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# Pharmacognostical study and development of quality control parameters for *Sarcostemma brevistigma* Wight & Arn.

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**Abstract**

Objective: To develop pharmacognostical and quality control parameters for drug *Sarcostemma brevistigma* Wight and Arn. (Family: Asclepiadaceae) commonly known as Somlata, is rasayana drug. Materials and Methods: Stems of *S. brevistigma* were collected from Jamnagar, Dwarka and Bhuj in the month of November 2008 and studied for macroscopical and microscopical characters. Quantitative microscopy and physico-chemical parameters were also performed. A pregnane derivative mono-*O*-acetyl bregenin was isolated from stem extract and characterized by U.V., IR, NMR, Mass spectroscopy. HPTLC method was developed to quantify mono-*O*-acetyl bregenin and generate fingerprint profile for the drug. HPTLC was performed using precoated TLC plate (silica gel 60 $F_{254}$) as a stationary phase and Chloroform : Methanol (9.4 : 0.6) as a mobile phase. The plate was scanned at 366nm. Results: Macroscopically and microscopically all the samples of stems were found to be similar but differed in quantitative parameters. Stems were green, cylindrical in shape having longitudinal ridges and nodes. T.S. of stems showed papillose epidermis, rosettes of calcium oxalate, non lignified fibres and latex cavities in cortex, striated cells of inner cortex and perimedullary phloem. Stone cells and abundant rosettes of calcium oxalate were found in section passing through the node. Powder showed stone cells, rosettes, fragments of pitted xylem vessels and non lignified fibres. Mono-*O*-acetyl bregenin was isolated by column chromatography. HPTLC chromatogram was developed and blue fluorescent spot was visualised at $R_f$ 0.5 under UV at 366nm. Mono-*O*-acetyl bregenin content was found to be 0.0339% w/w, 0.0399% w/w, 0.0227% w/w in samples collected from Jamnagar, Dwarka and Bhuj respectively.

**Key words:** *Sarcostemma brevistigma,* stone cells, mono-*O*-acetyl bregenin, HPTLC.

## 1. Introduction

*Sarcostemma brevistigma* Wight and Arn. belonging to family Asclepiadaceae, is commonly known as Soma (Sans), Somvel, Chirodi (Guj), Somlata (Hindi) and Moon Creeper (Eng.). It is found in countries like India, Burma, Baluchistan, Himalayas, arid rocks of Konkan, in stony places, and Kashmir. The plant is a perennial leafless, twining trailing shrub, with

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green cylindrical, fleshy, glabrous,green pendulous stems exuding milky white latex, exhibiting longitudinal ridges and nodes [1, 2, 3, 4, 5].

Previous phytochemical studies include reports of presence of sugars like sarcobiose, brevobiose, tigmobiose; and pregnane derivative sarcogenin; lignans like sacidumols A and B [6, 7, 8, 9, 10]. In Ayurvedic medicine, the plant is a Rasayana drug. It cures 'Tridosha', biliousness and thirst. It is found to be bitter, acrid, cooling, narcotic, emetic (dried stem), antiviral rejuvenator, and useful in curing diabetes and rheumatic pain. The fresh juice is used by Ayurvedic physicians in the control of allergic disorders especially bronchial asthma [1, 3, 11, 12].

The present study deals with the development of identification parameters for the plant which include morphological and microscopical evaluation, physicochemical parameters, isolation, characterization and estimation of mono-*O*-acetyl bregenin from ethyl acetate extract.

## 2. Materials and Methods

### *2.1 Plant material*

Fresh fully grown flowering plants of *S. brevistigma* (Somlata) from three different places of Gujarat like Jamnagar (Sample A), Dwarka (Sample B) and Bhuj (Sample C) in the month of November 2008. Authentification was done by the taxonomist of Jamnagar Ayurvedic University, Jamnagar. The stems were dried under shade and coarsely powdered separately and stored in airtight containers.

### *2.2 Pharmacognostical and physicochemical study*

Morphological and microscopical studies were carried out for stem and powdered drug. Quantitative microscopy was performed and physicochemical parameters such as ash values and extractive values were determined [13].

### *2.3 Chemicals*

All chemicals and reagents used for present study were of AR grade. Methanol, ethyl acetate, 0.5N HCl, anhydrous $Na_2SO_4$, aqueous $NH_3$ solution, chloroform and precoated silica gel 60 $F_{254}$ plate thickness 0.2mm (20 x 20cm) (E. Merck).

