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High-performance thin layer chromatography based assay and stress study of a rare steroidal alkaloid solanopubamine in six species of Solanum grown in Saudi Arabia

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Abstract
The present study describes a method developed for quantification and stability study of a rare steroidal alkaloid solanopubamine (SPN) in aerial parts of six different species of genus Solanum extracted with two different solvents. The Solanum species selected for investigation include S. schimperianum (SS), S. villosum (SV), S. coagulans (SC), S. glabratum (SG), S. incanum (SI) and S. nigrum (SN). The estimation of SPN was done by a validated high-performance thin layer chromatography method. The developed chromatographic system was found to give a sharp spot for solanopubamine at \(R_f = 0.39 \pm 0.01\). The steroidal alkaloid SPN was observed to be present only in extracts of aerial parts of S. schimperianum. The sensitivity of developed method produced 40 ng and 115 ng band \(^{-1}\), respectively as LOD and LOQ values. The percentage yield of SPN in aerial...
1. Introduction

Solanaceae is one of the therapeutically important plant families possessing a broad spectrum of chemical entities and always been an attractive target for the researchers. Solanum being the largest genera of family Solanaceae possessing around 1700 species distributed throughout the world. In Saudi Arabia, the genus Solanum is represented by about 16 species found in West and Southwestern areas of the country (Chaudhary, 2001; Collenette, 1999). Solanopubamine (SPN) (Fig. 1), chemically named as 3β-amino-5α, 22αH, 25β-[H- solanidan-23β]-ol, is a rare steroidal alkaloid and till now reported only in aerial parts of two species of Solanum. The first reporting of Solanopubamine was done by Kumari et al., 1985 in S. pubescence followed by Al-Rehaily et al., 2013 in S. schimperianum. Solanum is a medicinally useful genus and particularly known for therapeutically active alkaloids. Ethyl acetate extract of leaves of Solanum pubescens exhibits significant anti-inflammatory activity (Niyogi et al., 2012). The plant is also used as anti-lice (Hemamalini et al., 2011a), anthelminitic (Hemamalini et al., 2011b), antioxidant (Peddi et al., 2013), anticonvulsant, sedative (Suvarchala et al., 2013), antibacterial (Haseebur et al., 2014), gastroprotective (Hemamalini et al., 2011c), and hepatoprotective agent (Pushpalatha and Ananthi, 2012). The literature survey revealed that S. schimperianum possesses potent antitrypanosomal (Abdel-Sattar et al., 2009), antibacterial (Al-Oqail et al., 2012) and antifungal activity (Al-Rehaily et al., 2013).

There are a variety of compounds reported in S. pubescens and some of them are myricetin methyl ethers (Kumari et al., 1984), solanopubamine (Kumari et al., 1985), and solanopubamides A & B (Kumari et al., 1986). The compounds reported from S. schimperianum are solanopubamine (Al-Rehaily et al., 2013), esculentin, astragalin, isouqueretin, kaempferol 3- diglucoside or 3-diglucosidekaempferol, rutin, solamarine and solamarine (Coune and Denuel, 1975; Angenot, 1969); lupeol, β-sitosterol, β-sitosterol glucoside, oleanolic acid, tefiderin, tetnerinin, 5-hydroxy-3,7,4',3-trimethoxyflavone, retusin and kaempferol-3-O-β-D-glucopyranoside (Al-Oqail et al., 2012). Steroidal alkaloids are well known for their therapeutic potential as antifungal (Chen et al., 1998), antiviral (Wang et al., 2010), antiestrogen (Chang et al., 1998) and antitumor (Kupchan et al., 1965; Lee et al., 2004; Nin et al., 2009) activities. During combination chemotherapy for treating multidrug resistant cancer with the conventional cytotoxic drugs, steroidal alkaloids are used as chemosensitizers (Lavie et al., 2001).

