

Chapter

Detailed Pharmacognostical Standardization Studies on *Calotropis Procera* (Aiton) Dryand Fruit

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Abstract

Calotropis procera (Aiton) Dryand (Asclepiadaceae) is very well-known latex wild plant, it is covered in tropical Asia and Africa, traditionally as well as medicinally the plant has been used for insecticidal, antimalarial, antiviral, antimicrobial, analgesic, antifertility, antitumor, antihyperglycemic, hepatoprotective, anti-inflammatory, antidiarrhoeal, anticonvulsant, oestrogenic, antidiabetic and anthelmintic activity. For this reason, the main theme of research work was carried out on detailed pharmacognostical studies for quality control and standardization and this study provides the basis for the herbal remedy and provide physiological information about plant species. This research is helpful to identify and estimate the purity of the drug and can also be used to screen for adulteration and gives a drug quality. This comprehensive research work is for identification, collection, authentication and it is easy to identify the adulterants. Detailed macroscopical, T.S, L.S, Powder microscopy, Physicochemical parameters, Extractive values, Fluorescence, Preliminary Phytochemical studies were performed as per the standard. It is a very important research work and the author had has been established the evaluation standards for a medicinal plant. This evaluation is for the authentication of a fruit plant of *Calotropis procera* and the fruit physiology is useful for both qualitative and quantitative estimation of the medicinal herbal drugs.

Keywords: *Calotropis procera* fruit, pharmacognostical standardization, quality control

1. Introduction

Calotropis procera (Aiton) Dryand has a powerful insecticidal, antimalarial, antiviral, antimicrobial, analgesic, antifertility, antitumor, antihyperglycemic, hepatoprotective, anti-inflammatory, antidiarrhoeal, anticonvulsant, oestrogenic, antidiabetic and anthelmintic activity. Actually where ever observe, the plant is available on road sides

[1]. *C. procera* is a flowering plant and it belongs to *Asclepiadaceae* family. The *C. procera* is native to Africa, Arabian Peninsula, Western Asia, the Indian Subcontinent and Indo-China. The plant is a most powerful latex plant, and it attracts insects. In this plant plenty of medicinally useful secondary metabolites are present, those are cardenolides, terpenoids and anthocyanins, for this reason, collected fresh fruit and coarse powder for detailed anatomy of T.S, L.S RLS, LRS, powder microscopy, Extractive, Fluorescence and Preliminary Phytochemical studies. Therefore, present work was carried out to study the Pharmacognostical studies on the fruit of *Calotropis procera* (Aiton) Dryand.

2. Methods

2.1 Material and methods for pharmacognostical studies

2.1.1 Collection of plant materials for authentication and for anatomical studies

The collected fruit of *Calotropis procera* from Titupathi, at the area of nalla malai forest, India. *C. procera* collected in the month of December, 2018. The *C. procera* fruit was authenticated by Prof. K. Madhavachetty, Department of botany, Srivenkateswara University, Andhra Pradesh, India. The given voucher for specimen number is 1221, deposited at College of Pharmaceutical Sciences, Andhra University.

2.1.2 Collection of specimens

The collected plant species are too healthy for the proposed study, specimens of organs are cutted and soaked into 70% of 90 ml ethyl alcohol, 5 ml of Formalin and 5 ml of acetic acid [2]. The organs of specimens are dehydrated with graded series of tertiary butyl alcohol as per the guidelines given by sass, 1940. Specimens of infiltration were carried by the addition of 58–60°C paraffin wax until TBA solution attained super saturation.

2.1.3 Morphological features

These plant species grow in all types of soil, collected fresh fruit from 2.8 mts height of the plant early in the morning at 6 am from red loamy soil land. The fruit is 10 cm long and 6 cm width outer and inner layer are green in color and in every fruit contains a plenty of brown-colored seeds more over every seed length is 5.5 to 6.0 mm length and 3.7 mm width many of white silky hairs are present and each silky hair length is 40 to 50 mm in long. Fruit color of the *Calotropis procera* is green in color, strong pungent odor, taste is characteristic/bitter and gummy in nature. Soluble in benzene, ethyl acetate, ethanol, methanol and hydroalcoholic.

2.1.4 Sectioning

The specimens of paraffin-embedded were sectioned with the help of a rotary microtome, the thickness of each section was 10–12 μm [3]. As per the standard method the obtained sections were stained in to toluidine blue [4] because toluidine blue is a polychromatic stain. The obtained strains are good because some phytochemical reactions were obtained. The obtained dye changed pink color to mucilage blue for the protein bodies, other sections are stained with safranin, fast green and iodine [5].

For studying the stomatal number, the pattern of venation and distribution of trichomes, sections of paradermal (sections taken parallel to the surface of leaf) as well as clearing of the leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid [5] were prepared. Glycerine mounted temporary preparations were made for macerated /cleared materials. Different parts of powdered materials were cleared with NaOH and mounted in glycerine medium after staining. Measurement of different cell components was studied.

