

Research Article**QUANTITATIVE DETERMINATION OF β – SITOSTEROL FROM STEM BARK OF *KIGELIA PINNATA* LINN.**

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ABSTRACT

Kigelia pinnata grows to a medium size, with short thick trunk & horizontally spreading distorted branches, covered with thick & rough grayish - brown bark. Quantitative determination of β -sitosterol was undertaken to provide an easy and simple analytical method, which can be used as a routine quality control method. HPTLC was performed using chloroform as mobile phase. The detection and quantification was performed at a wavelength 254 nm. Linearity of detector response for β -sitosterol was between the concentrations 0.005% to 0.02%. The correlation coefficient obtained for the linearity was 0.994. The recovery value of standard β -sitosterol was 92.55%. The percentage of β -sitosterol in the stem bark was found to be 0.0462 %

KEYWORDS HPTLC, *Kigelia pinnata*, petroleum ether extract.

INTRODUCTION

Kigelia pinnata a plant belonging to family Bignoniaceae colloquially called the sausage tree on account of its large fruits has a variety of uses throughout Africa where it grows as an endemic species in many areas¹.

It is cultivated in many parts of India as an ornamental and roadside tree. It grows to a medium size, with short thick trunk & horizontally spreading distorted branches, covered with thick & rough grayish - brown bark. The phytochemical studies reveal the presence of Quercetin, Kaempferol, β - sitosterol, naphthaquinones, iridoids and flavonoids²⁻⁵

The stem bark and fruit extract showed activity against melanoma and carcinoma cell lines⁶. It is effective in the treatment of solar Keratosis, skin cancer & Kaposi Sarcoma. A number of companies are already producing skins creams, scalp Application & shampoos derived from *Kigelia* fruits. Extracts of rootbark and stembark exhibited antitrypanosomal

activity⁷. Most commonly, traditional healers have used the sausage tree to treat a wide a wide range of skin ailments, such as fungal infections, boils, psoriasis, & eczema & serious diseases, like leprosy, syphilis & Skin cancer. Several internal applications have also been employed in treatment of dysentery ringworm, tapeworm, pneumonia & toothache. Besides it is found to be active as an anti-ulcer and anti-rheumatic activity, it is necessary to explore and establish the analgesic activity of stembark of *K.pinnata*.

MATERIALS AND METHODS

The stem bark was collected from Nashik (MS) and authenticated by a botanist from Botany department of K.T.H.M.college, Nashik.

The stem bark was dried and powdered. The powder was extracted, successively with petroleum ether, chloroform and methanol using soxhlet extractor. The extracts were evaporated under vacuum. Extractive values (% w/w) of petroleum ether was 0.52.

Table 1- Validated data for the HPTLC method for estimation of β -sitosterol

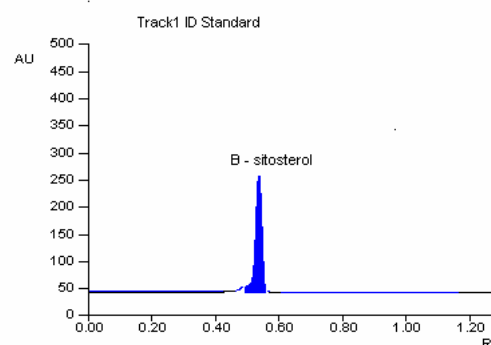
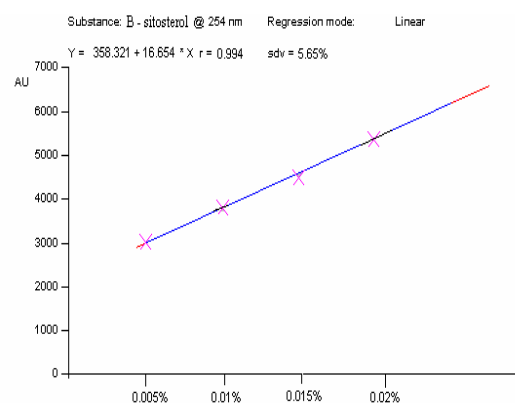
Parameter	Result
Linearity Range	0.005% - 0.02%
Instrumental Precision RSD (n=5)	0.445
Linearity Regression	0.994
Specificity	Specific

One milligram of standard β -sitosterol, was dissolved in 5 ml of chloroform. Three additional calibration levels were prepared by diluting this solution with chloroform, to obtain 0.005%, 0.01%, 0.015% and 0.02% concentrations, for studying the linearity. The precision of the method was also studied, by taking a solution of concentration 0.02% five times, and finding out the standard deviation and coefficient of variation.

A Camag TLC system comprising of Linomat V sample applicator (Camag, Muttlenz, Switzerland). TLC scanner III controlled by winCATS software version 1.3.0 was used for sample application and quantitative evaluation. Chromatography was performed on Merck silica gel G₆₀ F₂₅₄ TLC plates (10 cm X 10 cm) with chloroform as a mobile phase. Samples were applied as bands 8 mm long at 8 mm intervals, under a stream of nitrogen. Ascending development to a distance of 80 cm was performed in a 20 min presaturated 10 cm X 10cm Twin trough TLC developing chamber (Camag).

Chromatogram was evaluated by scanning in absorbance mode at wavelength of 254 nm. Slit dimensions were 6.00 X 0.45 mm. Data of each band was recorded. Densitogram and the linear calibration curve with the related statistical parameter are given in FIG. 1, 2

respectively. The overly spectra of Standard β -sitosterol and the corresponding peak from the extract are given in Fig.3.

**FIG.1. Typical Densitogram of standard β - sitosterol.****FIG.2. Calibration curve of β - sitosterol.**

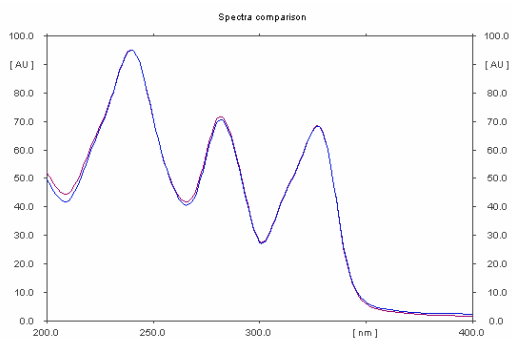


FIG. 3. Overlay absorption spectra of standard β - sitosterol and corresponding peak from an extract of *Kigelia pinnata* taken on CAMAG TLC Scanner 3.

RESULT AND DISCUSSION

Standard β -sitosterol solutions of 0.005%, 0.01%, 0.015% and 0.02% concentrations were analyzed for studying the linearity. β -sitosterol showed good linearity in the concentration range of 0.005% - 0.02% with a correlation coefficient of 0.994. The precision of the method was also studied taking a single sample solution five times and finding out the standard deviation and coefficient of variation was found to be 0.445 and 0.673. Low value of standard deviation and coefficient of variation are indicative of high precision of the method. The percentage of β -sitosterol in the stem bark was found to be 0.0462 %.

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