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# Quantification of β- Sitosterol using HPTLC from *Capparis decidua* (Forsk.) Edgew

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## ABSTRACT

*Capparis decidua (Gudhapatra) is traditionally used for curing a variety of ailments such as toothache, cough, asthma, intermittent fever and rheumatism in India. There are no reports of quantification of β- Sitosterol from this plant. Hence a TLC densitometric method has been developed and validated for quantification of this marker compound. β-Sitosterol was quantified from methanolic extract using the Solvent System of Toluene: Methanol (9: 1, v/v). The method was validated using ICH guidelines in terms of precision, repeatability and accuracy. Linearity range for β-Sitosterol was 80–480 ng/spot and its content was 0.0244 % w/w. This simple, precise and accurate method gave good resolution from other constituents of extract. It can be adopted for routine quality control of herbal material and formulations containing Capparis decidua.*

**Key words:** *Capparis decidua*; HPTLC; β- Sitosterol; Densitometric method.

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## INTRODUCTION

*Capparis decidua* (Forsk.) Edgew. commonly known as kair, karel, karer, karil, karu, gudhapatra, nigudhapatra, titali is a small much branched tree or shrub of the Thar desert.[1] It is also found in the subtropical and tropical zones and other arid regions in southern Asia with a mass of slender, leafless branches, the small caducous leaves being found only on young shoots. It rarely exceeds a height of 5 meters (15 feet). The new flush of leaves appears in November-January. Red conspicuous flowers appear in March to April and August-September and ripe by May and October. It is extremely drought-resistant and tolerates some frost.

The fruit of the plant has been traditionally used as pickles, for preparing vegetables, curry. The plant is also used in folk medicine as insecticidal [2] and is well known for curing a variety of ailments such as toothache, cough, asthma, intermittent fever and rheumatism [3]. The seeds of *Capparis decidua* have antibacterial activity [4] against vibrio cholerae, vibrio inaba and vibrio

ettor, while the fruit has anti atherosclerotic [5], antidiabetic [6], anti hypertensive [7] and anti-hyperlipidemic [8] activity. The shoots and young leaves have rubifacient [9] and hypocholesterolemic activity [10]. Isocodonocarpine found in the root of the plant was found to be responsible for anti-inflammatory and anti asthmatic activity [11). The alcoholic extract of fruit pulp and root bark possesses anthelmintic activity [12].

The flower, fruit [13], stem [14] and seeds contains n-pentacosane, n-triacontane [15], n-triacontanol, 2-carboxy-1, 1-dimethylpyrrolodine, 6-(1-hydroxy-non-3-enyl) tetrahydropyran-2-one, β-sitosterol, β-carotene, ascorbic acid, proteins, total carbohydrates, calcium, potassium, phosphorus, zinc, iron, manganese, glucosinolates.
The root bark contains spermidine [16, 17] alkaloids, 14-N-acetyl isocodonocarpine, 15-N-acetyl capparisine, cadabicine, stachydrine, rutin, codonocarpine, isocodonocarpine [18], capparisine [19], capparadisine and capparisinine [20].

In the present paper, a HPTLC method for the quantification of β-Sitosterol has been developed. β-Sitosterol (Scheme 1) is reported to show antioxidant [21], anti-inflammatory [22], analgesic and anthelmintic [23] activity. It is also found to be effective in the treatment of benign prostatic hyperplasia [24].

## MATERIALS AND METHODS

**Collection of plant material:**
The stem of *Capparis decidua* was collected freshly from Loharu district Bhiwani (Haryana) in the month of August-September, 2008 depending upon its easy availability. It was authenticated by Dr. Minoo Parabia, Professor and Head, Department of Biosciences, Veer Narmad South Gujarat University against voucher specimen SA-1. The stem of *Capparis decidua* was subjected to shed drying and further crushed to powder, and then the powder was passed through the mesh 40.

**Standard compound:** β-Sitosterol (Purity: 97 % w/w) was purchased from Natural Remedies Pvt. Ltd, Bangalore, India.

**Chemicals:** All chemicals used were of analytical grade.

**TLC conditions:**

***TLC Plates*** **:** 20 ×10 cm, precoated with silica gel 60 F254 TLC plate (E. Merck) (0.2mm thickness)

***Spotting device*** **:** CAMAG Linomat V Automatic Sample Spotter; Camag (Muttenz, Switzerland)

***Syringe*** **:** 100 µL (Hamilton)

***Developing chamber*** **:** CAMAG glass twin trough chamber (20 × 10 cm)

***Densitometer*** **:** CAMAG TLC Scanner 3 linked to winCATS software; Camag

***Experimental condition*** **:** Temperature 25 ± 2ºC, relative humidity 40 %

***Derivatization*****:** TLC plates were dipped in freshly prepared anisaldehyde-sulphuric acid reagent, heated at 100ºC for 5 min. and immediately scanned.