2.1.5 Photomicrographs

Different magnifications of photographs are taken with the help of Nikon labphoto 2 microscopic unit, bright fields were used for normal observation. The polarized light was used for the identification of Different kinds of tissues, crystals, stone cells, starch grains, fibers, lignified and non-lignified cells. Since these obtained structures have fair finger print property, under polarized light they appear bright against the dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are given in the standard anatomy books [6].

2.1.6 Instrumentation and technique

The obtained extracts and powder had examined under UV-light (254 nm, 354 nm) [7, 8]. The total ash value, Determination of Acid Insoluble Ash, Determination of Alcohol Soluble Extractive value, and Determination of Water-Soluble Extractive values in the obtained samples were performed, and in Loss on drying measured the amount of water and volatile oils, Swelling index, Foaming index were performed according to the standard procedure, for Extractive values, 2gms of an air-dried coarse powder of drug macerated with 40 ml of solvents (Hexane, Ethyl acetate, Chloroform, Acetone, Methanol and Distilled water) in a glass stoppered conical flask with frequent shaking for 6 hrs and then allowed to stand for 2 days, then filtered carefully, taking care against loss of solvent. The filtrate was evaporated in a silica crucible to dryness in the water bath and then dried 105° for 6 hrs, cooled in a desiccator for 30 min and weighed without delay [9]. Fluorescence Analysis of the drug was observed in UV light using various extracts of the drug. The drug powder was treated separately with different solutions [10, 11] and for Preliminary phytochemical studies. The prepared extracts were tested for the different types of chemical constituents present by known qualitative tests. The following tests were carried out on the extracts to detect various phytoconstituents present in them [12–14] were performed according to the official methods described in the Indian pharmacopoeia (1996) and WHO guidelines on quality control methods -for medicinal plant materials.

3. Results and discussion

3.1 Anatomy of the fruit

The fruit of *Calotropis procera* is a double follicle that develops from bicarpellery apocarpous ovules. The mature fruit has fused stigma namely pentagonal

stigma and the ovaries are free from each other. Later the stigma breaks and the two carpels become free from each other. The ovules develop into cylindrical, elongated fruits called follicles. The follicles are thick, soft and cylindrical. The ovules are many and attached to marginal placentum. When the follicles become fully matured they dehisce longitudinally along with the marginal layer of placental tissue.

3.2 Structure of the carpel

The carpel consists of the outer epicarp, middle mesocarp and inner endocarp (**Figure 1a**). The epicarp consists of an epidermal layer and inner thick compact parenchymatous tissue in which fairly prominent vascular strands are located (**Figures 2a** and **3a**). The inner pericarp is also thick with small circular, thin-walled compact.

Parenchymatous tissue (**Figures 2b** and **3b**) in the inner pericarp, the laticiferous canals are seen spreading in all directions. The middle part of the pericarp has wide air chambers and thin reticulate layers of parenchyma cells (**Figures 3a, b**) at certain places, thick horizontal layers of cells occur in between the outer and inner pericarp.

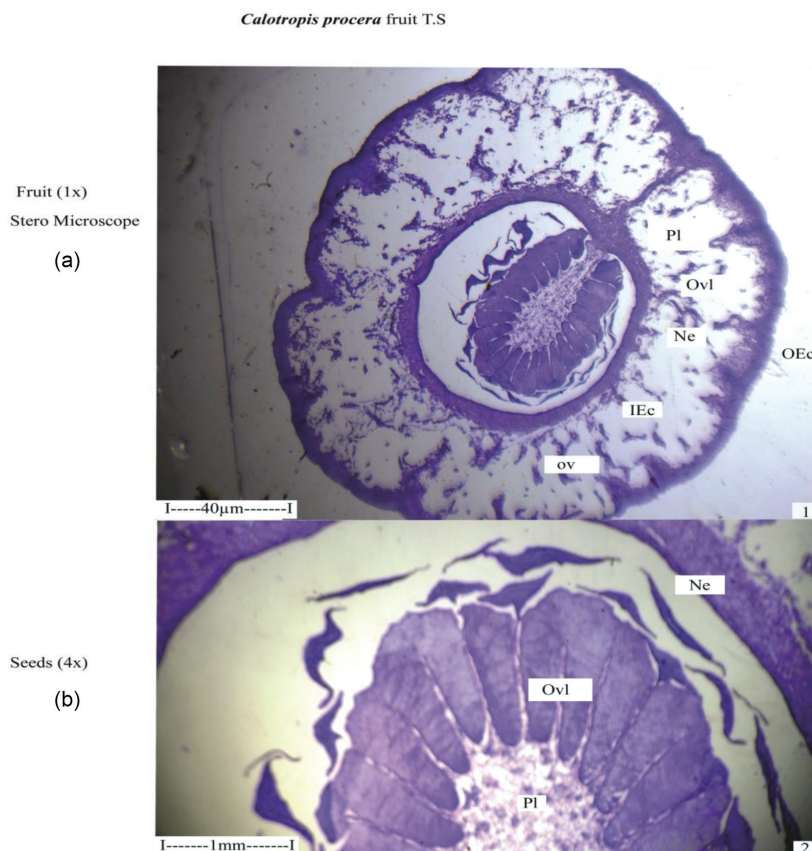


Figure 1.
(a) T.S. of a carpel with numerous ovules on marginal placentation; and (b) T.S. of carpel with ovule enlarged. IEP: inner epicarp; Ne: nectary; OEC: outer epicarp; Ov: ovary; Ovl: ovule; and Pl: placenta.