The clinical importance of steroidal alkaloids has drawn our interest to explore the presence of SPN and its quantification in six species of Solanum collected from Abha region of Saudi Arabia. These species include S. schimperianum (SS), S. villosum (SV), S. coagulans (SC), S. glabratum (SG), S. inca-num (SI) and S. nigrum (SN). Authors did not find literature related to the analytical and stability aspects of SPN. In recent years, high-performance thin-layer chromatography (HPTLC) gained much popularity for its analytical interventions in herbal drugs and formulations. This popularity is due to some of its special features such as simple, rapid, economic and multi-marker assessment capability (Siddiqui et al., 2015a). HPTLC technique is a multipurpose analytical tool and can be used for analyzing a broad range of chemical compounds. Formulations such as pharmaceuticals, cosmetics, and dietary supplements may also be analyzed for their contents and efficacy (Siddiqui et al., 2015b). This study was performed to explore SPN in some other species of Solanum as well as its quantification in S. schimperianum.

2. Materials and methods

2.1. Materials

The aerial parts of six Solanum species viz. S. schimperianum (SS), S. villosum (SV), S. coagulans (SC), S. glabratum (SG), S. inca-num (SI) and S. nigrum (SN) with voucher specimen numbers 15,038, 15,032, 15,101, 15,043, 15,102 and 15,149 respectively were collected from Abha region of Saudi Arabia in March, 2013, and identified by Dr. Mohammed Yusuf, Taxonomist, Department of Pharmacognosy, College of Pharmacy, King Saud University, Saudi Arabia. Specimens of the plant were deposited in the departmental herbarium.

2.2. Apparatus and reagents

Standard solanopubamine was obtained after isolation from aerial parts of S. schimperianum and characterized by UV,
IR, 1D and 2D NMR and MASS spectroscopic studies (Al-Rehaily et al., 2013). Reagents and solvents of analytical grade (chloroform, methanol, ethanol, ammonia, etc.) were purchased from WINLAB and BDH (U.K.). Glass-backed silica gel 60F254 HPTLC plates (20 × 10 cm) were purchased from Merck (Germany). An ethanol solution of standard and extracts was applied to chromatographic plates as bands, by an automatic TLC sampler-4 (CAMAG) and developed in automatic development chamber. HPTLC Plates were then documented and scanned by TLC Reprostar 3 and CATS 4, respectively.

2.3. HPTLC instrumentation and chromatographic conditions

The quantitative analysis of SPN was carried out on 20 × 10 cm HPTLC plate. Automatic TLC Sampler-4 fitted with Hamilton Gastight Syringe (1700 Series) of volume 25 µL was used to apply the samples as well as standard as bands on HPTLC plate. The application rate of the sample on the plate was 160 nLs\(^{-1}\). The plate was developed in previously saturated (saturation time 20 min at 25 °C with vapors of mobile phase) automatic developing chamber in linear ascending mode with chloroform:methanol:ammonium hydroxide (30:20:1.5 v/v/v) used as mobile phase. After development, the plate was dried at room temperature and derivatized with freshly prepared Dragendorff reagent, scanned, and quantified at optimized wavelength of 500 nm in absorbance mode with CATS 4 operated by Win CATS software (Version 1.2.0).

2.4. Preparation of samples

The aerial parts of all the Solanum species were air-dried and pulverized. The fruits were available only in S. schimperianum and S. villosum which were separated from the other aerial parts. 20 g of the powdered material of each species was macerated thrice for complete extraction by two extractive solvents.

2.4.1. Solvent A

The plant material was extracted only with ethanol (95%). Eight samples include:

- S. schimperianum leaves (SSAA), S. schimperianum fruits (SSFA), S. villosum leaves (SVAA), S. villosum fruits (SVFA), S. coagulans leaves (SCAA), S. glabratum leaves (SGAA), S. incanum leaves (SIAA) and S. nigrum leaves (SNAA).

2.4.2. Solvent B

Another lot of same plant material was extracted with a mixture of ethanol (95%) and ammonium hydroxide (6:4 v/v). Eight samples include:

- S. schimperianum leaves (SSAB), S. schimperianum fruits (SSFB), S. villosum leaves (SVAB), S. villosum fruits (SVFB), S. coagulans leaves (SCAB), S. glabratum leaves (SGAB), S. incanum leaves (SIAB) and S. nigrum leaves (SNAB).