**TLC fingerprinting profile:**

***Sample Solution*****:** Preparation of Sample Solution was optimized to achieve good fingerprinting and also to extract the marker compounds efficiently. Of these, the preparation of selected Sample Solution is given below:

***Methanolic extract***: Since the marker compound was soluble in methanol, we prepared a methanolic extract. Accurately weighed 1 g of the powdered drug was extracted with methanol (25 ml × 4) under reflux on a water bath. The methanolic extract was filtered through Whatman I filter paper, filtrates were combined, concentrated under vacuum and the volume was made upto 25 ml in a volumetric flask. This extract was used for TLC fingerprinting and co-chromatography with marker compound.

***Standard Solution of β-Sitosterol*****:** 2 mg of β-Sitosterol was dissolved separately in methanol and the volume was made upto 25 ml with methanol in volumetric flask.

***Solvent System*****:** Toluene: Methanol (9: 1, v/v) for co-chromatography with β-Sitosterol.

***Procedure*****:** For co-chromatography with β-Sitosterol, 10 µL of Sample Solution of methanolic extract along with the Standard was applied on a TLC plate and the plate was developed in Toluene: Methanol (9: 1, v/v) Solvent System to a distance of 8 cm. The plates were dried at room temperature in air and derivatized with anisaldehyde-sulphuric acid reagent and heated at $105^0$ for 5 min. The Rf values and colour of the resolved bands were noted.

**Quantification of β-Sitosterol using HPTLC:**

***Preparation of Standard Solutions of β-Sitosterol*****:** A Stock Solution of β-Sitosterol (50 µg/ml) was prepared by dissolving 5 mg of accurately weighed β-Sitosterol in methanol and making up the volume of the solution to 100 ml with methanol in a volumetric flask. The aliquots (1.6 to 9.6 ml) of the stock solution were transferred to 10 ml volumetric flasks and the volume of each was adjusted to 10 ml with methanol to obtain Standard Solutions containing 8µg/ml, 16µg/ml, 24µg/ml, 32µg/ml, 40µg/ml and 48µg/ml of β-Sitosterol respectively.

***Preparation of calibration curve of β-Sitosterol*****:** 10 µl each of the Standard Solutions of β-Sitosterol (80 to 480 ng/spot) were applied (band width: 6 mm, distance between the tracks: 12 mm) in triplicates on a TLC plate using automatic sample spotter. The plates were developed in a twin trough chamber (20×10 cm) upto a distance of 8 cm using a Solvent System of Toluene: Methanol (10 ml) (9: 1, v/v) at 25 ± 2°C and 40% relative humidity. After development, the plates were dried at room temperature in air, derivatized with anisaldehyde-sulphuric acid reagent, heated at $105^0$ for 5 min. and scanned densitometrically at 525 nm in absorbance mode using tungsten lamp. The area of the resolved peaks was recorded. Calibration curve of β-Sitosterol was obtained by plotting peak areas *vs* concentrations of β-Sitosterol applied.

***Quantification of β-Sitosterol in the sample*****:** 15 µl of suitably diluted Sample Solution of methanolic extract was applied in triplicates on a TLC plate. The plate was developed and scanned as mentioned above. The peak areas were recorded and the amount of β-Sitosterol was calculated using the calibration curve.

**Validation of the Method:** ICH guidelines were followed for the validation of the analytical method developed (CPMP/ICH/281/95 and CPMP/ICH/381/95) for precision, repeatability and accuracy. Instrumental precision was checked by repeated scanning (n = 7) of the same spot of β-Sitosterol (160 ng/spot) and expressed as relative standard deviation (% R.S.D.). The repeatability of the method was affirmed by analyzing 160 ng/spot of β-Sitosterol individually on TLC plate (n = 5) and expressed as % R.S.D. Variability of the method was studied by analyzing aliquots of standard solution containing 80, 160, 240 ng/spot of β-Sitosterol on the same day (intra-day precision) and on different days (inter-day precision) and the results were expressed as % R.S.D.