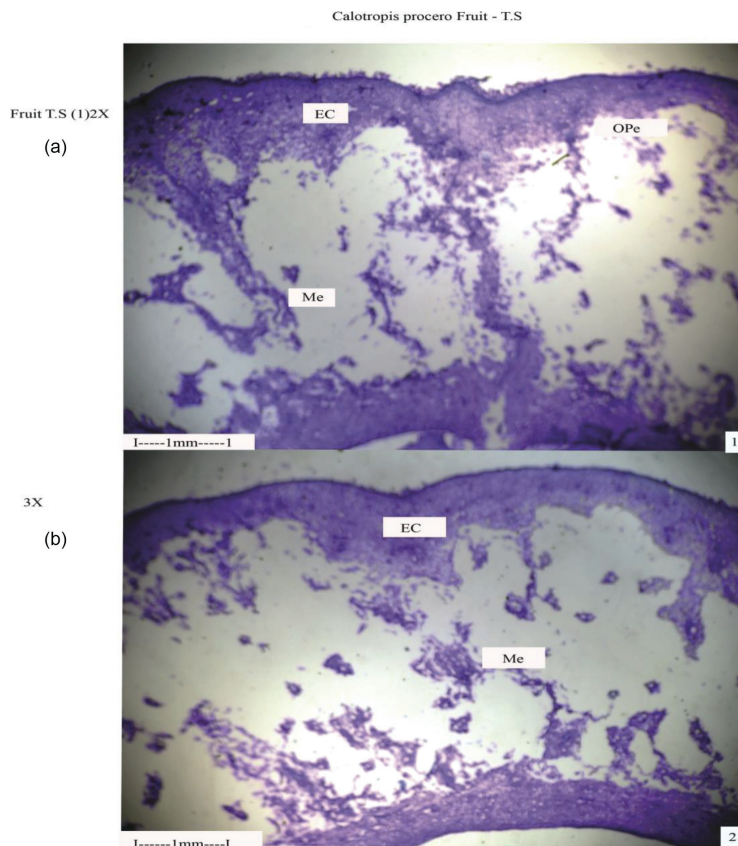


Figure 2.
 (a) T.S. of pericarp: outer part; and (b) T.S. of pericarp: inner part. Ec: epicarp; IPe: inner pericarp; OPe: outer pericarp; and Me: mesocarp.

This horizontal plate of cells possesses small scattered vascular strands. In the outer pericarp also, there are numerous small vascular strands dispersed profusely in the parenchymatous ground tissue (**Figure 4a, b**).

3.3 Ovules and seeds

The ovules occur in marginal placentation a thick massive placental tissue occurs along one side of the carpel. On this elongated cylindrical placental tissue occurs, numerous club-shaped ovules (**Figures 1b, 5, and 6a**).

The ovules are broad along the outer part and narrow towards the inner part (**Figure 5**). On the outer part of the carpel, there are numerous, curved elliptical, darkly stained appendages which are the nectarines (**Figures 5 and 6a, b**).

3.4 Powder microscopy

In the powder preparation, fragments of tissues of the pericarp, seed coat and cell inclusions are seen. The inner surface of the pericarp consists of elongated, rectangular, thin-walled cells (**Figure 7a**). These cells have straight cell walls. The cells are parallel to each other forming a dense mat (**Figure 7a**). The outer surface of the pericarp

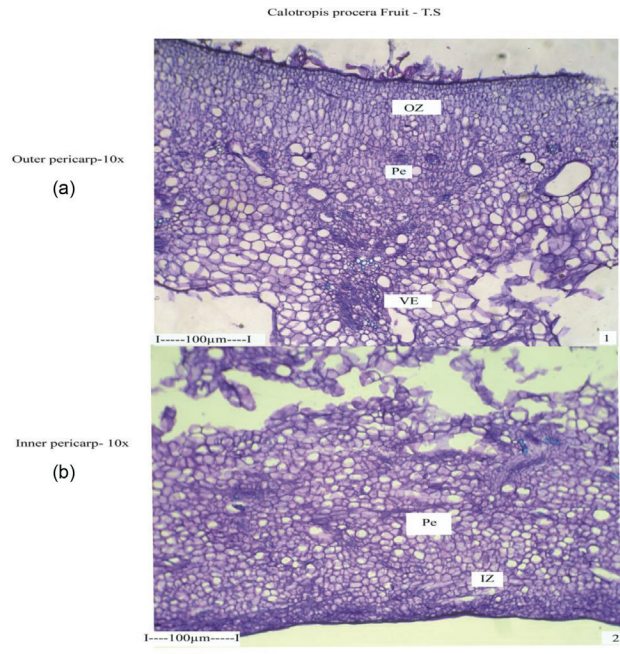


Figure 3.
(a) T.S. of pericarp outer part showing vascular elements; and (b) T.S. of pericarp inner part. IZ: inner zone; OZ: outer zone; Pe: pericarp; and Vs: Vascular strand.