The obtained extracts were concentrated by using rotary evaporator and finally dried. The percent yield of SSAA, SVAA, SCAA, SGAA, SIAA and SNA A extracts of ethanol (95%) was found to be 9.5%, 6.0%, 5.7%, 5.5%, 3.5% and 8.0% respectively. The extract of SSAB, SVAB, SCAB, SGAB, SIAB, SNAB and SSFA using C\(_2\)H\(_5\)OH:NH\(_4\)OH (6:4 v/v) as solvent was produced as 14.5%, 19.3%, 24.8%, 23.6%, 10.66%, 17.14% and 10.1% respectively. These extracts were used for detection and quantification of SPN. Since the solubility of SPN was found to be in methanol hence, the same was used as a vehicle for sample preparation from the extracts.

2.5. Preparation of standard stock solution

A stock solution of standard (SPN) (1 mg mL\(^{-1}\)) was prepared in methanol. 1 ml of the stock solution was again diluted with 09 mL of methanol to make the concentration 100 ng mL\(^{-1}\). For calibration, SPN standard solutions (1, 2, 3, 4, 5, 6, 7, 8, 9, 10 µL) were applied on a HPTLC plate to accomplish the final concentration in the range 100–1000 ng SPN per spot. The concentration–response curve was made with respect to height and area versus amount per spot.

2.7. Method development

In order to develop the appropriate chromatographic system, the primary concern was the absolute separation of the desired marker from other components of the extracts. Apart from these criteria, parameters such as the sensitivity of the method, appropriateness for the stability studies, \(R_l\) value, peak symmetry, and the stability of the mobile phase were also considered while selecting the eluents for this study. On the basis of these criteria, solvents and their various combinations such as methanol (MeOH), chloroform (CHCl\(_3\)), ammonium hydroxide (NH\(_4\)OH), MeOH: CHCl\(_3\), CHCl\(_3\): NH\(_4\)OH, CHCl\(_3\): NaOH, MeOH:NaOH were investigated. After trial and error method of mobile phase selection, the combination of chloroform:methanol:ammonium hydroxide (30:20:1.5 v/v/v) was preferred as the optimized eluent for this study (Fig. 2). Samples of six species extracted by two solvents along with ten spots of standard SPN were applied on two 20 × 10 cm HPTLC plates. The saturation time (optimized) was observed for 20 min and scanning was done at optimized wavelength of 500 nm in absorbance mode for densitometric analysis.

2.8. Method validation

The proposed method for the quantification of SPN was validated according to International Conference on Harmonization (ICH) guidelines for various parameters such as linearity, precision, accuracy, robustness of the method, LOD, and LOQ (ICH, 2006).

2.8.1. Linearity range

For determination of the linearity range of marker SPN, a sequence of spots of different volumes (1–10 µL) was applied so as to get 100–1000 ng SPN per band. The graph was plotted...
between concentration and peak area for linearity. Linearity data were statistically treated using least square linear regression analysis.

2.8.2. Precision

Precision (inter and intraday) was evaluated by performing replicate analyses \( n = 6 \) of samples at three concentration levels low, medium and high of 150, 300 and 600 ng/spot of SPN. The precision and accuracy for inter-day were assessed by repeating the intra-day assay on three different days. Precision was expressed as % RSD of measured concentrations for each calibration level.

2.8.3. Accuracy

Accuracy was determined by standard addition method. The pre-analyzed samples of SPN (300 ng/spot) were spiked with extra 0%, 50%, 100% and 150% of SPN and the solutions were reanalyzed in six replicates by the proposed method. Accuracy was interpreted as percentage recovery and percent relative standard deviation (% RSD).