### *2.4 Isolation and characterization*

700g of dried stem powder was extracted with 95% methanol by hot percolation. Methanol was evaporated and the residue was obtained. The residue was suspended in 0.5N aqueous HCl. The aqueous acidic solution was neutralized with $NH_3$ and it was extracted with ethyl acetate. The ethyl acetate soluble fraction was washed with water, dried over anhydrous $Na_2SO_4$, and evaporated under reduced pressure to yield 300mg extract.

This extract was subjected to column chromatography. 300mg of ethyl acetate extract was loaded on a glass column (50cmX2cm) packed with 30g silica gel G (60-120#, LOBA Chemie Pvt. Ltd) as a stationary phase. Gradient elution was performed using different proportion of Toluene :Ethyl acetate (100:0 to 60:40). Fractions 79-90 eluted using Toluene : Ethyl Acetate (60:40), when concentrated yielded solution which gave blue fluorescent spot at $R_f$ 0.5 when subjected to TLC using mobile phase, Chloroform : Methanol (9.4:0.6). The compound was recrystallized and characterized as mono-*O*-acetyl bregenin by comparing its M.P., UV, IR, NMR and Mass spectral data with the data given in literature [10]. It was used as a working standard to develop HPTLC method for its quantification in *S. brevistigma* stem extract.

*2.5 Fingerprinting of mono-O-acetyl bregenin*

Chromatogram was developed using precoated silica gel 60 $F_{254}$ TLC plates (E.Merck) as stationary phase and Chloroform : Methanol (9.4 :0.6) as mobile phase. Spots were visualised under UV light at 366nm.

*2.5.1 Sample preparation*

Ethyl acetate extracts of *S. brevistigma* collected from three different places were prepared and used for the present study. Mono-*O*-acetyl bregenin was used as standard to develop HPTLC method for its quantification in *S. brevistigma* stem extract.

*2.5.2 Estimation of mono-O-acetyl bregenin in S. brevistigma stem by HPTLC method.*

A calibration curve of mono-*O*-acetyl bregenin was obtained by plotting the peak area of mono-*O*-acetyl bregenin against the concentration of mono-*O*-acetyl bregenin. Accurately weighed 5mg of working standard mono-*O*-acetyl bregenin was taken in a 5ml hexane in volumetric flask (1.0 mg/ml).

A fixed volume of standard solution (6, 10, 14, 18, 22ml) and sample solutions were spotted on the plate. The plate was then developed in a twin trough chamber containing mobile phase, Chloroform: Methanol (9.4:0.6). After development the bands were scanned at 366nm. After applying suitable dilution factor and comparing peak height and peak area of standard and sample solution, the amount of mono-*O*-acetyl bregenin in stems was calculated.

*2.5.2.1 Validation of HPTLC method*

The method was validated in terms of linearity, precision, repeatability, specificity, limit of detection (LOD) and limit of quantification (LOQ).

*Precision-Repeatability*

Precision under same condition (same analyte, same apparatus, short interval of time and identical reagent) using the same sample was measured.

*Interday and Intraday Precision*

The intraday precision was determined by analyzing mono-*O*-acetyl bregenin for three times on the same day. The interday precision was determined by analyzing mono-*O*-acetyl bregenin daily for 5 days.

*Linearity*

The range of concentration of the standard compound was determined for the linearity. The obtained test results must be in direct proportion to the concentration of analyte in the sample calibration curve for the analyte. The results were expressed in terms of correlation coefficient of the linear regression analysis.

*Accuracy*

The accuracy of an analysis was determined by calculating systemic error involved. Accuracy of the above method was ascertained by adding known concentration of mono-*O*-acetyl bregenin to the prequantified sample solution and estimating the quantity of mono-*O*-acetyl bregenin using the proposed method.