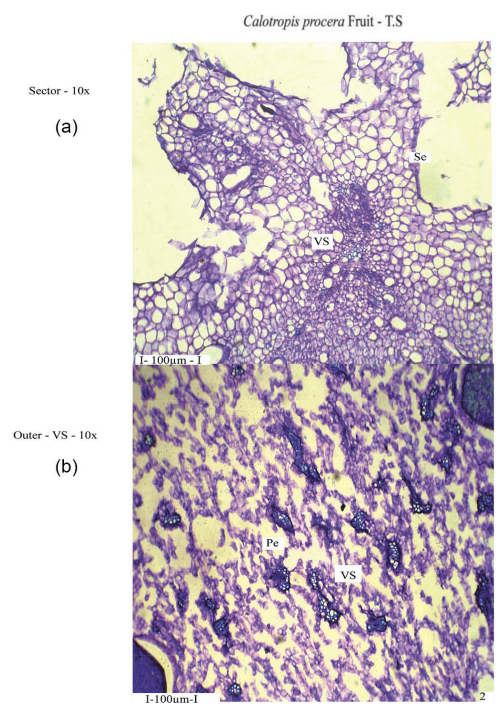


Figure 4.
(a and b) T.S. of pericarp with scattered vascular strands. Pe: pericarp; Se: segment of fruit wall; and Vs: vascular strand.

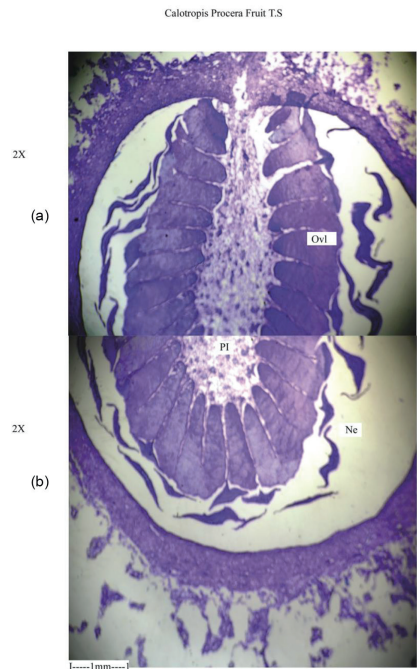


Figure 5.
T.S. of carpel enclosing a bundle of ovules in marginal placentation. Ne: nectary; ovl: ovule; Pl: placenta.

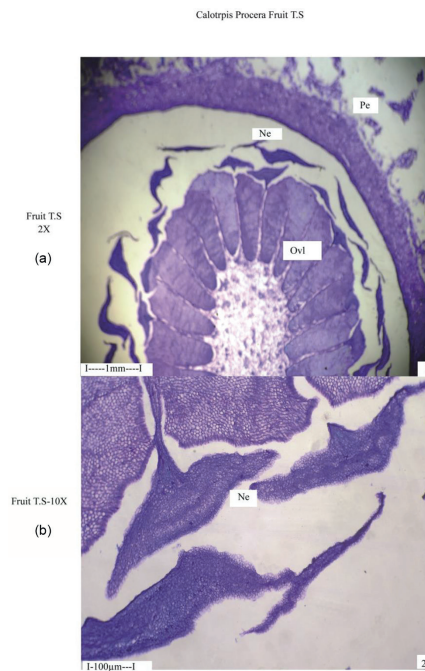
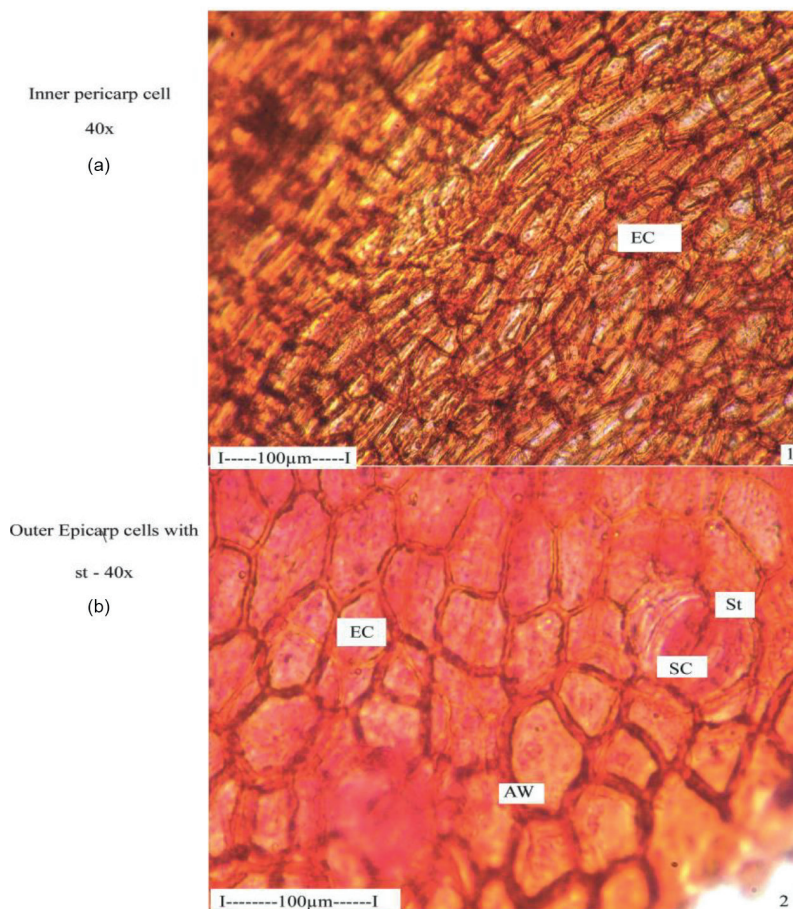