2.8.4. Robustness

Small deliberate changes were made to mobile phase composition, mobile phase volume, and duration of mobile phase saturation for analyzing robustness of the method by triplicate study at 500 ng band\(^{-1}\). The results were evaluated in terms of relative standard deviation (% RSD). Mobile phases prepared from chloroform:methanol:ammonium hydroxide (30:20:1.5) in different proportions (29:20:1.5, v/v/v; 30:19:1.5, v/v/v; 30:20:1, v/v/v) were used for analyses. Small fluctuation in mobile phase volume \( 20 \pm 2 \text{ mL} \) (18, 20, and 22 mL) and duration of saturation \( 20 \pm 10 \text{ min} \) (10, 20, and 30 min) was made to investigate the robustness of the method.

2.8.5. LOD and LOQ

The calculation of LOD and LOQ was made on the basis of the standard deviation (SD) of the response and the slope (S) of the calibration curve. The standard deviation of y-intercepts of regression lines was used to determine the standard deviation of the response. The following formulae were used to calculate the LOD and LOQ:

\[
\text{LOD} = 3.3(\text{SD}/S) \quad \text{and} \quad \text{LOQ} = 10(\text{SD}/S),
\]

[LOD = 3.3(\text{SD}/S) and LOQ = 10(\text{SD}/S)].

2.9. Application of developed method for the assay of solanopubamine

Standard SPN and known concentration of test samples from six species of *Solanum* were applied on two HPTLC plates. On one plate eight test samples extracted with solvent A along with reference compound (1–10 \( \mu \text{L} \)) were applied for quantification. On another plate, eight test samples extracted with solvent B have been implemented along with standard (1–10 \( \mu \text{L} \)) for quantification. The quantity of SPN present in test samples was determined by measuring the peak area for the reference standard and test samples. Thereby the percentage of SPN was calculated for all the samples exhibited the presence of SPN (Table 1).

| S. no. | Sample | Extractive solvent | % Yield of extract | Content of solanopubamine (%) |
|-------|--------|------------------|--------------------|-----------------------------|
| 1.    | *S. schimperianum* (Leaves) (SSAA) | Ethanol | 9.5 | 1.03 |
| 2.    | *S. villosum* (Leaves) (SVAA) | Ethanol | 6 | Absent |
| 3.    | *S. coagulans* (Leaves) (SCAA) | Ethanol | 5.7 | Absent |
| 4.    | *S. glabratum* (Leaves) (SGAA) | Ethanol | 5.5 | Absent |
| 5.    | *S. incanum* (Leaves) (SIAA) | Ethanol | 3.5 | Absent |
| 6.    | *S. nigrum* (Leaves) (SNAA) | Ethanol | 8 | Absent |
| 7.    | *S. schimperianum* (Leaves) (SSAB) | \( \text{C}_2\text{H}_5\text{OH}:\text{NH}_4\text{OH} \) (6:4) | 14.5 | 2.09 |
| 8.    | *S. villosum* (Leaves) (SVAB) | \( \text{C}_2\text{H}_5\text{OH}:\text{NH}_4\text{OH} \) (6:4) | 19.3 | Absent |
| 9.    | *S. coagulans* (Leaves) (SCAB) | \( \text{C}_2\text{H}_5\text{OH}:\text{NH}_4\text{OH} \) (6:4) | 24.8 | Absent |
| 10.   | *S. glabratum* (Leaves) (SGAB) | \( \text{C}_2\text{H}_5\text{OH}:\text{NH}_4\text{OH} \) (6:4) | 23.6 | Absent |
| 11.   | *S. incanum* (Leaves) (SIAB) | \( \text{C}_2\text{H}_5\text{OH}:\text{NH}_4\text{OH} \) (6:4) | 10.66 | Absent |
| 12.   | *S. nigrum* (Leaves) (SNAB) | \( \text{C}_2\text{H}_5\text{OH}:\text{NH}_4\text{OH} \) (6:4) | 17.14 | Absent |
| 13.   | *S. schimperianum* (Fruits) (SSFA) | Ethanol | 10.1 | Absent |
| 14.   | *S. villosum* (Fruits) (SVFA) | Ethanol | 8.4 | Absent |
| 15.   | *S. schimperianum* (Fruits) (SSFB) | \( \text{C}_2\text{H}_5\text{OH}:\text{NH}_4\text{OH} \) (6:4) | 5.88 | Absent |
| 16.   | *S. villosum* (Fruits) (SVFB) | \( \text{C}_2\text{H}_5\text{OH}:\text{NH}_4\text{OH} \) (6:4) | 6.2 | Absent |
2.9.1. Stability study of SPN

