

Comparative botanical and phytochemical evaluation of *Calotropis procera* Linn. and *Calotropis gigantea* Linn. Root

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ABSTRACT

The present study aimed to develop pharmacognostical and phytochemical descriptors (HPTLC) of *Calotropis procera* and *Calotropis gigantea*. β -sitosterol which is one of the common terpene content and a potent antioxidant, purgative, antispasmodic and expectorant, has also been studied through a simple and high-precision method using high performance thin layer chromatography (HPTLC). This may be utilized by pharmaceutical industries for quality evaluation, ensuring successful commercial exploitation of this drug. From the present study it has been observed that both *Calotropis procera* and *C. gigantea* have similar microscopic characteristics, physico-chemical parameters showed a little variation as total ash components and extractive values are little less in *C. gigantea*. HPTLC studies also showed similar qualitative profile with some quantitative variations in total β -sitosterol, which was higher in *C. gigantea* (2.79%).

INTRODUCTION

Calotropis procera Linn. and *Calotropis gigantea* Linn. (Family: Asclepiadaceae) known, as 'Ark or Aak' in Ayurveda are versatile medicinal plants used singly or in combination with other medicinal plants for treating a variety of ailments like irritation, different types of pain, leprosy, syphilis, intestinal worms, cough, and it also act like emetic, diaphoretic, alterative, purgative and antispasmodic activity (Caius, 1986; Das, 1996). *C. procera* possesses potent antioxidant and anti-inflammatory properties and has been evaluated for its hepatoprotective effect against carbon tetrachloride (CCl₄) induced hepatotoxicity in rats (Padhy *et al.*, 2007). The hypoglycaemic property of *C. procera* has also been assessed by an oral glucose tolerance test (OGTT) in streptozotocin-diabetic rat's (Bauer *et al.*, 1966). The root of *C. procera* is used as a carminative in the treatment of dyspepsia (Kumar and Arya, 2006). Further, the root barks and leaves of *Calotropis* species have been used by various tribes of Central

India as curative agents for jaundice (Samvatsar and Diwanji, 2000). The aqueous extract of the latex has been shown to inhibit cellular infiltration and provide protection against development of neoplastic changes in the transgenic mouse model of hepatocellular carcinoma (Choedon *et al.*, 2006). The chloroform extract of the root exhibits protective activity against CCl₄ induced liver damage (Basu *et al.*, 1992).

On chemical studies, flavonoids (Chopra *et al.*, 1956; Singh and Rastogi, 1972), triterpenoids (Pal and Sinha, 1980), volatile long chain fatty acids (Sen *et al.*, 1992), glycosides and proteases (Kitagawa *et al.*, 1992) have been isolated from the various parts of the plant *Calotropis* species. Due to the various uses of *Calotropis procera* and *C. gigantea*, the present study was aimed to develop botanical and phytochemical descriptors for roots of both species. β -sitosterols is widely available and easy to detect and quantify among these species through HPTLC. Thus as one of the active constituent β -sitosterols, a major terpenes, has also been quantified through a simple and high-precision method using high performance thin layer chromatography (HPTLC), which may be one of the identifying parameter used by industries for quality evaluation.

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MATERIALS AND METHODS

The roots of *C. procera* and *C. gigantea* were collected at flowering stage from Lucknow, India. Materials were authenticated, and deposited in the Institute (please name the institute) herbarium (LWG No. 92906, 93248). Roots were preserved in 70% alcohol for histological studies. Microtome sections were cut and stained with safranin and fast green and photographed with Nikon F70X camera (Johansen, 1940). Physicochemical and phytochemical studies *viz.* total ash, acid insoluble ash, extractive values, total sugar, starch and tannin contents were carried on the shade dried powdered materials as per method described in AOAC (Anonymous, 1984; Peach and Tracy, 1955).

HPTLC Studies

Reagents were from Merk (Germany) and standard β -sitosterol was procured from Sigma-Aldrich (Steinheim, Germany).

Preparation of root extracts:

Air dried (45-55 °C) powdered root of *C. procera* and *C. gigantea* (1.0 g) in triplicate were extracted separately with 3×10 ml petroleum ether extract. Extracts were concentrated under vacuum, re-dissolved in methanol, filtered and finally made up of 100 ml with petroleum ether extract prior to HPTLC analysis.

