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Pharmacognostic study of *Calotropis procera* (Aiton) W.T. Aiton root and stem

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Calotropis procera (Aiton) W.T. Aiton, belonging to the family Apocynaceae is an important medicinal shrub. It is widely used in folklore and Ayurvedic system of medicine. Various parts of this plant were used to treat human diseases such as *Pama* (Scabies), *Vicharchika* (Eczema), *Kasa* (Cough), *Mukhakrishnatwa* (Hyperpigmentation of the face), *Pleehodara* (Splenomegaly) and preparation of Ayurvedic as well as traditional medicines like Arkalavana and Arkataila. The present communication deals with a detailed account of the pharmacognostic study of *C. procera* root and stem. The study includes macroscopy, microscopy, powder microscopy studies, physicochemical, phytochemical analysis, heavy metal tests, screening of microbiological parameters and development of High-Performance Thin Layer Chromatography (HPTLC) fingerprints profile of *C. procera* root and stem. HPTLC fingerprints profile of ethanolic extract was done by using mobile phase toluene: ethyl acetate: formic acid for root and stem.

Keywords: *Calotropis procera*, HPTLC fingerprinting, Pharmacognostic study, Phytochemical investigation.

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Introduction

Calotropis procera (Aiton) W.T. Aiton, belonging to the family Apocynaceae is an important medicinal plant found in dry, wastelands, roadside throughout India and also distributed in China, Java and tropical Africa. It is known as Arka, Shwetaarka in Sanskrit, Aak, Madar, Akavana, Akada, Safed Aak in Hindi, Madar, Dead See apple, Maddar tree, Milkweed, Swallow-wort in English, Madar, Aak in Urdu[1,2]. *C. procera* plant is erect, perennial shrub, 1.5-2.5 meter high with milky latex. Leaves-thick, ovate to oblong-elliptic or obovate, 6 to 24 cm long and 5 to 10 cm wide, entire, mucronate, both surface cottony pubescent, become glabrous and smooth on drying due to easily detachable hairs, veins prominent on lower surface, symmetrical, petiole short, stout, 1 to 3 mm long, cylindrical and grooved on upper surface, pale yellowish brown, taste bitter and astringent, odour strong and characteristic. Inflorescence umbellate, cymes, flowers buff-pink, turning to bluish purple, 3-4 cm across, corona of 5 fleshy, laterally compressed lobes spurred at base. Fruit two ovoid follicles, each 4-7x2.5-5.5 cm long, generally one of them abortive. Seeds many broadly ovate, flat, narrowly margined, brown with 2-3.5 cm long cottony hairs. It is widely used in folklore and ayurvedic system of medicine. Various parts of this plant are used to treat human diseases such as *Pama* (Scabies), *Vicharchika* (Eczema), *Kasa* (Cough), *Mukhakrishnatwa* (Hyperpigmentation of the face), *Pleehodara* (Splenomegaly), leprosy, piles, tumours, ulcers, spleen and liver disease, neuromuscular blocking, blood pressure, respiratory disorders and preparation of ayurvedic as well as traditional medicines likewise Arkalavana and Arkataila[3,4]. The plant showed antimicrobial, anticoagulant, anti-inflammatory, analgesic, purgative and antipyretic activities[5-8].

C. procera plant has several medicinal properties due to the presence of numerous secondary metabolites[9]. These compounds are valuable for various medicinal activities. Different types of phytochemicals such as sterol, resin and fatty acid, flavonoids, triterpenoids[10] and alkaloids are found in the root bark[11]. Root is reported as antiulcer and anti-fertility[12] activities. It is also rich in cardiac glycosides[13,14] which are working as antitumor agents. Flowers were found to possess anti-fungal[15], hepatoprotective[16,17], antimicrobial, analgesic,

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antipyretic, and anti-larvicidal activities. Latex is reported to have wound healing[18], analgesic, antimicrobial, and anti-inflammatory activity[19]. *C. procera* plant contains various types of phytoconstituents like root bark contains benzoylisolinelone, cardenolide, lupeol, flavanols like quercetin-3-rutinoside and benzoylinesolone[20-21]. Stem and leaf contain muradine active constituents as well as a bitter yellow acid, calotropagenin, resin, uscharin, calotoxin and calotropin[22]. Latex contains a very powerful bacteriolytic enzyme, terpenol, uzarigenin and toxic glycoside calactin and flower contains ultiflavenol and calotropenyl acetate. The whole plant of the *C. procera* contains gigantin, taraxasterol, giganteol, isogiganteol, alpha-amyrin, beta-amyrin, beta-sitosterol and wax[23].