For the evaluation of limit of detection and limit of quantification different concentrations of the standard solutions of β-Sitosterol were applied along with methanol as blank and determined on the basis of signal to noise ratio.

The accuracy of the method was assessed by performing recovery study at three different levels (50 %, 100 % and 125 % addition of β-Sitosterol). The percent recoveries and the average percent recoveries were calculated.

## RESULT & DISCUSSION

There is no report of quantification of β-Sitosterol in *Capparis decidua.* Hence we developed a simple and precise method for quantification of this marker compound.

**TLC fingerprint and co-chromatography**

Quality control and quality assurance of herbal drugs remains a challenge as they contain a myriad of compounds in complex matrices in which no single active constituent is responsible for the overall efficacy [25]. Hence a systematic consideration of all its constituents is as important as the quantification of the active constituents present in it. TLC fingerprint profile of herbal drugs represents a comprehensive qualitative approach for the purpose of species authentication, evaluation of quality and ensuring the consistency and stability of herbal drugs and their products. In the present study, we developed TLC fingerprint profile for *Capparis decidua* and carried out co-chromatography with marker compound β-Sitosterol.

β-Sitosterol was resolved at Rf 0.62 (Table 1, Fig. 1) from Sample Solution of methanolic extract when the plate was developed in Solvent System and derivatized as mentioned above.

**TLC densitometric quantification of β-Sitosterol using HPTLC:**

The simplicity of the sample preparation, and the possibility of analyzing several sample of herbal products simultaneously in a short time, make HPTLC the method of choice. In the present method β-Sitosterol was quantified from *Capparis decidua* by TLC densitometric method using HPTLC.

The TLC densitometric method was validated in terms of precision, repeatability, and accuracy (Table 2, 3 and 4). The linearity range for β-Sitosterol was 80–480 ng/spot with correlation coefficient (*r* values) of 0.995. The TLC densitometric method was found to be precise with R.S.D for intraday in the range of 0.15–0.52 and for interday in the range of 0.26–0.61 for different concentrations of β-Sitosterol (Table 3). This indicates that the proposed method was precise and reproducible. The limit of detection (LOD) value for β-Sitosterol was found to be 20 ng, and limit of quantification (LOQ) value was 80 ng (Table 2). The average percent recoveries at 3 different levels of β-Sitosterol were found to be 99.92 % (Table 4).

The content of β-Sitosterol quantified using TLC densitometric method was found to be 0.044 % w/w (Table 5, Fig. 2).

**Table 1: TLC fingerprinting profile of *Capparis decidua* stem (Sample Solution 1 and Standard Solution; Solvent System) under UV 525 nm**

| S. No. | Rf value | Colour of the band |
|---|---|---|
| 1 | 0.12 | Deep Blue |
| 2 | 0.32 | Blue |
| 3 | 0.40 | Blue |
| 4 | 0.62 (β- sitosterol) | Blue |

**Table 2: Method validation parameters for the quantification of β-Sitosterol by the proposed TLC densitometric method**

| S. No. | Parameter | β-Sitosterol |
|---|---|---|
| 1 | Instrumental precision (% CV, n = 7) | 0.85 |
| 2 | Repeatability (% CV, n = 5) | 1.51 |
| 3 | Accuracy (average % recovery) | 99.92 |
| 4 | Limit of detection (ng) | 20 |
| 5 | Limit of quantification (ng) | 60 |
| 6 | Specificity | Specific |
| 7 | Linearity (Correlation coefficient) | 0.995 |
| 8 | Range (ng/spot) | 80-480 |

**Table 3: Intra-day and Inter-day precision of β-Sitosterol**

| Marker | Concentration (ng/spot) | Intra-day precision* | Inter-day precision* |
|---|---|---|---|
| β-Sitosterol | 80 | 0.15 | 0.26 |
| | 160 | 0.52 | 0.43 |
| | 240 | 0.46 | 0.61 |

** % R.S.D.; Mean (n=3)*

**Table 4: Recovery studies of β-Sitosterol at 50 %, 100 % and 125 % addition by the proposed TLC densitometric method**

| Marker | Amount of marker present (µg) | Amount of marker added (µg) | Amount of marker found (µg) | Recovery* (%) | Average Recovery (%) |
|---|---|---|---|---|---|
| β-Sitosterol | 45 | 22 | 67.54 ± 4.71 | 100.81 ± 1.05 | 99.92 |
| | 45 | 45 | 89.75 ± 7.14 | 99.72 ± 1.19 | |
| | 45 | 54 | 98.23 ± 3.13 | 99.22 ± 0.87 | |