Chromatographic conditions

Chromatography was performed on Merk HPTLC precoated silica gel 60GF₂₅₄ (20×20 cm) plates. Petroleum ether solutions of samples and standard compound β -sitosterol of known concentrations were applied to the layers as 6 mm-wide bands positioned 15mm from the bottom and 15 mm from side of the plate, using Camag Linomat 5 automated TLC applicator with nitrogen flow providing a delivery speed of 150nl/s from application syringe. These conditions were kept constant throughout analysis of the samples.

Detection and quantification of β -sitosterol

Following sample application, layers were developed in a Camag twin trough glass chamber that had been pre-saturated with the mobile phase of Toluene: ethyl acetate: Methanol (8.5: 1.5: 0.5) till the proper separation of bands up to 8cm height. After development, layers were dried with a dryer and derivatised with anisaldehyde-sulphuric acid reagent and β -sitosterol was simultaneously quantified using Camag TLC scanner model 3 equipped with Camag Wincats IV software. Following scan conditions were applied: slit width 6x0.45mm; wavelength, 320nm; absorption-reflection mode. In order to prepare calibration curves, stock solution of β -sitosterol (1mg/ml each) was prepared and various volumes of these solutions were analyzed through HPTLC exactly as mentioned above, calibration curves of peak area vs. concentration were also prepared. β -sitosterol (yield, 0.534% dry basis) had following values: R_f 0.49 and r^2 0.9899.

RESULTS AND DISCUSSION

Macroscopic and microscopic characters of the *C. procera* and *C. gigantea* root

Macroscopic and microscopic characters of roots both the species *viz.* *C. procera* and *C. gigantea* were similar and there were no marked differences which could be projected as key identifying parameters (Table1 and Fig. 1 and 2).

Table 1 Comparative Macroscopic and microscopic characters of the roots of *Calotropis procera* and *C. gigantea*

Characters	<i>Calotropis procera</i>	<i>Calotropis gigantea</i>
Macroscopic	Root-rough, fissured longitudinally, measuring 3 to 8 cm in length, 2 to 7 cm in diameter: corky and soft, externally yellowish-grey while internally white, central zone cream colored.	Root cylindrical, tortuous, often branched, measuring 3 to 10 cm in length, 2 to 10 cm in diameter; surface rough, longitudinally wrinkled and fissured, externally yellowish white internally paler.
	Odor, characteristic: taste, bitter and acrid.(Fig 1A)	Odor, characteristic: taste, bitter and acrid.(Fig 2A)
Microscopic	Transverse section of root shows outer most cork tissue consisting of 6-9 rows of tangentially elongated and radially arranged cells followed by 3-6 rows of moderately thick-walled parenchymatous cells, (Fig 1B)	Transverse section of root shows stratified cork, consisting of 8 to 12 layers, cells of the inner layers, containing phellogen, 2 to 3 layered, phelloderm, comparatively narrow, of thin walled parenchymatous cells; phelloderm and phellogen contains laticiferous tubes.(Fig 2B)
	Vascular cambium present just within the phloem consisting of 2-5 rows of thin-walled, tangentially elongated cells xylem forms the central part of root composed of vessels. Tracheids, fibres and xylem parenchyma, vessels present throughout xylem region and arranged radially in groups of 2-7, sometime single vessels also occur, (Fig 1C, 1D, 1E)	Vascular cambium, well developed; xylem consist of large vessels, mostly isolated, radially arranged (Fig 2C, 2D, 2E), parenchyma thick walled; medullary rays uniseriate, rarely bi-to triseriate running almost parallel, diverging and getting broader in the phloem.
	Parenchymatous cell rectangular to polygonal with thick walls filled with numerous starch grain.(Fig 1F)	Parenchymatous cells rectangular to polygonal with fairly thick and pitted walls, filled with starch grain.(Fig 2F)