Despite the numerous medicinal uses attributed to this plant, there are no systematic pharmacognostical studies on the root and stem of this plant have so far been carried out. Hence the present work deals with morphological, anatomical evaluation, physicochemical tests, preliminary phytochemical screening, heavy metals test, microbiological screening and High-Performance Thin Layer Chromatography (HPTLC).

Materials and Methods

Sample collection and identification

The fresh plant samples of *C. procera* (root and stem) were collected from the Arogyadham campus, Chitrakoot, Satna, Madhya Pradesh in the month of March 2019. The plant was identified and authenticated by a Senior Scientist, Deendayal Research Institute Chitrakoot. The voucher specimen (AD/AS/347/2019) was prepared as per standard procedure[24] and maintained in the herbarium of the Institute for further reference. Fresh material was used for anatomical studies whereas shade dried material was powdered in electric grinder for physico-chemical study, phytochemical investigation and development of HPTLC fingerprint profile.

Macroscopic study

Macroscopic or organoleptic characters (like appearance, colour, odour, and taste) of *C. procera* root and stem were evaluated.

Microscopic study

Fresh root and stem sections were cut by free hand sectioning and numerous sections were examined Microscopically[25]. Photographs of the microscopical sections were captured with the help of Olympus Trinocular Research Microscope CX- 21l with Digi-eye camera using Caliper plus version 4.2 software.

Powder microscopic study

The dried root and stem were powdered separately and completely passed through 355 μm IS Sieve (old sieve number 44) and not less than 50% passel on through 180 μm IS Sieve (old sieve number 85). About 2 g of root and stem powder separately washed thoroughly with potable water, poured out the water without loss of material. Mounted small portions in glycerin were used to study all characters of the *C. procera* root and stem. Small quantity of samples was cleared by heating with chloral hydrate solution. It was properly washed and mounted with glycerin. Another small quantity of samples stained with sudan red solution and mounted with glycerin. All mounted slides were seen under at 10x and 40x magnification of Trinocular Research Microscope[26-27].

Physico-chemical parameters

Physico-chemical parameters such as moisture content (loss on drying at 105^{o}C), water-soluble extractive value, hexane soluble extractive; alcohol soluble extractive value, total ash value and acid insoluble ash value were calculated separately for root and stem powder[28,29].

Preliminary phyto-chemical investigation

Preliminary phyto-chemical tests were carried out on ethanolic and water extracts of *C. procera* root and stem for the presence\absence of phyto-constituents like alkaloids, flavonoids, tannins, resins, carbohydrates, proteins, and saponins[30-32].

HPTLC fingerprint profile

For HPTLC, 5 g of each root and stem powdered samples were extracted overnight with 100 mL of ethanol, then filtered, and concentrated. It was applied by spotting extracted sample on pre-coated silica-gel aluminium plate 60 F_{254} (5x10 cm with 0.2 mm layer thickness Merk Germany) using Camag Linomat -5 sample applicator and a 100 μL Hamilton syringe. The samples, in the form of bands of length 6 mm, were spotted 15 mm from the bottom, 15 mm from left margin the plate and 10 mm part. Plates were developed using mobile phase consisting of toluene: ethyl acetate: formic acid (7.0: 2.5: 0.5 v/v). Linear ascending development was carried out in 10x10 cm twin through glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 30 minutes at room temperature.