## 3. Results and Discussion

*3.1 Morphological evaluation*

All the stem samples of *S. brevistigma* collected from three different places of Gujarat showed almost similar morphological characters. Stems ware cylindrical in shape 3.7-13.3cm in length and 2-6mm in diameter; the surface was glabrous showed presence of longitudinal ridges (9-28) and nodes (3.5-9 cm long). The fresh stems were greenish externally and creamish internally with slightly bitter taste and no characteristic odour. Fig: 1

Fig. 1 *Sarcostemma brevistigma*

Table 1. Quantitative microscopy.

| **Microscopical Characters** | | **Samples of *S. Brevistigma*** | |
|---|---|---|---|
| (Diameters) | A (µ) | B (µ) | C (µ) |
| Rosettes | 17.26-26.13 | 24.57-33.33 | 17.88-24 |
| Mesocortical fibers | 21.16-29.24 | 28.47-48 | 17.67-26 |
| Pericyclic fibers | 12.62-20.57 | 16.80-29.60 | 18.67-30 |
| Latex Cavity | 21.87-41.60 | 24-34.67 | 17.43-24.67 |
| Xylem Vessels | 28.29-44 | 31.27-54.24 | 34.25-61 |
| Stone Cells· | | | |
| Length | 27-40 | 29.56-41 | 32.30-41.60 |
| Breadth | 12.24-22 | 13-24.10 | 15.20-26 |

*3.2 Microscopical evaluation*

TS of the stem shows typical cortex and well developed stelar region. Epidermis is the outer single layer of tangentially elongated or cubical to rectangular suberised cells with anticlinal walls covered with papillose cuticle. The cortex layer consists of outer longitudinally elongated parenchymatous cells, which show presence of rosettes of calcium oxalate. Rosettes are seen just below the epidermis layer also. The cells of inner cortex are somewhat round to elongated showing striations and wavy walls. Both the outer and inner cortical cells show presence of non lignified fibres and latex cavities. It shows presence of bicollateral vascular bundles. Xylem is radiating and encircles the pith. Phloem is well developed and shows both stone cells and lignified phloem fibers. Medullary rays are uni-biseriate, pitted and penetrate the cortex. Cells of pith are round and parenchymatous (Fig. 2).

TS passing through the nodal region shows presence of abundant stone cells and rosettes.

Powder is fibrous, light greenish brown in colour and bitter in taste. It shows presence of stone cells from cortex; rosettes of calcium oxalate; fragments of pitted and spiral vessels; cork in surface view and fibres (Fig. 3).