For evaluation of stability study property of newly developed method, a solution of biomarker (SPN) was exposed to various stress factors such as ultraviolet radiations, alkaline, and acidic conditions. The sample was also exposed to H₂O₂ (30%) and thermal stress. The selected concentration (100 μg/mL) of SPN was freshly prepared. An aliquot (1 mL) of this solution was mixed with 4 mL of 0.1 mol L⁻¹ HCl and 4 mL of 0.1 mol L⁻¹ NaOH, respectively. The obtained mixture (25 μL) equivalent to 500 ng was exposed to different stress conditions for 48 h. and subsequently analyzed by the newly developed method. For analyzing the sensitivity of sample for oxidation an aliquot (1 mL) of the solution was mixed with an aliquot (1 mL) of H₂O₂ (30%) in 1:4 ratios. The resultant mixture was stored for

Figure 3   Chromatogram of standard Solanopubamine (1000 ng/spot) scanned at 500 nm with mobile phase: chloroform:methanol: ammonium hydroxide (30:20:1.5).

Figure 4   Chromatogram of sample SSAA scanned @ 500 nm.
48 h. and then analyzed by the proposed method. Thermal stability of SPN was assessed by analyzing the samples (500 ng) which were kept on 40 °C and 60 °C for 48 h. The samples of SPN (500 ng) were exposed to sunlight for 48 h to observe the photo-oxidation.

3. Results and discussion

Results of the present study have been shown in the form of tables and figures as well as discussed below. When methanol, chloroform, and ammonium hydroxide were employed individually as mobile phase for observing the migration of SPN on silica stationary phase, none of the eluents showed the capacity to take SPN along with it to substantial distance (insignificant $R_f$) and SPN stayed over at the starting point while little migration was observed in case of methanol and chloroform combination but the peak was asymmetrical. It is obvious from the above discussion that obtaining the required substantial mobility of SPN was not achieved by any single component mobile phase system studied. Hence, different ratios of a mixture of these solvents have to be used to obtain the sufficient mobility of SPN. When various combinations of
these solvents were used as mobile phase except chloroform: methanol:ammonium hydroxide, all other possible combinations gave unsymmetrical peak with a high tailing factor (2.47) and a less number of theoretical plates. The mixture of chloroform:methanol:ammonium hydroxide was found to be chromatographically superior to all other eluent systems studied in providing better peak and suitable Rf value. However, the problem of peak broadening was observed with this mobile phase which may be due to the interaction between solute (SPN) and the stationary phase. This problem was overcome by the addition of a little amount of NH₄OH (1% aq.). Based on these results, a combination of chloroform:methanol:ammonium hydroxide in the ratio of 30:20:1.5 v/v/v was selected as the eluent for the method development of SPN and its analysis in herbal formulations containing SPN (Fig. 3).

There was a remarkable difference found in the yield of extracts due to the difference of extractive solvents. The yield of extracts is mentioned in Table 1. In general, the yield of

![Figure 7 3D-display of chromatogram of Solanopubamine scanned at 500 nm.](image)

### Table 2

| Parameter                  | Solanopubamine |
|----------------------------|----------------|
| Rf (ng band⁻¹)             | 0.39 ± 0.01    |
| Linear range (ng band⁻¹)   | 100–900        |
| Regression equation        | Y = 4.442X + 239.618 |
| Correlation coefficient (r²)| 0.998          |
| Slope ± SD                 | 4.442 ± 0.015  |
| Intercept ± SD             | 239.618 ± 0.42 |
| Standard error of slope    | 0.022          |
| Standard error of intercept| 2.16           |
| LOD                        | 40 ng band⁻¹   |
| LOQ                        | 115 ng band⁻¹  |