The length of chromatogram run was 8 cm. 20 mL of the mobile phase. Subsequent to the development, Thin Layer Chromatography plates were dried with the help of a Hot Air Oven. The peak area was recorded with the Camera photo documentation system Camag Reprostar 3. Visualization of spot was made before and after derivatization (with anisaldehyde - sulphuric acid reagent) at 366 nm, after derivatization at 366 nm, and UV light with Win cat software and R_f values noted[33-36].

Microbiological limit tests

Microbial limit tests are employed for the estimation of the number of viable aerobic micro-organisms present and for detecting the presence of designated microbial species in pharmaceutical substances. Following tests were carried out as per[37,38,39] to determine the microbial load in three samples of *C. procera* root and stem powder. Enumeration of *Staphylococcus aureus*/g, enumeration of *Salmonella sp.*/g, enumeration of *Pseudomonas aeruginosa*/g, enumeration of *Escherichia coli*, determination of total microbial count (TBC), determination of yeast & mould. The microbiological tests were determined using specified agar media and enrichment media from Himedia, Pvt. Ltd. Mumbai.

Results

Macroscopic characters

C. procera root- Cylindrical varies in length, 1 to 3 cm in diameter, surface, smooth longitudinally striated, lateral, tortuous or scars left by them shows cracks and transverse lenticels at places, fracture short in the bark and fibrous in the wood; fractured surface shows central wide yellowish cream wood and peripheral creamish narrow bark, externally pale yellowish brown in colour. odour not characteristic; taste is bitter and acrid (Fig. 1a-b).

C. procera stem- Cylindrical rough shows longitudinally running anastomosing, broad, elevated ridges of the cork embedded with a furrow and fine transverse striations emerging from it. Fracture short, externally yellowish grey in colour, taste- slightly bitter, astringent odour- odourless (Fig. 1c).

Microscopic characters

TS of the root is circular in outline with irregular outer narrow margin shows an outer stratified cork consisting of 2 to 4 rows of tangentially running narrow compressed lignified cells alternating with a row of big sized rectangular cells, interrupted at places with lenticels making the margin uneven, underneath this lie tangentially running 3 to 4 rows of pale brown coloured cells of phelloderm, followed by a wider parenchymatous zone of cortex. Phloem tissue lying underneath this almost of the consisting of irregularly running uni-to biseriate medullary rays slightly getting wider towards the peripheral region, laticiferous vessels, rosette crystals of calcium oxalate, simple starch grains and rarely isolated lignified fibres traverse throughout the parenchymatous cells of the cortex and phloem. Cambium is distinct. Xylem consists of isolated or groups of vessels and tracheids associated with thin-walled fibres and pitted parenchyma; medullary rays uni- to bisereates (Fig. 2a-e).

TS of the stem is circular in outline and shows outer wide spongy sinuously running cork, almost occupying one third the area of the section, consisting of irregularly arranged lignified cells of various sizes, shapes and thickness, embedded with few cluster crystals of calcium oxalate, a narrow band of tangentially running pale yellowish brown cells of

Fig. 1 — a) *C. procera* plant; b) Dried pieces of root; and c) Dried pieces of stem.