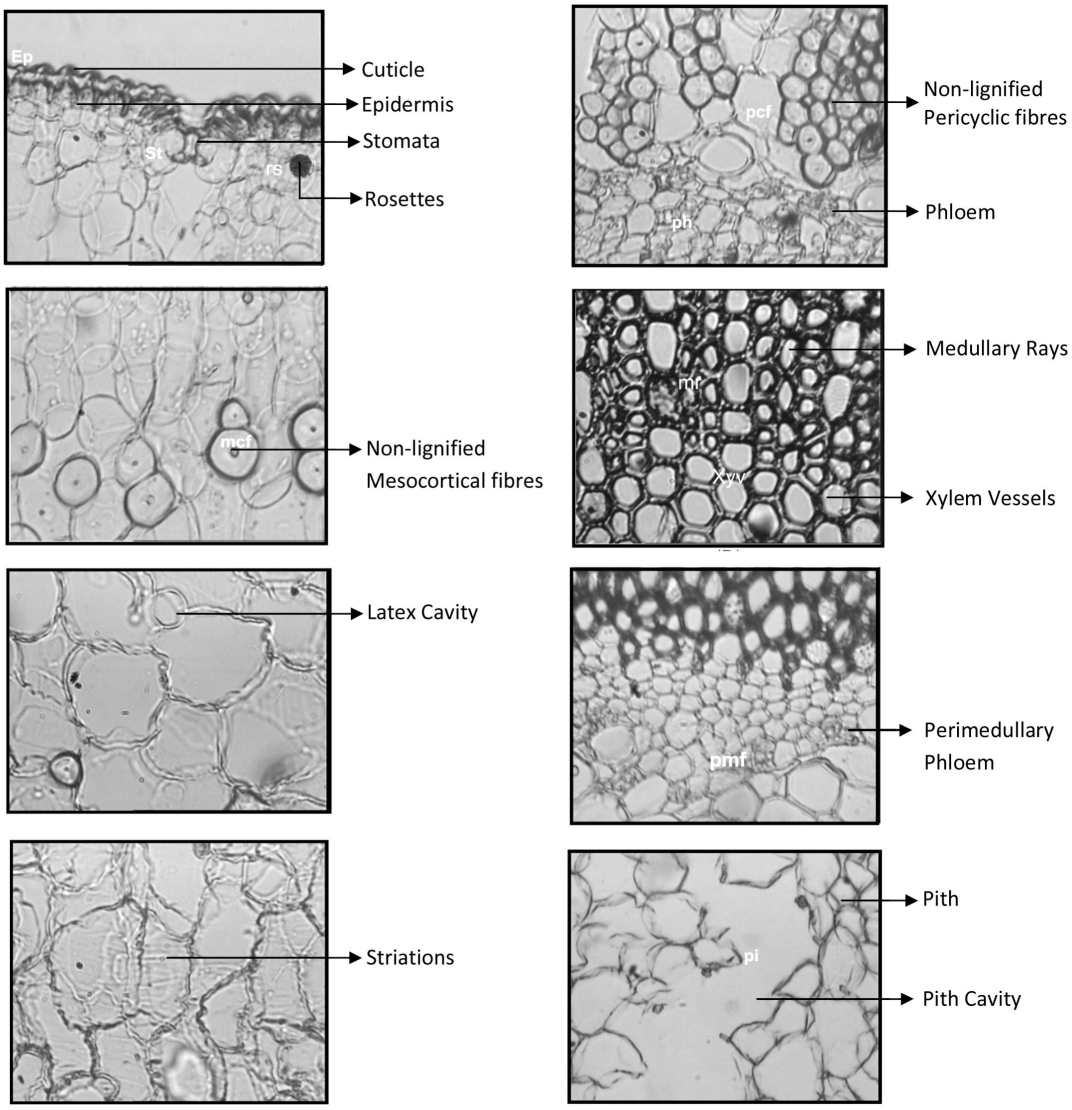
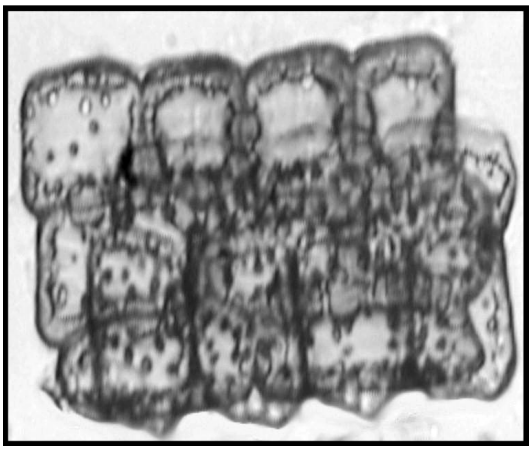
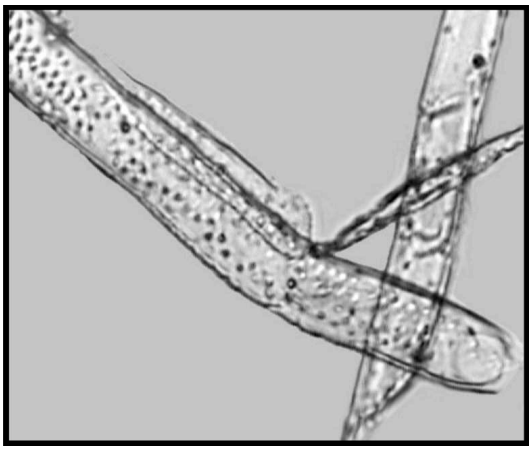