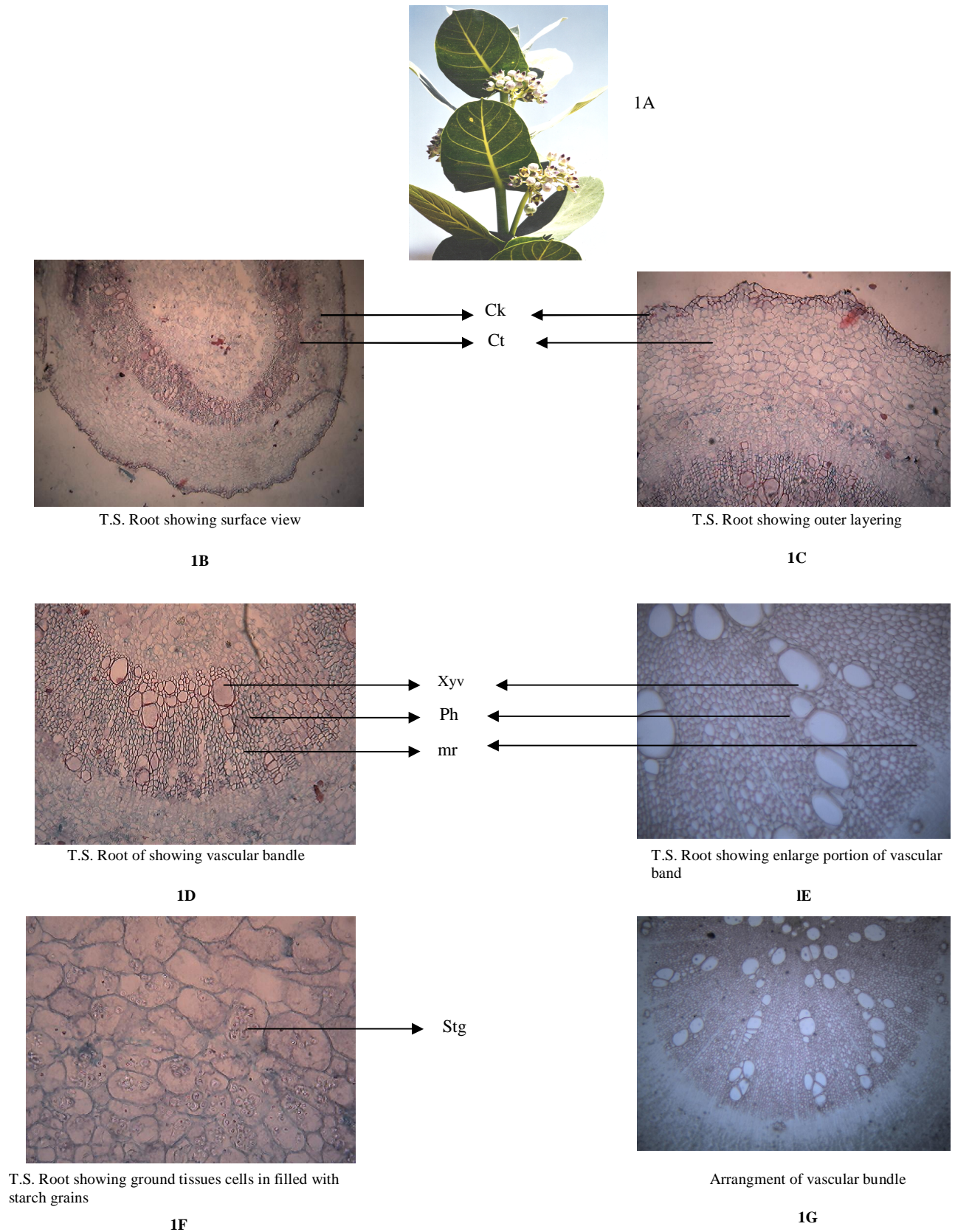


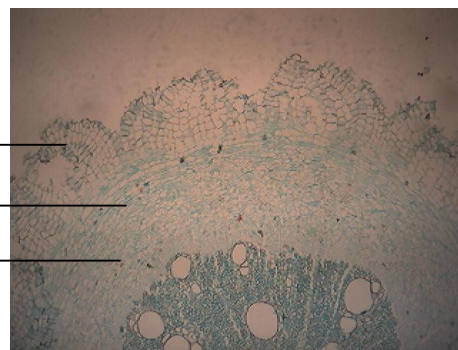
Fig.1: T.S. Root of *Calotropis procera* (x 10X, x 40X) Abbreviation:- CC-Cork cell; CT-Cortex; MR-Medullary ray; PH-Phloem; Vs-Vessels; Xyv-Xylem vessels; Stg-Starch grain