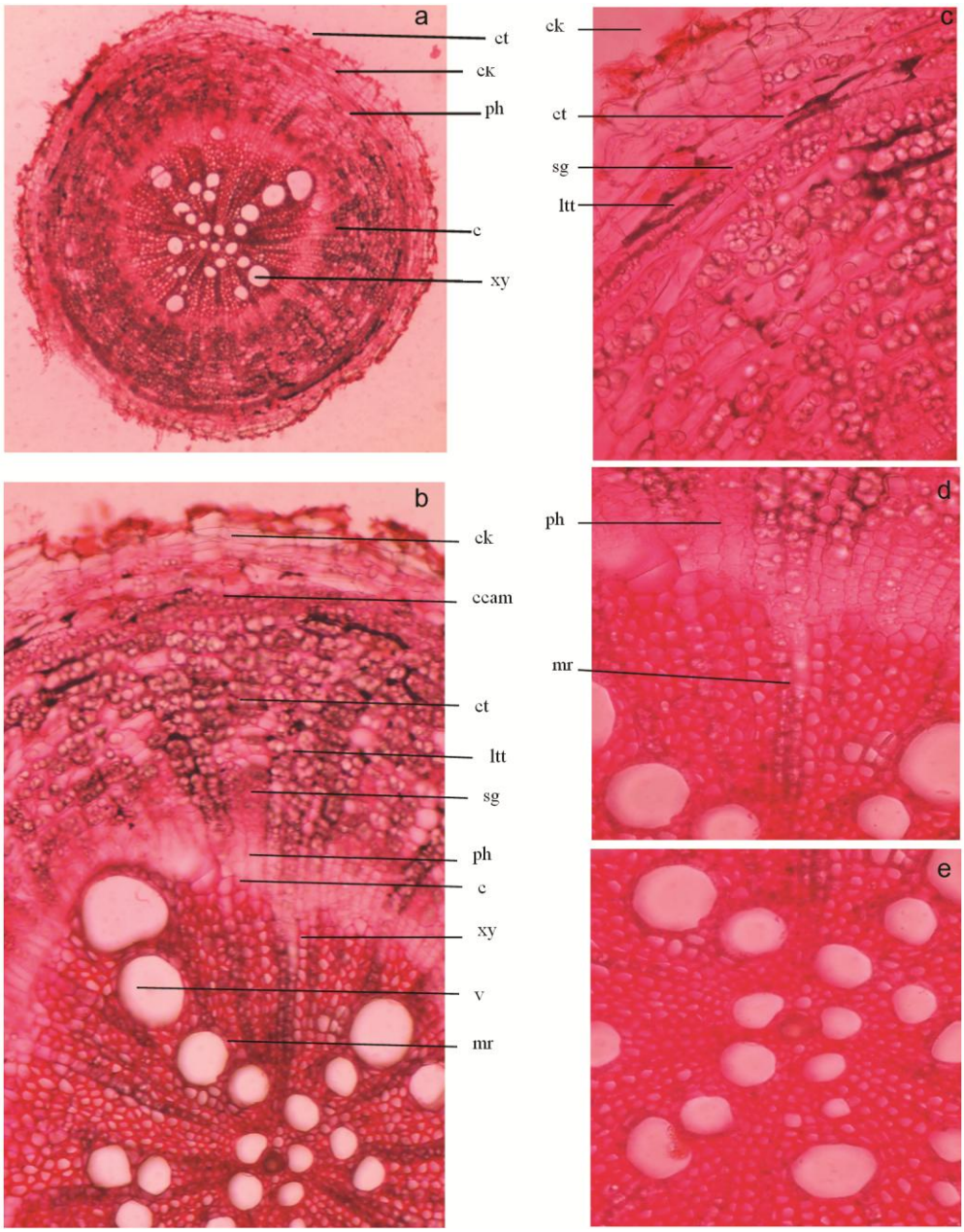

Fig. 2 — a) TS of root (diagrammatic); b) TS of root (detailed); c) Cork & cortex (enlarged); d) Cortex & phloem (enlarged); and e) wood region (enlarged).
Abbreviations: *c, cambium; ccam, cork cambium; ck, cork; ct, cortex; ltt, laticiferous tubes; mr, medullary rays; ph, phloem; sg, starch grains; v, vessel; xy,* xylem.

phelloderm being located underneath it followed by a wider parenchymatous zone of cortex, embedded with laticiferous vessels and sclereids. A narrow pericyclic zone lying underneath is also embedded with groups of sclereids and laticiferous vessels but they are very small in size. Phloem is a wider parenchymatous zone devoid of fibres or lignified elements consisting of uni-to biseriate medullary rays (Fig. 3a-d).