### Table 3

| Concentration (ng band⁻¹) | Intra-day precision (Repeatability) | Inter-day precision (Reproducibility) |
|---------------------------|-------------------------------------|---------------------------------------|
| Avg. Conc. ± SD; (n = 6)  | % RSD                               | Avg. Conc. ± SD; (n = 6) % RSD        |
| 150                       | 148.52 ± 1.53                       | 1.03                                  |
| 300                       | 301.19 ± 2.98                       | 0.98                                  |
| 600                       | 600.22 ± 5.43                       | 0.90                                  |

### Table 4

| Excess drug added to analyte (%) | Theoretical concentration (ng) | Experimental concentration (ng) | % Recovery | % RSD |
|----------------------------------|-------------------------------|-------------------------------|------------|------|
| 0                                | 300                           | 295.1 ± 3.27                  | 98.1       | 1.12 |
| 50                               | 450                           | 443.2 ± 4.69                  | 98.4       | 1.06 |
| 100                              | 600                           | 594.1 ± 5.98                  | 98.8       | 1.01 |
| 150                              | 750                           | 743.3 ± 7.49                  | 99.1       | 1.01 |

### Table 5

| Optimized conditions | Intentional changes in optimized conditions | Solanopubamine SD % RSD |
|----------------------|--------------------------------------------|-------------------------|
| Mobile phase composition | Chloroform: methanol: ammonium hydroxide (31:21:1.5 v/v/v); (29:20:2) v/v/v; (30:20:1) v/v/v | 2.16 0.421 |
| Mobile phase volume   | (18, 20, and 22 mL)                        | 1.66 0.326 |
| Duration of saturation | (10, 20, and 30 min)                      | 2.08 0.403 |
all Solanum species extracted with solvent B was found to be more than extracted by solvent A (Table 1) and of course, the yield of SPN extracted by solvent B was found to be more than double than samples extracted from solvent A.

3.1. Method development

The development of chromatogram for reference compound SPN was done under the optimized condition of chamber saturation (20 min) with mobile phase chloroform:methanol:ammonium hydroxide in a ratio of 30:20:1.5 v/v/v (Fig. 3). The chromatograms for samples of Solanum extracts were also developed using the same mobile phase (Figs. 4 and 5). Scanning of the developed chromatographic plate and densitometric analysis was performed at 500 nm in the absorbance mode. Well separated, sharp, symmetrical and high-resolution bands of SPN were obtained at \( R_f 0.39 \pm 0.001 \). The successful resolution of biomarker along with its degradation products was observed in the proposed method. The confirmation of identity for the selected biomarker (SPN) in the sample extracts was made by superimposing their absorption spectra with those of the reference standard (Figs. 6 and 7). The findings of this experiment proposed a maiden method to quantify the steroidal alkaloid SPN in plant species of genus Solanum by a viable, accurate, rapid, cost-effective and validated HPTLC method.

3.2. Method validation

The linearity of compound SPN was validated by the linear regression equation and correlation coefficient. The ten-point calibration curve for SPN was found to be linear in the range of 100–1000 ng. Regression equations and \( r^2 \) values for the reference compound SPN were observed as \( Y = 4.442X + 239.618 \) and 0.998 which revealed a good linearity response for developed method (Table 2). The mean value with ±SD of the slope and intercept was 4.442 ± 0.013 and 239.618 ± 0.012 respectively. No significant difference was observed on the slopes of standard plots (\( P > 0.05 \)). Table 3 presents intra-day and inter-day precision for SPN at triplicate quality control (QC) levels (150, 300 & 600 ng band\(^{-1}\)). Both intraday and inter-day precisions were determined in terms of percent of relative standard deviation (% RSD). Intraday and inter-day precisions (\( n = 6 \)) for SPN were found to be 1.01–1.30 and 2.15–2.60, respectively which demonstrated the excellent precision of proposed method. However, the accuracy values of the

