate was developed at 25 ± 2°C with 40% relative humidity up to 8 cm in solvent system containing n-butanol: ethyl acetate: acetic acid 10% (10:7:3). Following development, plate underwent air drying, derivatization, and 530 nm wavelength scan. Area of peak had been recorded. Peak area vs concentration was then plotted for generating calibration curve.

#### 2.5.5.2 Precision

By scanning the same solasodine spot (250 ng/spot) six times (n=6), the instrument's precision was evaluated. Aliquots of solasodine standard solution at 250, 500, and 750 ng/spot have been examined daily and over numerous days for evaluating technique variability (interday and intraday precision). Data is given utilizing percentage relative standard deviation (% RSD).

#### 2.5.5.3 Accuracy

Spiking samples at three distinct concentrations: 200, 250, and 300 ng accuracy has been evaluated employing conventional addition method. Calculations have been conducted to determine recovery percentage and the mean recovery %.

#### 2.5.5.4 Robustness

Robustness of proposed HPTLC densitometric method has been investigated by making small deliberate modifications to chromatographic parameters that could affect method's performance, including volume, composition, and duration of mobile phase saturation in addition to interval within derivatization and scanning. Mobile phase n-butanol: ethyl acetate: 10 % acetic acid was used at various concentrations, viz., 11:8:2 v/v, 10:7:3 v/v, and 9:7:4 v/v, for developing chromatograms. The robustness of the approach has been assessed for solasodine at a concentration level of 550 ng/spot.

#### 2.5.5.5 Specificity

By comparing R<sub>f</sub> (retardation factor) values in sample and standard tracks, technique specificity has been examined. Bands in chromatogram derived from samples that resembled solasodine had been verified through comparing their R<sub>f</sub> values, and peak purity had been determined.

#### 2.5.5.6 Limit of detection (LOD) and limit of quantification (LOQ)

LOD is a representation of the lowest detectable amounts of solasodine. The LOQ, on the other hand, represents the lowest concentrations that may be estimated with a respectable degree of accuracy and precision. The linear regression equations 3.3 σ/S and 10 σ/S, respectively, were used to calculate LOD and LOQ, where S represents slope of linearity plot, and σ response's standard deviation (y-intercept).

### 2.6 Quantification of solasodine in *S. trilobatum*

To make extracts of *S. trilobatum* leaves, a measured amount of the extract had been dissolved in methanol to attain working concentration of 100 mg ml<sup>-1</sup>. Prior to HPTLC examination, 0.45 µm membrane filter "Millipore, Burlington, MA, USA" had been employed for filtering samples. Seven distinct volumes: 1, 2, 3, 4, 5, 6, and 7 µl were utilized for duplicate analysis. The plates were developed and scanned following standard procedures, with identification achieved by comparing R<sub>f</sub> values of standards and samples. Area and concentration of standard marker have been employed for measuring solasodine, and results have been expressed as a percentage of dry weight of powdered sample (Amir *et al.*, 2018).

### 2.7 Fourier transform infrared spectrometer (FT-IR) analysis of *S. trilobatum* extract

FT-IR analysis was carried out employing Thermo Nicolet 6700 FT-IR (Thermo Fischer Scientific Inc., USA) for analyzing the crude extract of *S. trilobatum*. The scan was conducted between 4000  $\text{cm}^{-1}$  and 400  $\text{cm}^{-1}$  after the liquid sample had been placed inside the cell.

## 3. Results

### 3.1 Extraction yield percentage and phytochemical screening

Extraction yield percentage of *S. trilobatum* ethanolic extract in the present study is 2.29% and the result of phytochemical screening displayed in Table 1.

**Table 1: Qualitative phytochemical screening of *S. trilobatum***

S. No.	Phytoconstituents	Name of the test	Inference
1.	Carbohydrates	Benedict's test	Negative
		Molisch's test	Negative
2.	Terpenoids	Salkowski's test	Positive
3.	Alkaloids	Mayer's test	Positive
		Dragendroff's test	Positive
		Wagner's test	Positive
		Hager's test	Positive
4.	Phenols	Ferric chloride test	Positive
5.	Tannins	Braymer's test	Negative
6.	Cardiac glycosides	Keller-killani test	Negative
7.	Flavonoids	Shinoda's test	Positive
		Alkaline reagent test	Positive
8.	Saponins	Foam test	Positive
9.	Glycosides	Borntreger's test	Positive
10.	Proteins	Millon's test	Negative

### 3.2 Estimation of total phenolic, flavonoid and alkaloid content

Table 2 illustrates the total amount of phenolic, flavonoid, and alkaloid content in *S. trilobatum* leaves.