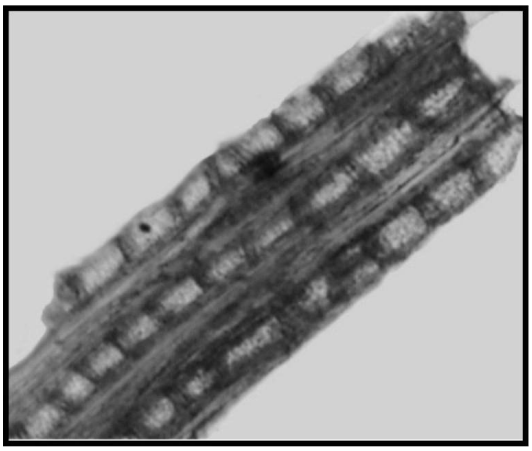

**Fig. 2** Detailed TS of stem of *Sarcostemma brevistigma*



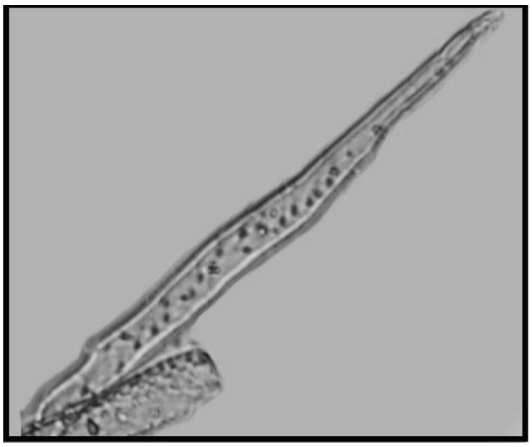
Stone cells



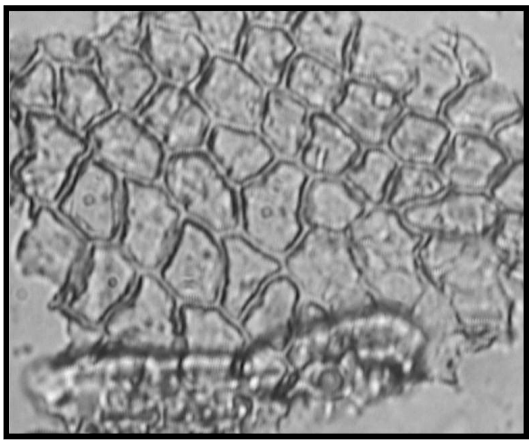
Vessels showing pits



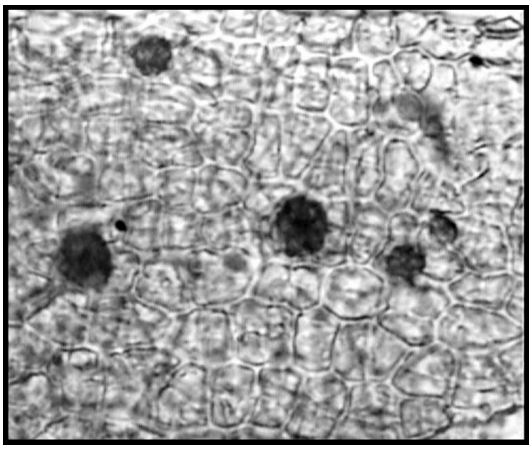
Fibres with pitted Parenchyma



Non lignified cortical fibres



Epidermis



Rosettes of calcium oxalate

**Fig. 3** Powder study of *Sarcostemma brevistigma*

### *3.3 Physicochemical parameters*

Limits of physical parameters such as ash values and extractive values of *S. brevistigma* are mentioned in table 2

Table: 2 Physicochemical parameters

| **Quality Parameters** | **Literature** | **Experimental values % w/w** | | |
|---|---|---|---|---|
| | **Value [13]** | **Sample - A** | **Sample - B** | **Sample - C** |
| **Loss on Drying** | ——- | 73.65 | 76.09 | 71.1 |
| **Ash value** | | | | |
| Total ash value | 6.10-7.81 | 9.61±0.44 | 9.87±0.71 | 9.66±0.2 |
| Acid insoluble ash | 0.37-1.30 | 0.27±0.05 | 0.4±0.06 | 0.337±0.03 |
| water soluble ash | 1.05-2.45 | 4.935±0.22 | 4.795±0.018 | 4.298±0.018 |
| **Extractive value** | | | | |
| Water soluble | 7.60-26.59 | 15.056±0.67 | 14.6±0.17 | 15.544±0.48 |
| Alcohol Soluble | 9.68-14.6 | 13.78±0.3 | 12.66±0.09 | 14.21±0.42 |
| Petroleum ether | ———— | 3.652±0.11 | 3.122±0.3 | 4.122±0.66 |
| Solvent ether | | 1.86±0.2 | 1.49±0.31 | 2.23±0.24 |

Standard deviation (SD) ± SD
Number of readings (N) =3

### *3.4 Phytochemical screening*

Preliminary phytochemical analysis showed the presence of flavonoids, phenolics, carbohydrates, sterols and triterpenoids (Table 3).