2 A

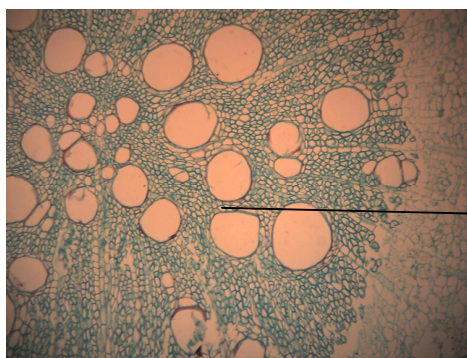


T.S. Root showing surface view
2 B

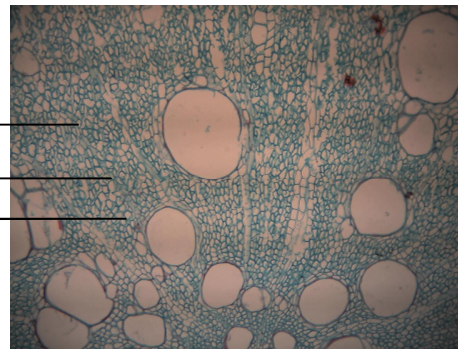


T.S. Root showing outer layering
2 C

← Ck ←
← Ct ←
← Xy ←



T.S. Root showing vessels
2 D



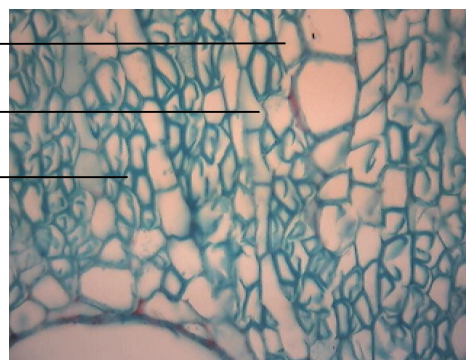
T.S. Root shows enlarge portion of vascular bundle surface view
2 E

← Xy ←
← pith ←
← mr ←



Ground tissue with xylem and Phloem

2 F



Arrangement of vascular bundles

2 G

← Xy ←
← Ph ←
← mr ←

Fig.2: T.S. Root of *Calotropis gigantea* (x 10X, x 40X)Abbreviation:- CC-Cork cambium; CK-Cork cell; CT-cortex; MR-Medullary ray; PH-Phloem; Vs-Vessels; Xyv-Xylem vessels

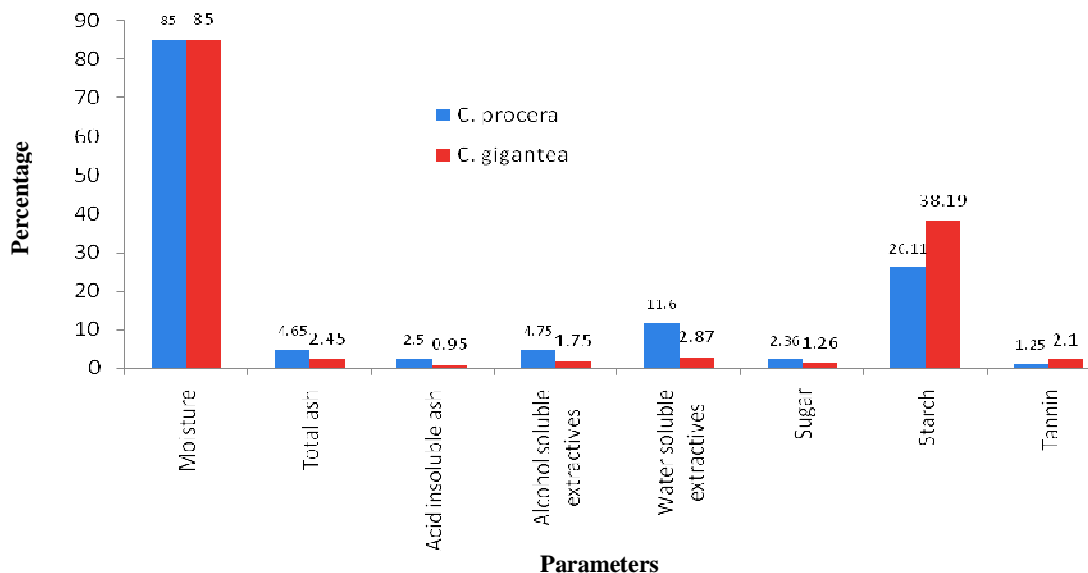


Fig. 3 Comparative physicochemical values of *C. procera* and *C. gigantea*.