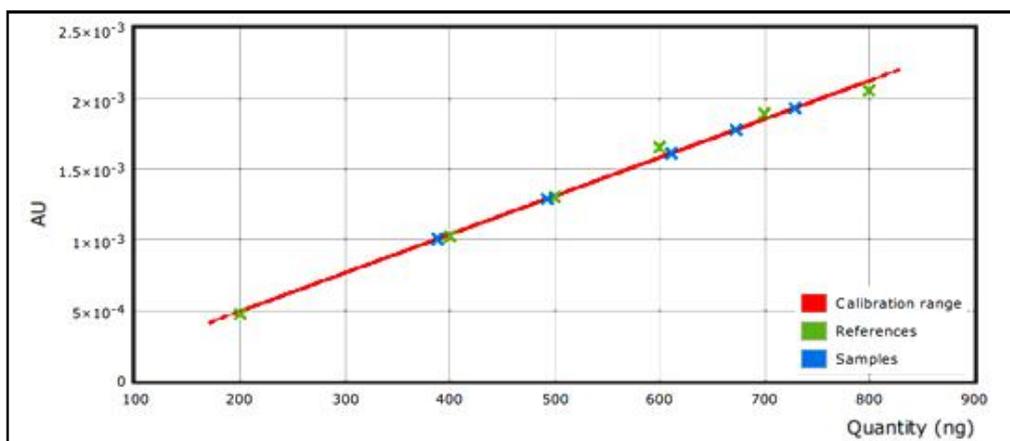
**Table 2: Quantitative phytochemical screening of *S. trilobatum***

Total flavonoid content	$67.15 \pm 0.18 \text{ mg RE g}^{-1}$
Total phenolic content	$2.161 \pm 0.57 \text{ mg GAE g}^{-1}$
Total alkaloid content	$3.69 \pm 0.23 \text{ mg g}^{-1}$

### 3.3 HPTLC studies for the estimation of solasodine from *S. trilobatum*

#### 3.3.1 Linearity of solasodine

With a correlation value ( $r^2$ ) of 0.996, standard solution solasodine concentration and peak response in concentration ranging 200-800 ng/spot demonstrated a substantial connection ( $r^2$ ) applying linear regression (Figure 3). For further validation linearity of suggested method, residual plot curve of standards area vs. concentration had been displayed. A linear model fits the data rather well, according to residual plot's positive random pattern (Figure 4).



**Figure 3: Linearity of solasodine.**

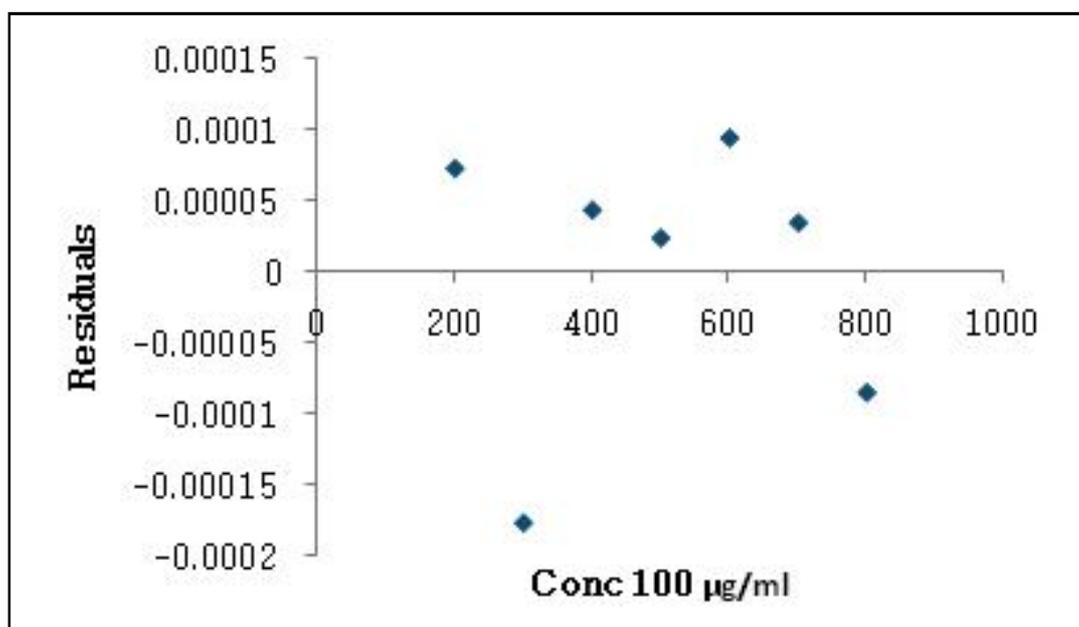


Figure 4: Residual plot of regression analysis for the calibration of solasodine.