**Mean ± SD (n=3)*

**Table 5: β-Sitosterol content estimated in *Capparis decidua* by proposed TLC densitometric method**

| S. No. | Sample/Standard Solution | β-Sitosterol (% w/w)* |
|---|---|---|
| 1 | 1 | 0.0244 ± 0.031 |

**Mean ± S.D. (n = 3).*

**Scheme 1: Structure of β-Sitosterol**

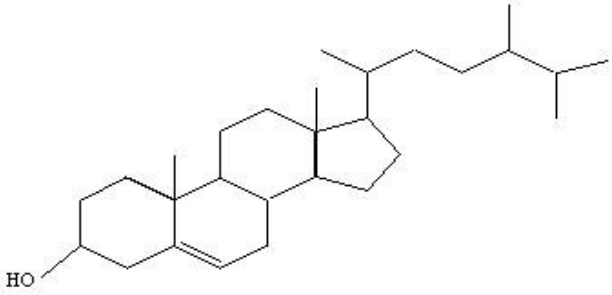



**Fig. 1: TLC profile of *Capparis decidua* stem after derivatization under 525 nm**
*Spot 1 & 2 indicates sample solution in duplicate and Spot 3 indicates Standard β-sitosterol*

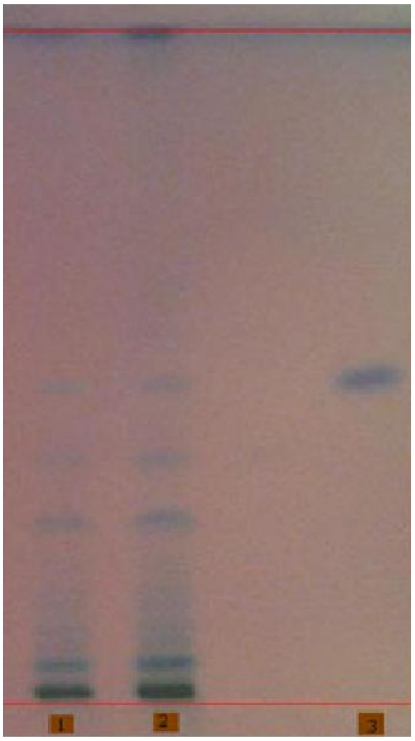
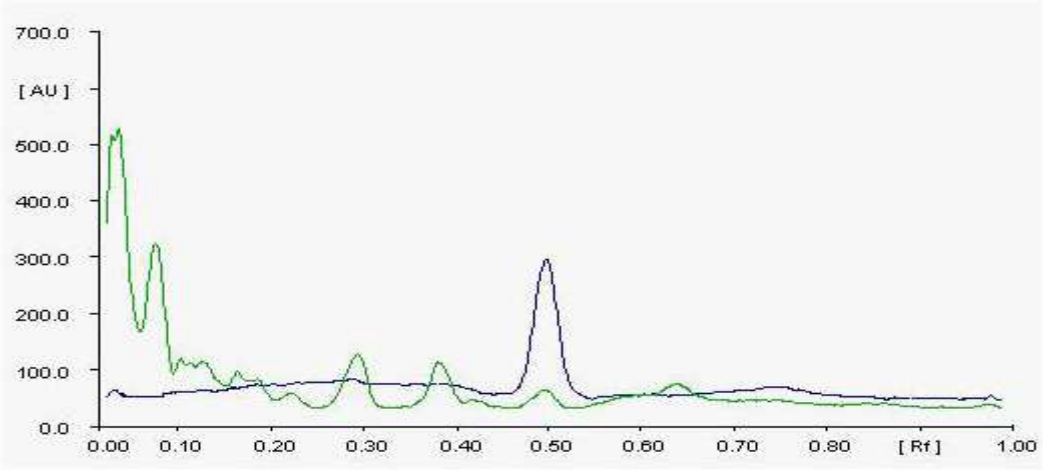



**Fig. 2: TLC densitometric scan at 525 nm of test solution of *Capparis decidua stem***
*β-sitosterol standard solution (Blue line); Test/sample solution (Green line)*



## CONCLUSION

We established TLC densitometric method for the quantification of β-Sitosterol from stem of *Capparis decidua* using HPTLC. The method was found to be simple, precise, specific sensitive and accurate and can also be used for the quantification of β-Sitosterol in the herbal raw materials. It can also be used in routine quality control of herbal materials as well as formulations containing any or all of these compounds.

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