Figure 6.
(a) T.S. of carpel enclosing the ovules with peripheral nectarines; and (b) Nectarines enlarged. Ne: nectary; Ovl-ovule; and Pe: pericarp.

Calotropis procera Fruit

**Figure 7.**

(a) A piece of inner pericarp showing elongated compact parallel parenchyma cells; and (b) Surface view of the outer pericarp where the cells are polygonal with thick straight anticlinal walls and stomata. Aw: anti clinal wall; Ec: epidermal wall; Sc: subsidiary cell; and St: stomata.

consists of wide polygonal cells with thick, straight anticlinal walls. Stomata are also frequently seen on the outer epicarp. The stomata have narrow stomatal aperture and elliptical guard cells. The stomata appear to be paracytic type (**Figure 7b**).

Broken pieces of seed coats are frequently seen in the powder. The abundance of epidermal trichomes of non-glandular type are seen on the seed coat. The trichomes are thick at the base and become narrowly pointed at the tip. The cell walls of the trichome are thick and lignified and the cell lumen is narrow and canal like (**Figure 8a, b**). The trichomes are 130 μm long and 15 μm thick.

The epidermal cells of the seed coat are angular, polygonal and compact (**Figure 8a and 9b**). Laticiferous canals are commonly present in the pericarp of the fruit. The laticiferous are long, canal-like, non-septate and unbranched. They contain dense white latex secreted from the laticifer (**Figure 9a**). The seeds have dense accumulation of different sizes of oil bodies. They appear white spherical bodies floating in water (**Figure 10a**). Prominent spherical or elliptical starch