### 3.3.2 Precision

For instrumental precision investigated at a single concentration of 250 ng/spot, relative standard deviation (RSD) (%) value was 0.14.

Intra-day and inter-day precision investigations have been evaluated employing three distinct solasodine concentrations, and Table 3 displays results as RSD (%) values.

Table 3: Intra-day and inter-day precision of solasodine

Standard	Nominal concentration (ng)	Concentration obtained (ng)		% RSD	
		Intra-day	Inter-day	Intra-day	Inter-day
Solasodine	250	249.82	249.62	0.135	0.167
	500	499.36	498.72	0.180	0.280
	750	749.06	748.56	0.126	0.156

### 3.3.3 Accuracy

By spiking samples at three distinct concentrations: 200, 250, and 300 ng accuracy has been evaluated employing standard addition

method. Recovered solasodine percentage is determined to be between 99.96 and 100.01%, with RSD values between 0.026 and 0.039. Average recovery for solasodine was 99.98%, according to recovery of analysis (Table 4).

Table 4: Accuracy of solasodine

Concentration taken (ng)	Concentration added (ng)	Concentration found (ng)	% Recovery	% RSD
250	200	449.89	99.97	0.039
250	250	499.84	99.96	0.029
250	300	550.01	100.01	0.026

### 3.3.4 Robustness

Only slight variations in the peak regions were seen when the method's resilience was verified by deliberately modifying chromatographic parameters, that includes mobile phase concentration, chamber saturation duration, and delay in digital scanning following derivatization. When the analysis was conducted within 30 min of

derivatization, the quantitation was significantly impacted by the time interval between derivatization and scanning. Under laboratory circumstances, it stayed stable for 24 h following a 30 min derivatization period. Percentage RSD <2% following SD of peak areas for each deliberate alteration to mobile phase's composition had been calculated. The results are shown in Table 5, and these low percentage RSD values demonstrated the method's robustness.

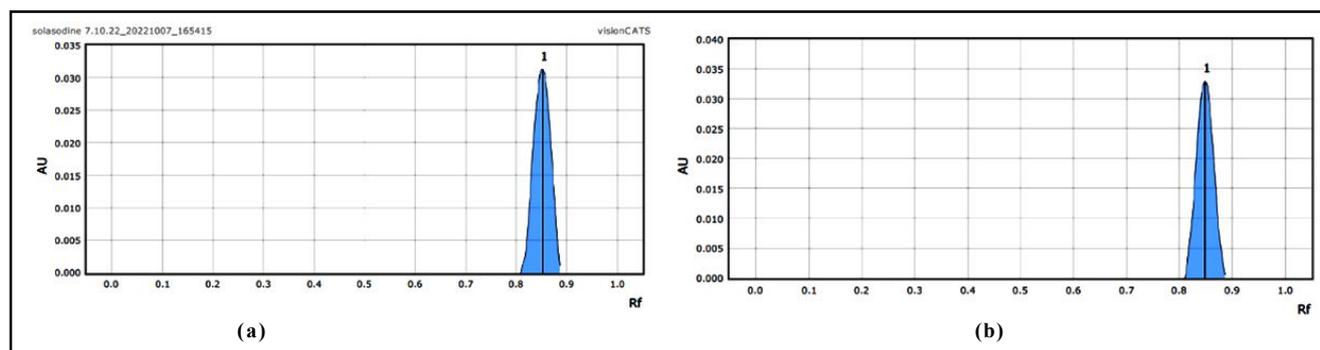
**Table 5: Robustness of solasodine**

Concentration (ng/spot)	Mobile phase composition (n-butanol: ethyl acetate: acetic acid)		Results (n = 6)		
	Original (v/v)	Used (v/v)	Concentration $\pm$ SD	% RSD	$R_f$
550	10:7:3	11:8:2	547.59 $\pm$ 0.46	0.08	0.85
		10:7:3	549.88 $\pm$ 0.17	0.03	0.86
		9:7:4	547.82 $\pm$ 1.10	0.20	0.86