Table: 3 Estimation of phytoconstituents in stems of *S. brevistigma parameters.*

| | | **Samples % w/w** | | |
|---|---|---|---|---|
| **Sr. No** | **Phytoconstituents** | **A** | **B** | **C** |
| 1. | Flavanoids | 2.76 | 2.65 | 2.32 |
| 2. | Phenolics | 0.81 | 0.61 | 0.74 |
| 3. | Alkaloids | 0.084 | 0.078 | 0.0639 |
| | Carbohydrates: | | | |
| 4. | A. Sugar | 13.9 | 12.78 | 13.17 |
| | B. Starch | 4.3 | 4.08 | 5.12 |

Standard deviation (SD) ± SD
Number of readings (N) =3

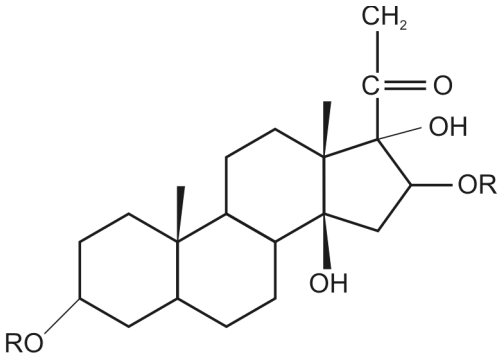
*3.5 Identification Parameters*

*Spectral analysis:* The results of IR, NMR and Mass spectra of the isolated pregnane derivative by column chromatography was found to be mono-*O*-acetyl bregenin (Table 4, Fig. 4).

Data of M.P and *Spectral analysis* of isolated Compound Mono-*O*-acetyl bregenin

| Parameter | Mono-*O*-acetyl bregenin |
|---|---|
| **M.P.** | 94-97 °C |
| **UV ($\lambda_{max}$)** | 293nm |
| **IR ($cm^{-1}$)** | 3400 ~ -OH group; 2983, 2941 ~ -$CH_3$ (s); 1739, 1731 ~ very broad peak indicative of more than one C=0 group (Str. of –$COCH_3$), no aromaticity, no conjugation; 1479, 1463, 1446 ~ C-H bending of alkane; 1392-1371 ~ broad peak but possibility of gem dimethyl; 1240, 1047 ~ C-O |
| **$^1$H NMR($\delta$)** | 0.83-0.89 ~ $CH_3$ (two triplets) (Cyclohexane ring system); 1.29 ~ -$CH_3$ ; 1.5 ~ -$CH_3$ + remaining cyclohexane; 3.9 ~ $COCH_3$ (s); 4.2 ~ -OH; 5.5 ~; 6.8-6.85, 6.8-7.0 ~ -CH (t) adjacent to a C=O but IR does not indicated conjugation |
| **MASS** | 402 $^{M+1}$ Molecular ion peak |

**Fig: 4**

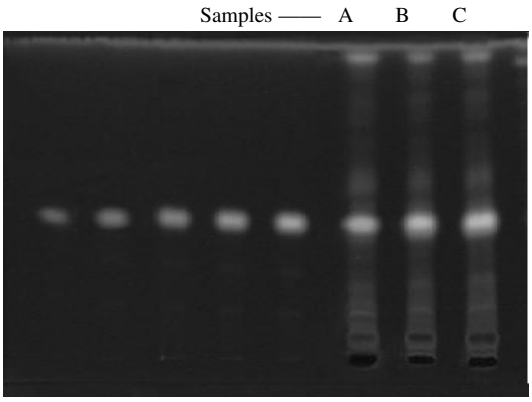



R = Ac, $R^1$ = H

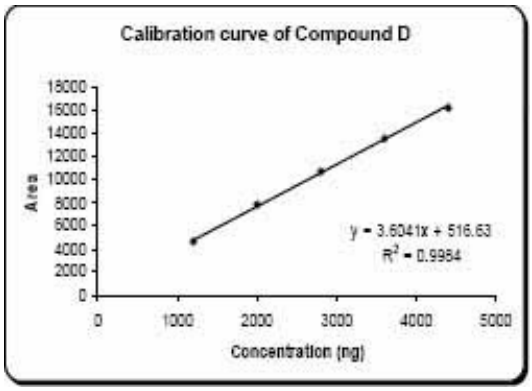
*3.6 Estimation*

A new compound mono-*O*-acetyl bregenin was quantified by HPTLC densitometric method using Chloroform : Methanol (9.6:0.4) as a mobile phase and precoated TLC silica gel 60 G $F_{254}$ plate. The compound resolved as a single spot at $R_f$ 0.5 under UV light (Fig. 5). By single level HPTLC analysis, mono-*O*-acetyl bregenin content was found to be 0.0339% w/w (Sample A), 0.0399% w/w (Sample B) and 0.0227% w/w (Sample C) in stems (Table 5). The HPTLC densitometric method was validated for specificity, linearity, accuracy, precision, repeatability and reproducibility (Table 6).