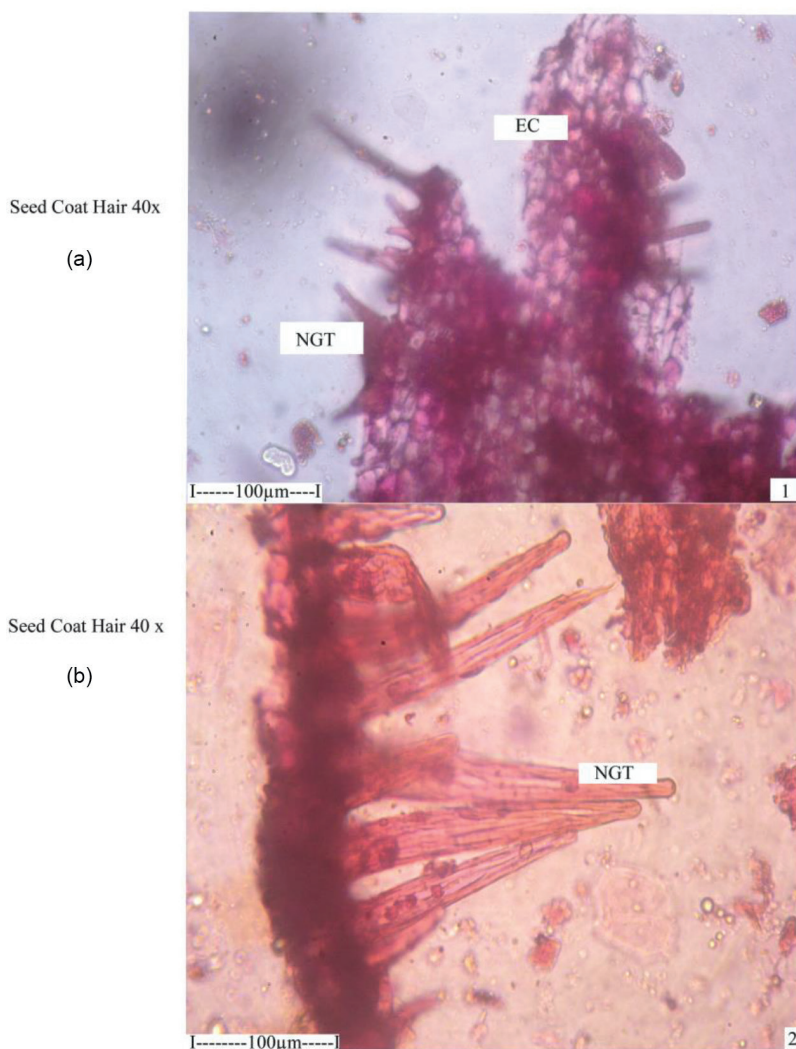
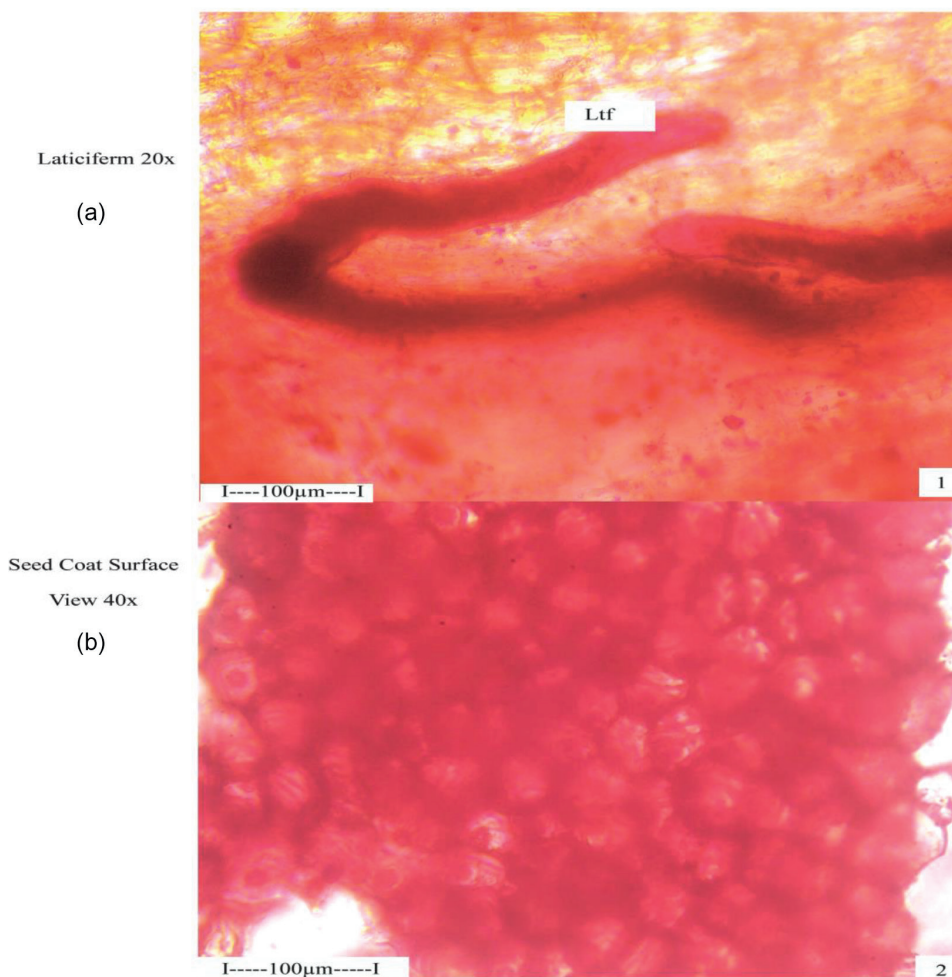


Figure 8.
(a) A broken piece of seed coat showing polygonal epidermal cells and non-glandular epidermal trichomes; and (b) Clusters of non-glandular trichomes seen in powder. Ec: epidermal cells; and NGT: non-glandular trichomes.

grains are common in the powder. When stained with IKI, the starch grains appear black or dark blue (**Figure 10b**). The seeds of *Calotropis* are flat, obovate and thin. One end of the seed is narrow and pointed. This pointed end of the seed bears a tuft of thin, white, soft trichomes. This tuft of trichomes is called coma. The trichomes of coma are also seen in the powder. These trichomes have thick walls and wide lumen. They are unicellular and unbranched. The trichomes are 30 µm thick (**Figure 10c**).

Calotropis Procera Fruit

**Figure 9.**

(a) A piece of pericarp tissue with unicellular, unbranched laticiferous canal; and (b) Seed coat fragment showing thick-walled epidermal cells. Ec: epidermal cells; and LtF: laticifer.