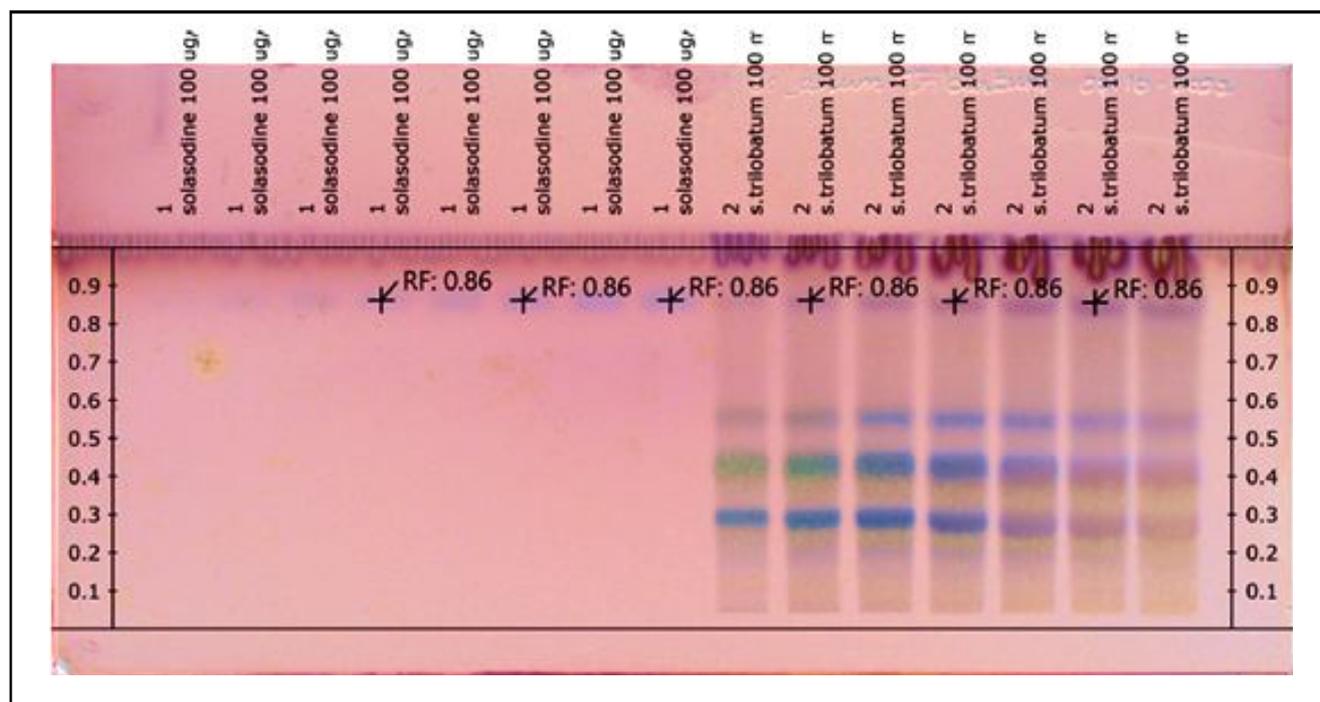
### 3.3.5 Specificity

By contrasting  $R_f$  of solasodine in standard and sample tracks, technique specificity has been examined. Since the developed plate was derivatized using anisaldehyde - sulfuric acid, the solasodine

bands appeared bluish. Therefore, specificity obtained upon comparing  $R_f$  values ( $0.86 \pm 0.01$ ) and blue color bands in standards and sample, which make sure the purity of solasodine peaks in the sample track and is represented in Figure 5. and Figure 6.



**Figure 5: Specificity of solasodine showing densitogram of standard (a) and sample (b).**



**Figure 6: Developed and derivatized chromatographic plate of solasodine showing blue color bands in standard (represented as 1) as well as a sample (defined as 2) at  $R_f$  0.86.**

### 3.3.6 Limit of detection and limit of quantification

Sensitivity of method has been determined as  $3.3 \sigma/S$  for LOD and  $10 \sigma/S$  for LOQ, where S represents slope of linearity plot and  $\sigma$  is SD

of response (y-intercept). LOD and LOQ measured for solasodine: 110.4 and 334.5 ng/spot, respectively. This implied that the new method's sensitivity for measuring solasodine was good.