| S. no. | Exposure conditions          | Time (h) | Figure | Drug remained ng/500 ng | % Recovery (±SD, \( n = 6 \)) |
|--------|-------------------------------|----------|--------|-------------------------|-------------------------------|
| 1.     | Treated with UV (254 nm)      | 48       | 8      | 495.45                  | 87.09                         |
| 2.     | Treated with base             | 48       | 9      | 00.00                   | 00.00                         |
| 3.     | Treated with acid             | 48       | 10     | 193.00                  | 38.6                          |
| 4.     | Treated with 40 °C            | 48       | 11     | 430.85                  | 86.17                         |
| 5.     | Treated with 60 °C            | 48       | 12     | 210.60                  | 42.12                         |
| 6.     | Treated with H2O2 (30%)       | 48       | 13     | 00.00                   | 00.00                         |

Table 6 Results of stress study of solanopubamine.
proposed method for estimation of SPN were observed as 98.1–99.1 and 1.01–1.12 in terms of % recovery and % RSD, respectively (Table 4). The small value of SD and % RSD obtained after introducing intentional changes in the method indicates that the method was robust (Table 5). LOD and LOQ were found to be 40 ng band\(^{-1}\) and 115 ng band\(^{-1}\), respectively for SPN (Table 2). This indicated that the proposed method exhibited an excellent sensitivity for the quantification of SPN.

In the present study, we performed the comparative estimation of SPN in six species of Solanum extracted by two different methods. The findings of the present study revealed its presence only in S. schimperianum out of the six species investigated for the targeted marker compound. The results of the present experiment proved the superiority of solvent B over A for extraction and yield of SPN. Method B has shown 152% more yield of extract than method A. Also the quantity of SPN in aerial parts extracted by method B was found to be more than double (2.09% w/w) than from method A (1.03% w/w) but altogether absent in fruits of SS and SV. SPN is a rare steroidal alkaloid and possesses potent antifungal activity. The literature has revealed the potency of SPN (MIC 12.50 g/ml).
against *Candida albicans* and *Candia tenuis* (Al-Rehaily et al., 2013). The concentration of SPN (2.09% w/w) in aerial parts of *S. schimperianum* strongly indicates the need to explore the *S. schimperianum* as emerging antifungal agent.

### 3.3. Stability study of solanopubamine

The degradation-indicating character and specificity of the proposed method were evaluated by analyzing the selected concentration of SPN samples exposed to various stress conditions. Stability results revealed that compound SPN is quite stable under UV exposure as well as for photo-oxidation, and no significant degradation was observed (Fig. 8) but exhibited absolute destruction to basic pH (Fig. 9). Partial destruction (61.4%) was seen in acid treated samples (Fig. 10). To some extent solanopubamine showed moderate sensitivity (13.83–57.88%) with a gradual rise in temperature (Figs. 11 and 12). Stress study exhibited that SPN is highly sensitive to oxidation and completely degraded (100%) when mixed with H₂O₂ (Fig. 13). It can be inferred

![Figure 11](image1.png) Chromatogram of solanopubamine (500 ng) exposed at 40 °C.

![Figure 12](image2.png) Chromatogram of solanopubamine (500 ng) exposed at 60 °C.
from the stability study that SPN was quite stable under UV, photo-oxidation and at room temperature conditions but found to be highly sensitive to alkaline and the oxidative environment (Table 6). The developed HPTLC method is precise, accurate, robust and specific for the determination of SPN. The statistical analysis and selectivity proved the reproducibility character of the developed method.

4. Conclusion

The findings of this experiment revealed some significant facts such as S. schimperianum possesses a significant amount of SPN which is responsible for its anti-fungal activity and can be recommended for Candida infection, the better yield of SPN can be obtained by applying method B for extraction, and the stability aspect may provide better storage conditions and efficacy of the drug. As the proposed method successfully separated the drugs from other constituents present in the extract, it can be used to assure the quality of products during processing as well as final products in the market with regard to SPN content. It is suggested for the further study of SPN as antifungal drug and its determination in biological samples.

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