Powder microscopic characters

C. procera root powder is brownish white in colour, taste-slightly bitter, astringent and odour not characteristics. Under microscope powder shows cork cells in surface view, cork cells in sectional view, parenchymatous cells embedded with starch grains and rosette crystals of calcium oxalate, simple starch grains, prismatic crystals of calcium oxalate, pitted

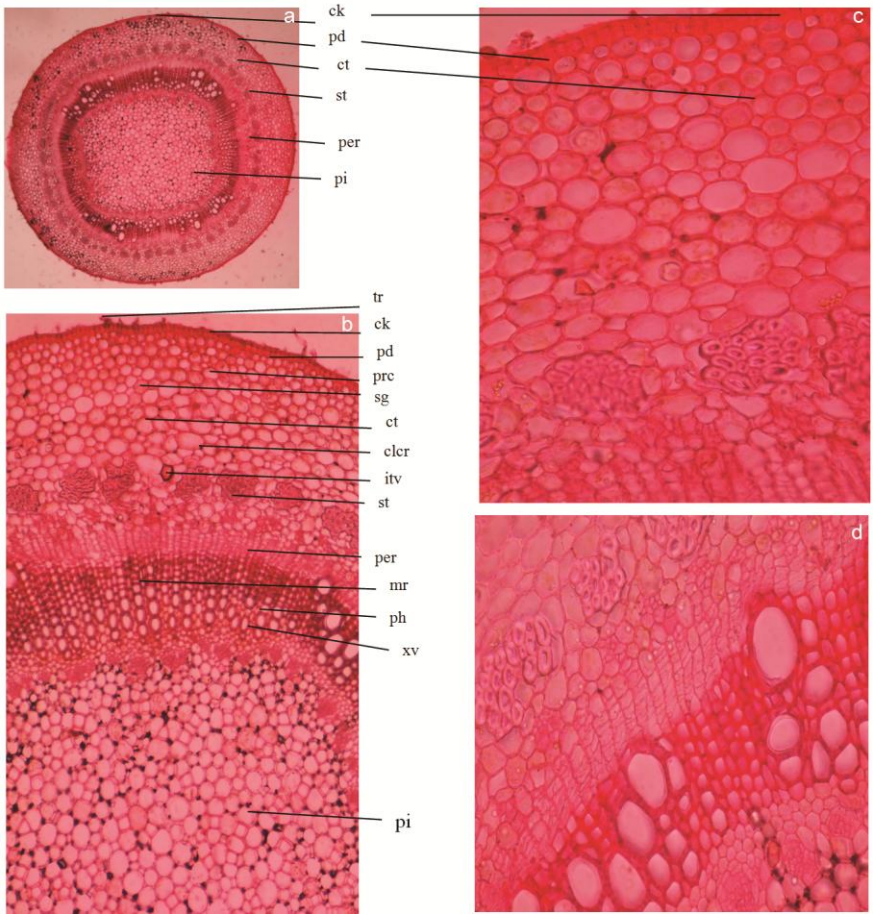

Fig. 3 — a) TS of stem (diagrammatic); b) TS of stem (detailed); c); Cork & cortex (enlarged); and d) Wood region (enlarged). Abbreviations: *ck, cork; ct, cortex; clcr, cluster crystal of calcium oxalate; ltv, laticiferous vessel; pd, phelloderm; per, pericycle; pi, pith; prc, prismatic crystals of calcium oxalate; st, stone cells; mr, medullary rays; tr, trichomes; ph, phloem; sg, starch grains; v, vessel; xy, xylem.*

vessels & tracheids, radially cut medullary rays crossing the xylem or phloem fibres, fragments of laticiferous vessels and pitted fibres (Fig. 4).

Stem powder colour is yellowish brown or greenish grey, taste-astringent and odour not characteristics. Under microscope, powder shows cork cells in surface view, stone cells and pitted sclereids, cork cells in surface view, fragments of thin-walled long xylem fibres or their fragments with oblique pits, Rosette crystals of calcium oxalate, parenchymatous cells filled with starch grains and crystals, fragments of laticiferous vessels (Fig. 5).

Physico-chemical analysis

The physico-chemical parameters such as extractive values are useful for the determination of exhausted or adulterated drug; ash values of the drug gave an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Three samples of each *C. procera* root and stem powder physico-chemical results are given in Table 1.

Heavy metals tests

C. procera root and stem powder three sample each heavy metal elements (Pb, Cd, As, and Hg) test were performed and found under limits as per guideline WHO and results are given in Table 2.