Therefore, analytical features of verified HPTLC method for determining solasodine content in *S. trilobatum* are given in Table 6.

### 3.4 Quantification of solasodine

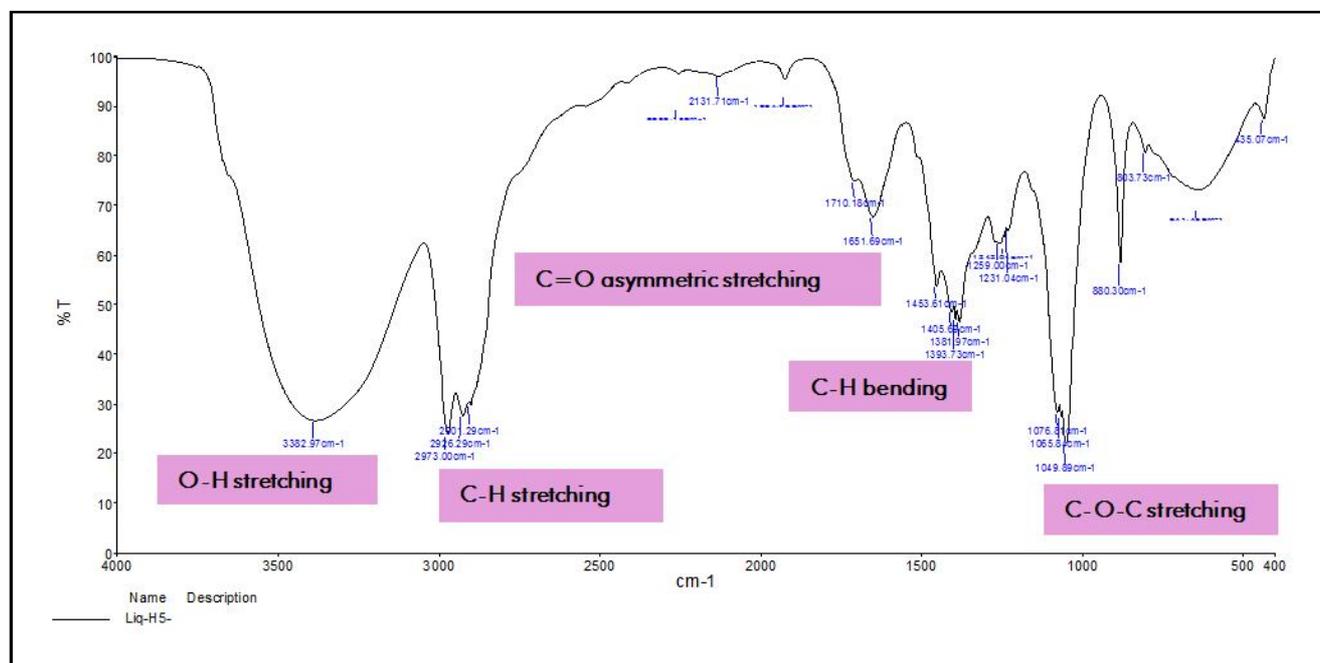
Solasodine was identified at  $R_f$   $0.86 \pm 0.01$ , and its quantification was based on area versus concentration of solasodine. In the leaves of *S. trilobatum*, 0.5168% of solasodine was found on dry wt. basis.

### 3.5 FT-IR analysis

FT-IR analysis of *S. trilobatum* extract showed peaks at 880.30, 1049.89, 1259, 1393.73, 1453.61, 1651.69, 1710.18, 2131.71, 2973.49 and 3382.97  $\text{cm}^{-1}$  and is shown in Figure 7. It is associated to following: amines (N-H bend), alcohol, phenol (O-H stretch), alkynes ( $-\text{C}=\text{C}-$  stretch, C-H stretch), carboxylic acid (C=O stretch), epoxide (C-O-C stretching), primary alcohol (C-O stretching), aromatic group (C-H out of plane bending), and unsaturated ester (C=O stretch).