The ash is mainly for the identification of carbonates, silicates, phosphates, sodium, potassium, calcium and magnesium without an organic matter. The obtained total ash value (11.4%), Water soluble ash (7.5), Acid insoluble ash (1.8), Foaming Index (<100), Swelling Index (52%), Loss on Drying (16.7%) And the extractive values are mainly for the estimation of primary and secondary metabolites in Petroleum ether (5.65%), it might be terpenoids, sterols, lipids and waxes and apart from Ethyl acetate extract (2.25%), Acetone extract (3.17%), Methanolic extract (4.53%) contains terpenoids, phenols, and glycoside containing components may be present. Distilled Water (10.11%) and Hydro-alcohol (6.05%) contains polyphenols, tannins and alkaloids present (**Tables 1–3**).

For herbal drugs, fluorescence analysis is very primary tool for the determination of constituents, this fluorescence analysis provides an informative data on nature of constituents [12]. The chemical reagents were used for the powdered drug analysis and

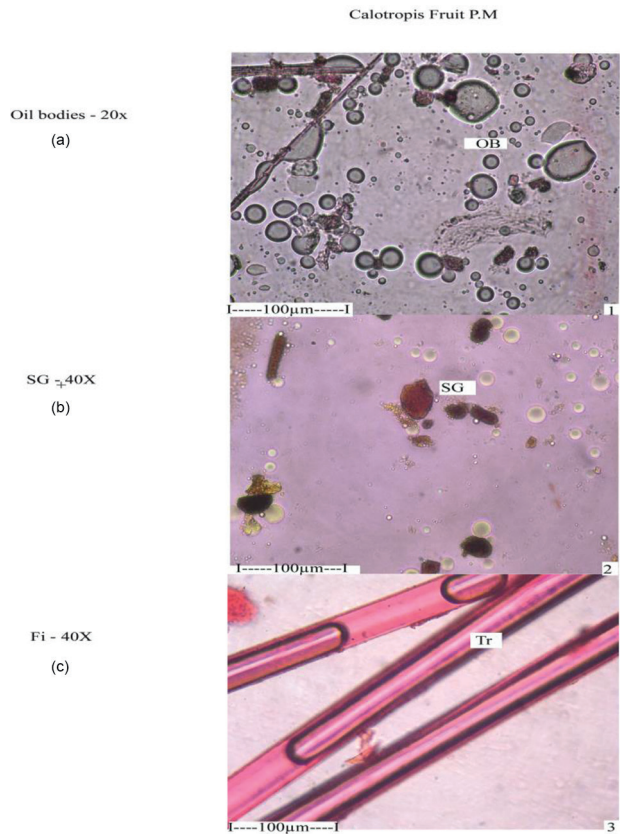


Figure 10. Oil globules of different sizes found in the powder; (b) Starch grains stained with potassium iodide; and (c) Trichomes from coma of the seed. OB: oil bodies; SG: starch grains; and Tr: trichomes.

Parameters	Fruit
Color	Green
Shape	Bean
Size	length10cm
Odor	Characteristic
Powdered drug study	
Color	Green
Odor	Characteristic
Taste	Strong Bitter
Filter paper test	

Table 1. Morphological characters of *Calotropis procera* fruit.

observations are available in visible light and UV lights of Short and long wave lengths (Table 4).

The preliminary phytochemical screening on *Calotropis procera* was done by using hexane, n-butanol, ethanol, acetone, ethyl acetate, methanol and hydro alcoholic

Parameter	Percentage (%)
Total Ash	11.4
Water soluble ash	7.5
Acid insoluble ash	1.8
Foaming index	<100
Swelling index	52
Loss on drying	16.7

Table 2.
Results for physicochemical parameters.

Solvents	Percentage (%)
Petroleum ether	5.65
Ethyl acetate	2.25
Acetone	3.17
Methanol	4.53
Distilled water	9.11
Hydro-alcohol	6.05

± Calculated as SEM of three reading.

Table 3.
Results of extractive values.

Chemical treatment	Day light	Fluorescence	UV longer (365 nm)	UVShort (254 nm)
Drug powder	Peanut Brownish green	Green	Dark green	Green
Hexane	Yellow	Yellowish green	Green	Green
Ethyl acetate	Yellowish green	Greenish yellow	Reddish green	Light reddish green
Methanol	Yellowish green	Yellow	yellow	light green
Water	Brown	Dark brown	Light green	Green
Drug powder+1 N NaOH in methanol	Yellowish green	Yellowish green	yellow	Light green
Drug powder+1 N NaOH in water	Brownish green	Brownish green	green	light green
5% NaOH	Light Yellowish	Yellowish	Yellowish	Yellowish
10% NaOH	Yellowish	Yellowish	Yellowish	Yellowish
Powder+1 N Hcl solution	Dark brown	brownish	Light green	Colorless
Powder+50% HNO ₃	Yellow	Reddish yellow	Dark green	Light green
Powder+50%H ₂ SO ₄	Light green	Reddish yellow	Light green	Light green
Powder+50% HCl	Dark brown	brownish	Light green	Colorless
5% FeCl ₃	Greenish peanut	Brownish green	Darkish yellowish red green	yellowish red green
Powder + picric acid	Orange yellowish green	Yellowish green	Darkish yellowish red green	yellowish red green
Powder + Dil. NH ₄	Dark green	Greenish peanut	Greenish peanut	Greenish peanut
Powder + acetic acid	Dark yellowish green	yellowish green	Dark green	Light green

Table 4.
Fluorescence analysis on Calotrophis procera fruit.