Physicochemical studies

Quantitative determination of different physicochemical and phytochemical parameters revealed that:

C. procera root contains- moisture, 85%; total ash, which is considered to be an important and useful parameter for detecting the presence of inorganic substances was 4.65%; acid insoluble ash, 2.5%; extractive values are indicators of the total solvent soluble component; containing alcohol soluble extractive 4.75%; water soluble extractive, 11.6%; sugar, 2.36%; starch, 26.11% and tannins, 1.25%. However *C. gigantea* composition showed some differences: moisture, 85%; total ash, 2.45%; acid insoluble ash, 0.95%; alcohol soluble extractive, 1.75%; water soluble extractive, 2.87%; sugar, 1.26%; starch, 38.19% and tannins, 2.1% (Fig. 3).

Physicochemical studies thus indicate more potentiality of *C. procera* as compared to *C. gigantea*, containing higher amount of extractive values.

HPTLC Studies

A densitometric HPTLC analysis was also performed for the development of characteristic fingerprint profile, which may be used as markers for quality evaluation and standardization of the drug. The percentage of total β -sitosterol was estimated through HPTLC methods and was higher in roots of *C. gigantea* (2.79%) than in roots of *C. procera* (1.07%) at R_f value 0.49 and r^2 0.9899 (Figs. 4-6).

It is observed that, both *Calotropis procera* and *C. gigantea* have similar microscopic characteristics, physicochemical studies showed a little variation; ash components and total extractive values are less in *C. gigantea*. However, HPTLC studies also showed similar qualitative profile with minor quantitative variations in β -sitosterol content, which was higher in *C. gigantea*.

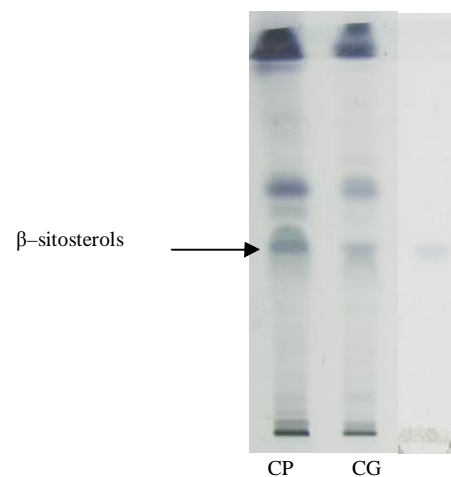
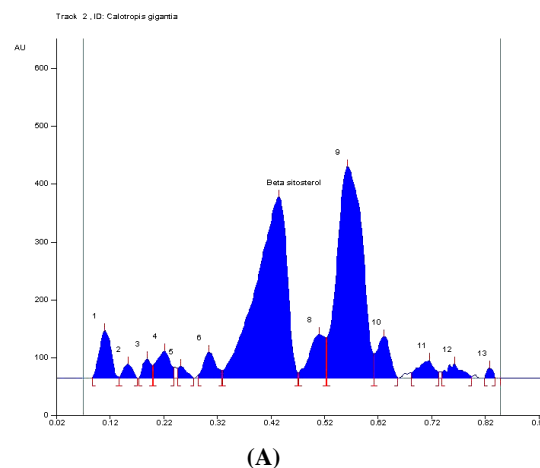


Fig. 4 HPTLC scan profile of *Calotropis procera* (CP) and *Calotropis gigantea* (CG) (petroleum ether extract) with Reference (REF) β -sitosterols under visible light. (Plate sprayed with Anisaldehyde sulfuric acid).



(A)

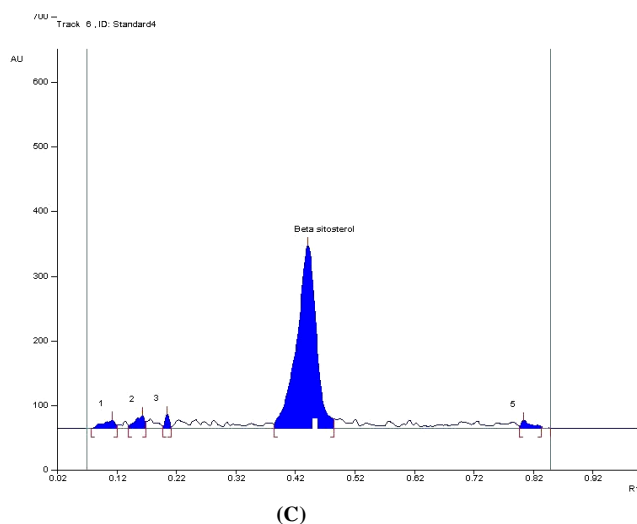
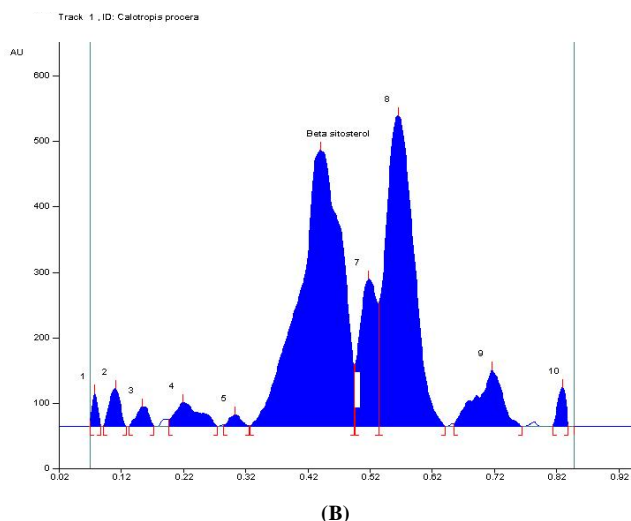