**Table 6: Validated HPTLC parameters for the estimation of solasodine in *S. trilobatum***

Parameters	Results
Linearity range	200 to 800 ng/spot
Limit of quantification	334.5 ng/spot
Accuracy (Recovery)	99.98%
Limit of detection	110.4 ng/spot
Correlation coefficient	0.996
Specificity	Specific
Robustness	Robust
Retardation factor ( $R_f$ )	$0.86 \pm 0.01$
Regression equation	$Y=2.725 \times 10^{-9} x$
Slope	2.89642857142857E-06
Intercept	-0.000171071
Standard deviation	0.0000969013980930149



**Figure 7: FT-IR spectrum of ethanolic extract of *S. trilobatum* leaves showing the presence of solasodine.**

## 4. Discussion

Antimicrobial resistance is a major global health concern, highlighting the need for alternative therapies like phytomedicine, which leverages plant-derived compounds with diverse mechanisms of action (Akhtar,

2024). Phytochemicals are worthy bioactive components that provide immense health benefits to human beings as well as to livestock. The Deccan peninsula of India is home to the straggler climber *Solanum trilobatum* L., which contains a variety of phytochemicals,

including the significant glycoalkaloid solasodine. Therefore, the main phytochemical profiling of *S. trilobatum* leaves is covered in this work, with a focus on estimating solasodine content using HPTLC. The extractive yield percentage for *S. trilobatum* in this study is in good agreement with Rajkumar *et al.* (2018) in their earlier research, which found a yield of 2.98 per cent. In line with the findings of Balakrishnan *et al.* (2015), preliminary screening of phytochemicals showed the presence of a variety of bioactive components, such as terpenoids, alkaloids, phenols, carbohydrates, and flavonoids. Despite a few small variations, the results pertaining to phytochemical quantitation are largely consistent with earlier studies. Conversely, quantifications reported by Parasuraman *et al.* (2016) and Priya and Chellaram (2014), which reported 1.21 mg GAE/g and 1.43 mg/g, respectively, current study found a higher concentration of phenolics and alkaloids in *S. trilobatum*. Priya *et al.* (2012) reported a lower concentration of flavonoid content (41.7 mg RE/g).

Many factors, such as variations in geographic location, plant material, solvent selection, extraction duration, temperature, harvesting time and extraction methods, analytical techniques, and environmental conditions, may be responsible for the slight variations in yield percentage, qualitative, and quantitative phytochemical screening results in current study with previous research (Kanerla *et al.*, 2012; Shaikh and Patil, 2020).

The lack of standardization in polyherbal formulations hinders their acceptance, making the development of SOPs and physicochemical standardization essential to ensure batch-to-batch efficacy, safety, and ingredient integrity (Beg *et al.*, 2022). Among the most advanced instrumental methods for evaluating herbal medicines qualitatively and quantitatively is HPTLC (Vidhyatai *et al.*, 2022). Quantitative, semi-quantitative, and qualitative phytochemical analysis of herbal medicines and formulations additionally discovered HPTLC to be an effective technique. This includes the estimate of biomarkers and the development of TLC fingerprinting profiles. Thus, for the quantitative and qualitative evaluation of solasodine in *S. trilobatum*, the HPTLC densitometric approach was selected. According to the findings, all of the parameters that were validated in this study, including linearity, precision, accuracy, robustness, specificity, LOD, and LOQ, fall within the acceptable ranges specified in the ICH standards. The present study's findings on the solasodine content of *S. trilobatum* leaves differed slightly from those of Anirudhan and Nair (2009), who reported 0.232% solasodine as evaluated by HPLC. This could be because of a number of causes as previously mentioned.

FT-IR analysis of *S. trilobatum* extract in current research confirms results of Narendhran *et al.* (2019), showing presence of compound classes including alkynes (C=C stretch, C-H stretch), alcohol/phenol (O-H stretch), unsaturated ester (C=O stretch), carboxylic acid (O-H stretch), alkanes (C-H rock), amines (N-H bend), and aliphatic amine (C-N stretch). Additionally, the results are in accordance with authentic solasodine peaks that Subroto *et al.* (2007) reported. These peaks, that have been identified at 3448.9, 2940.5, 1640.8, 1675.2, 1452.9, 1136.5, and 1070.6 cm<sup>-1</sup>, demonstrate that *S. trilobatum* extract contains solasodine.

## 5. Conclusion

The quantity of solasodine in *S. trilobatum* leaves could be determined by employing an approved HPTLC method. The proposed method for quantifying solasodine from *Solanum* species has been

demonstrated to be simple, linear, accurate, reproducible, robust, and precise. Solasodine presence has also been confirmed by spectroscopic studies. Thus, the standardization of herbal drugs and formulations is essential for ensuring its therapeutic efficacy and safety which can significantly contribute to quality assurance practices, especially in preventing adulteration in the herbal formulations containing solasodine.

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## Conflict of interest

The author declares no conflicts of interest relevant to this article.

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