Microbiological limit tests

Microbiological profile of the *C. procera* root and stem powder was found satisfactory under limits as per guideline WHO. Results are given in Table 3.

Preliminary phytochemical investigation

In *C. procera* root, qualitative phyto-constituents were screened in the extracts taken in water and ethyl alcohol. The screening exhibited presence of alkaloids, flavonoids, tannin, protein and saponin.

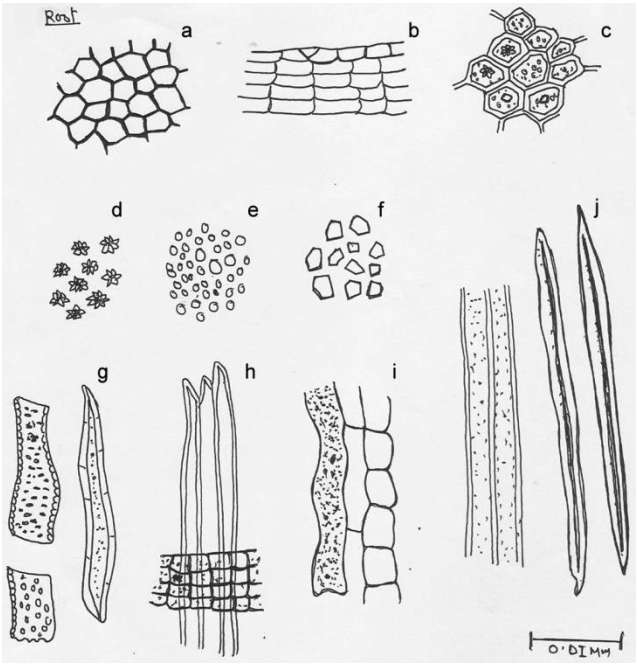

Fig. 4 — Powder microscopy of root, a) Cork cells in surface view; b) Cork cells in sectional view; c) Parenchymatous cells embedded with starch grains and rosette crystals of calcium oxalate; d) Rosette crystals of calcium oxalate; e) Simple starch grains; f) Prismatic crystals of calcium oxalate; g) pitted vessels & tracheids; h) Radially cut medullary rays crossing the xylem or phloem fibres; i) Fragments of laticiferous vessels; and j) Pitted fibres.

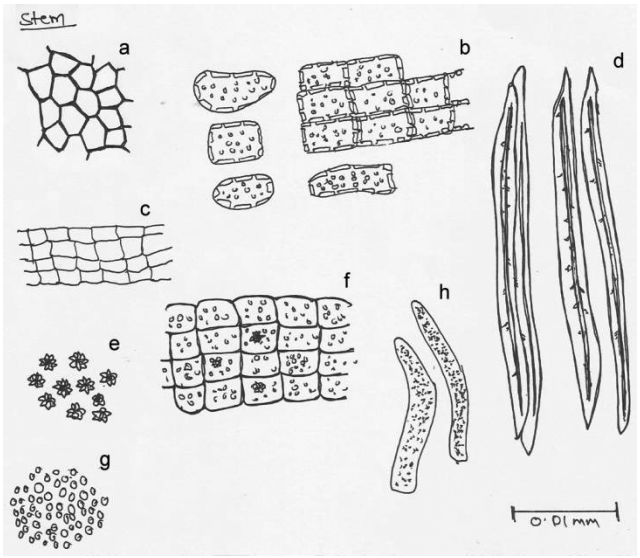

Fig. 5 — Powder microscopy of stem, a) Cork cells in surface view; b) Stone cells and pitted sclereids in various shape and size; c) Cork cells in surface view; d) fragments of thin walled long xylem fibres or their fragments with oblique pits; e) Rosette crystals of calcium oxalate; f) Parenchymatous cells filled with starch grains and crystals; g) Starch grains; and h) Fragments of laticiferous vessels.