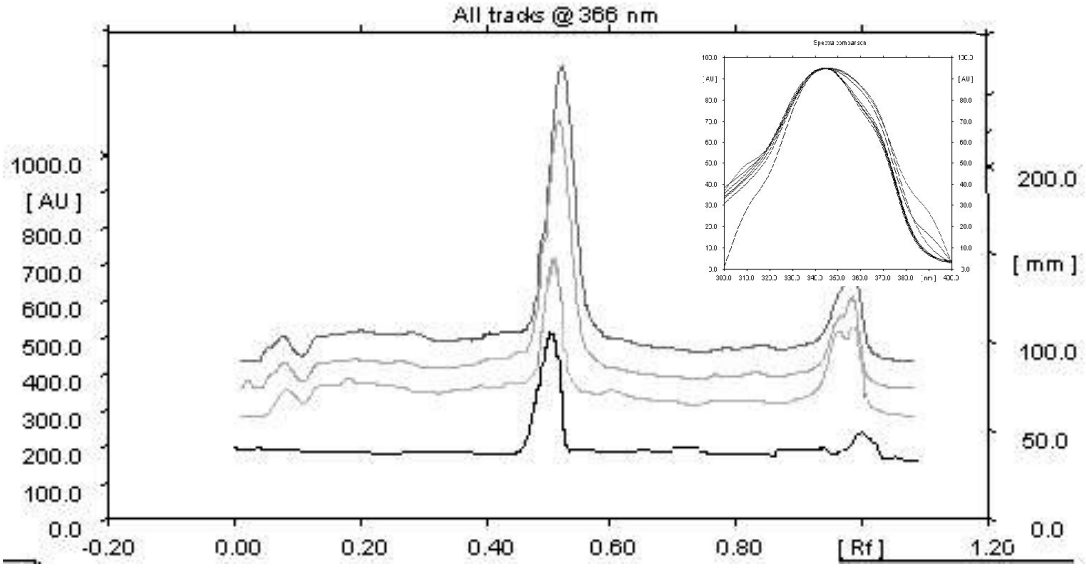
The aim of this investigation was to develop identification parameters for the stem and HPTLC densitometric method for estimation of mono-*O*-acetyl bregenin, which has been achieved.




[A] HPTLC Chromatogram




[B] Calibration curve




***[C] HPTLC Densitometric chromatogram scanned at 366 nm***

**Fig. 5**. HPTLC study of mono-*O*-acetyl bregenin

Table 5
Estimation of mono-*O*-acetyl bregenin in stems of *S. brevistigma*

| **Samples of *S. brevistigma*** | **Mean peak area (n=4)** | **Average amount of Mono-*O*- acetyl bregenin (ng/spot).** | **Average %w/w * of Mono-*O*- acetyl bregenin ± S.D.** | **% C.V.** |
|---|---|---|---|---|
| Sample A | 12592.12 | 3350.48 | 0.0339 ± 0.0002 | 3.5 |
| Sample B | 15092.65 | 4044.28 | 0.0399 ± 0.00015 | 3.7 |
| Sample C | 6676.86 | 1709.22 | 0.0227 ± 0.0006 | 2.46 |

Table 6. Method validation parameters for the estimation of mono-*O*-Acetyl bregenin

| Sr. No. | Parameters | Results |
|---|---|---|
| 1 | Linearity | 0.9984 |
| 2 | Precision (% C.V.) | |
| | *Repeatability of Measurement* | 0.68 % |
| | *Repeatability of Application* | 2.28 % |
| | *Interday* | 2.49-3.0 % |
| | *Intraday* | 1.97-2.5 % |
| 3 | Range | 1200-4400 ng/spot |
| 4 | Limit of Detection | 320.94 ng/spot |
| 5 | Limit of Quantification | 972.54 ng/spot |
| 6 | Accuracy | 96.62-102.98 % |
| 7 | Specificity | Specific |

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