Fig. 5 Densitometric scan profile of *Calotropis procera* (A) and *Calotropis gigantea* (B) (petroleum ether extract) with standard β -sitosterols (C).

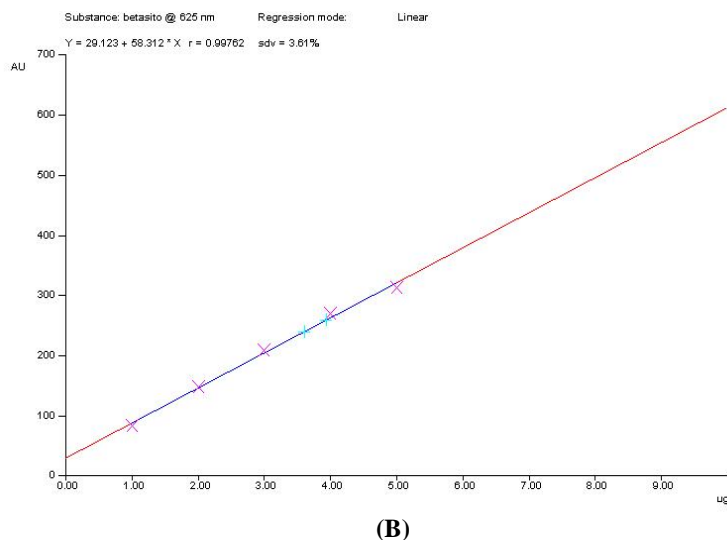
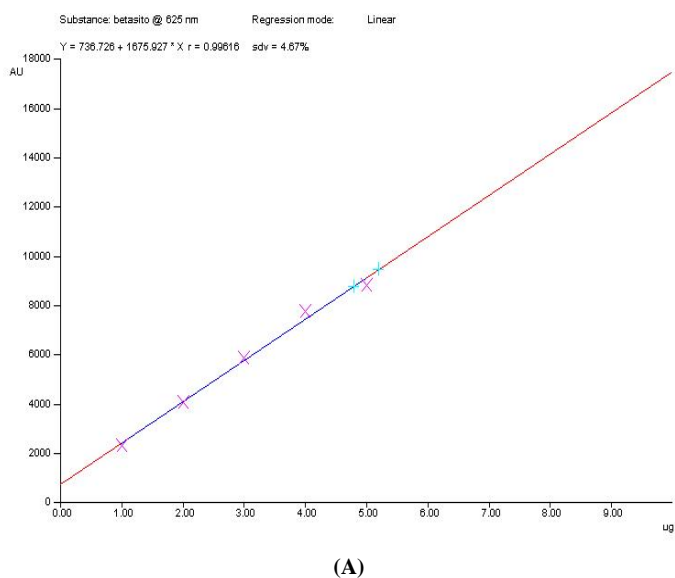


Fig. 6 Linear regression of *Calotropis procera* (A) and *Calotropis gigantea* (B) (petroleum ether extract) with standard β -sitosterols.

CONCLUSION

Above studies concludes that both the species have almost similar profile and falls in the range as mentioned in ayurvedic pharmacopoeia of India. Thus either of these species may be used in any formulations. These data may also be useful as supportive information for the exploitation of *C. gigantea* as a substitute to *C. procera* (ark). Physico-chemical and HPTLC parameters of both the species may also be useful to pharmaceutical industries for the authentication and batch to batch consistency of the commercial samples.

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