Table 1 — Physicochemical analysis of *C. procera*

Name of samples	LOD (% w/w)	ASE (% w/w)	HSE (% w/w)	WSE (% w/w)	Total ash (% w/w)	Acid insoluble ash (% w/w)
Root	5.55	12.20	5.52	25.29	9.63	3.48
Stem	6.74	14.55	4.44	31.66	9.36	2.41

Table 2 — Determination of heavy metal of *C. procera*

Name of metals	Root	Stem	WHO Limit
Lead (Pb)	6.7949 ppm	5.3412 ppm	10 ppm
Cadmium (Cd)	0.0624 ppm	0.0126 ppm	0.3 ppm
Arsenic (As)	7.2396 ppb	4.1564 ppb	03 ppm
Mercury (Hg)	5.4178 ppb	3.5421 ppb	01 ppm

Table 3 — Microbiological limit test in *C. procera*

Name of metals	Root	Stem	WHO Limit
Staphylococcus aureus/g	Absent	Absent	Absent
Salmonella sp./g	Absent	Absent	Absent
Pseudomonas aeruginosa/g	Absent	Absent	Absent
E. coli	Absent	Absent	Absent
Total microbial plate count (TPC)	245 cfu/g	255 cfu/g	10^5 cfu/g
Total Yeast and mould	40 cfu/g	50 cfu/g	10^3 cfu/g

In *C. procera* stem, qualitative phyto-constituents were screened in the extracts taken in water and ethyl alcohol. The screening exhibited presence of alkaloids, resin, flavonoids, tannin, protein, and saponin.

HPTLC fingerprint profile

HPTLC study of the ethanolic extract three spots of the *C. procera* (root and stem) separately samples extract applied in precoated TLC plate. Applied 10 µL of the test solution as 8 mm bands and develop the plate in a solvent system toluene:ethyl acetate:formic acid (7.0:2.5:0.5 v/v) to a distance of 8 cm. Dry the developed both plates in room temperature and examined. Derivatized the plate using anisaldehyde–sulphuric acid reagent and heating at 105°C till the bands are clearly visible. Major spots R_f values and colour were recorded at 366 nm before derivatization, and after derivatization at 366 nm of *C. procera* stem and root. Major spots Rf values and colour were recorded at 366 nm before derivatization, after derivatization at 366 nm and UV light. Chromatogram profile and R_f values are given (Fig. 6 & Table 4).

Discussion

Whole plant of *C. procera* is used to cure various disease such as diarrhea, jaundice, fever, teeth ache, dysentery[40] etc. and have antioxidant, antimicrobial, antitumor, inflammatory, analgesic, hepatoprotective activities[41]. All parts of the plant showed pharmacological properties due to presence of several phyto-chemicals like alkaloids, saponins, tannins,