Phytochemical analysis	Hexane	N- Butanol	Ethylacetate	Ethanol	Acetone	Methanol ic	Hydroalcoh olic
1. Alkaloids							
(a) Mayer's test	-	-	+	+	+	++	+
(b) Hager's test	-	-	+	-	-	++	++
(c) Dragendroff's test	-	-	++	-	-	++	++
2. Carbohydrates							
(a) Molisch's test	+	+	++	++	++	++	++
(b) Fehling's test	+	++	+	+	+	+	+
(c) Barfoed's test	+	++	++	++	++	++	++
(d) Benedict's test	+	+	+	+	+	++	++
3. Glycosides							
(a) Borntrager's test	+	++	++	+	++	++	++
(b) Legal's test	+	+	++	++	++	++	++
(c) Keller-Kiliani test	+	+	++	++	++	++	++
4. Phenols and Tannins							
(a) Ferric chloride test	+	++	++	++	++	+	++
(b) Gelatin test	-	++	++	++	++	+	++
(c) Lead acetate test	+	++	++	++	++	+	++
5. Flavonoids							
(a) Alkaline reagent test	-	+	+	+	+	+	+
(b) Shinoda test	-	++	++	++	++	++	++
(c) Zn + HCl test	+	++	++	++	++	++	++
6. Test for fixed oils							
(a) Spot test	++	++	++	++	++	++	++

Phytochemical analysis	Hexane	N- Butanol	Ethylacetate	Ethanol	Acetone	Methanol ic	Hydroalcoh olic
7. Saponins							
(b) Foam test	++	+	++	++	++	++	++
8. Proteins and Aminoacids	+	+	++	++	++	++	++
(a) Millon's test	+	+	+	+	+	+	+
(b) Biurettes test	+	+	+	+	+		+
(c) Ninhydrin test	+	+	+	+	+	+	+
9. Terpenoids/ Phytosterols							
(a) Libermann-Burchard test	++	++	++	++	+	+	+
10. Test for triterpenoids							
(a) Salkowski test	++	++	++	++	+	+	+
11. Gum and Mucilages							
(a) Alcoholic precipitation test	++	++	++	++	++	++	+
12. Test for lignin	+	++	++	++	++	++	+
(a) Lignin test	+	++	++	++	++	++	+
(b) Labat test	+	++	++	++	++	++	+
“-” indicates absent; “+” indicates presence; and “++” indicates more intense.							

Table 5.
Preliminary phytochemical tests for identification of the class of primary and secondary components in the extract of C. procera fruit.

extracts. N-butanol, ethyl acetate and acetone extracts gave positive tests for alkaloids, carbohydrates, proteins, glycosides and terpenoids. Hexane extract showed only for terpenoids. Hydro alcoholic extract showed the presence of Alkaloids, Carbohydrates, Tannins, and Glycosides. Methanolic extract showed the presence of Alkaloids, Carbohydrates, Phenols, Tannins, Flavonoids, Proteins and Glycosides (**Table 5**).

4. Conclusion

This plant species is available in overall tropical and subtropical countries, standardization of crude drug work is the primary reference to do further step. Herbal Drugs are useful for different ailments in different countries. Pharmacognostical, Preliminary Phytochemical evaluation studies provide us a basis to establish the quality protocols of any medicinal herbs. In the Introduction part, we described about the species of plant, macroscopical, microscopical, preliminary phytochemical, ash values, extractive values, fluorescence analysis are the main tools to identify the purity, presence and absence of certain chemical groups in a herbal drug. Pharmacognostic studies are mainly useful for quality of a herbal drugs [15]. Breakthroughs and Innovations of *Calotropis procera* is mainly useful for insecticidal, antimalarial antiviral, antimicrobial, analgesic, antifertility, antitumor, antihyperglycemic, hepatoprotective, anti-inflammatory, antidiarrhoeal, anticonvulsant, oestrogenic, antidiabetic and anthelmintic activity etc. reported. In Andhra Pradesh state in India no pharmacognostic research work was established. So, this work is a primary and important for further studies. *Calotropis procera* is helpful for establishing the correct identification of a plant and the plant will be main tool for standardization, characterization and identification of *Calotropis procera* fruit. The plant is also helpful for the other research studies.

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Conflicts of interests

Conflicts of interest is none.

Availability of data and material

Availability of data and material is our own, based on the literature we have written ourselves.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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