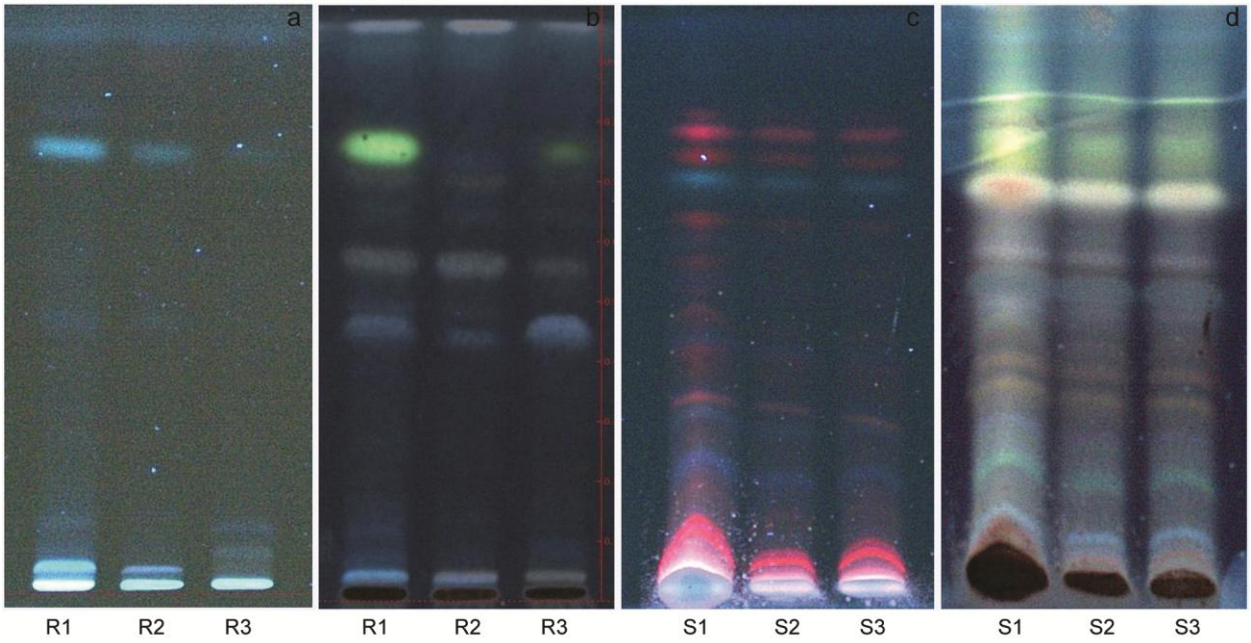

Fig. 6 — HPTLC fingerprints profile. a) at 366 nm before derivatization (root); b) at 366 nm after derivatization (root); c) at 366 nm before derivatization (stem); and d) at 366 nm after derivatization (stem).

Table 4 — R_f values of HPTLC fingerprints profile of *C. procera*

R_f value	Root		Stem	
	At 366 nm before derivatization	At 366 nm After derivatization	At 366 nm before derivatization	At 366 nm after derivatization
R_{f1}	0.04 (sky blue)	0.46 (blue)	0.08 (pink)	
R_{f2}	0.82 (sky blue)	0.54 (whitish blue)	0.20 (blue)	0.20 (sky blue)
R_{f3}	-	0.82 (yellow)	0.32 (pink)	0.32 (brown)
R_{f4}	-	0.90 (brown)	0.72 (blue)	0.50 (brown)
R_{f5}	-	-	0.80 (pink)	0.72 (white)
R_{f6}	-	-	0.82 (pink)	0.80 (light green)
R_{f7}	-	-	-	0.82 (light green)

flavonoids, glycoside, phenols, steroids, and bitter contents. The root bark is the major source of calotropoleanyl ester, proceroleanenol compounds[42,43]. While latex and leaves have major active compounds such as uzarigenin, calotropin, calotoxin, trypsin, calactin, voruscharin and caoutchouc[44,45]. The macroscopic, microscopic, and powder microscopic distinguished characters have been established to identify *C. procera* root and stem. The pharmacognostic and physicochemical parameters can be used for checking the adulteration and purity of this drug. HPTLC fingerprint profile helps in identification of various phytochemical constituents present in the crude drug thereby substantiating and authenticating of crude drug. The TLC profile also helps to identify the important phyto-constituents. The Aflatoxins were absent in the *C. procera* root and stem samples. Heavy metal elements are found below limits as per guidelines of WHO and microbial limits test of the *C. procera* (root and stem) were also found satisfactory. Total microbial plate count (TBC), Yeast & Moulds counts were reported less than the limit as per WHO standards and pathogenic bacteria i.e., *Staphylococcus aureus*, *Salmonella sp*., *Pseudomonas aeruginosa* and *Escherichia coli* were found to be absent in root and stem. All findings indicate that samples are genuine and free from adulterations. These findings could be helpful in identification and authentication of *C. procera* root and stem.

Conclusion

Various parts of *C. procera* plant are used to treat various ailments like *Pama* (Scabies), *Vicharchika* (Eczema), *Kasa* (Cough), *Mukhakrishnatwa* (Hyperpigmentation of the face), *Pleehodara* (Splenomegaly) and preparation of Ayurvedic as well

as traditional medicines like Arkalavana and Arkataila. Hence, there would be no exaggeration in concluding that this plant is accepted as most sacred for its high medicinal and spiritual values.

Conflict of interest

The authors state that they have no conflict of interest.

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