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Keerthana P
R & D Centre, Cholayil Private Limited, Ambattur, Chennai, Tamil Nadu, India

Babu K
R & D Centre, Cholayil Private Limited, Ambattur, Chennai, Tamil Nadu, India

Anoop Austin
R & D Centre, Cholayil Private Limited, Ambattur, Chennai, Tamil Nadu, India

Corresponding Author:
Babu K
R & D Centre, Cholayil Private Limited, Ambattur, Chennai, Tamil Nadu, India

Pharmacognostic and phytochemical evaluation of *Chrozophora rottleri* (Geiseler) A. Juss. ex Spreng.

Keerthana P, Babu K and Anoop Austin

Abstract
Chrozophora rottleri (Geiseler) A. Juss. ex Spreng. is an annual, monoecious, medicinal herb belongs to the spurge family Euphorbiaceae. The plant distributed throughout India, Myanmar, Thailand, Andaman Islands and Malesia, used for various ailments in traditional systems of medicine. The plant is reported to contain various phytochemicals and biological activities; however, there is lack of pharmacognostical study. Hence, the present study was undertaken and this paper provides detailed and distinctive anatomical characters for diagnosing the root, stem, leaf, petiole and phytochemical analysis of the afore mentioned drugs from their adulterants.

Keywords: *Chrozophora rottleri*, Euphorbiaceae, Pharmacognosy, Phytochemical analysis, medicinal plant

Introduction
Plants harbour a plethora of bioactive molecules which are being used in traditional medicine since time immemorial. The quest for novel herbal drugs against the modern age diseases is upsurging. In the recent times, the medicinal plants have caught the limelight because of their uses in treatments to even indelible diseases and for their safe utility, compared to synthetic counterparts which has allied side effects. So, the need of the hour is to look for such plants with active principles and evaluate their properties based on traditional knowledge which helps in further validation and development of potent drugs against various diseases.
However, a major obstacle that has hampered the acceptance of herbal medicines is the lack of documentation and stringent quality control. In all traditional systems, different botanical species are used under the same drug name and this is one of the major problems encountered. Further these are claimed to posses similar therapeutic efficacy and used by the physicians as the same drug and so they are termed as controversial drugs. In order to avoid adulteration of herbal drugs, it is extremely important to make an effort towards standardization of the plant material to be used as medicine. In such cases pharmacognostical studies help in identifying genuine plants used, by opting standardization of microscopical and phytochemical methods. Owing to this, the present work was undertaken to study the pharmacognostic characteristics and physicochemical evaluation of aerial parts and root of *Chrozophora rottleri.*
Chrozophora rottleri well known as Suryavarti in Sanskrit belongs to the family Euphorbiaceae. It is an annual, fulvous-tomentose, monoecious herb, up to 75 cm tall with ascending branches trichotomously forked from base. Leaves - alternate, broadly ovate, 2-6 × 1.5-7 cm, (sub) coriaceous, 3-nerved from base; vein deeply impressed, petiole up to 8 cm, biglandular at the base of lamina. Inflorescence - terminal raceme, tomentose up to 3 cm; ♂ flowers (sub) sessile, crowded in upper axils, stamens yellow; ♀ flowers pedicellate below. Tepals 5 +5, inner petaloid. Stamens 12-15, in 2 series, connate, inner long. Ovary 3 locular; ovules 3, axile; styles 3, red, bifurcate. Disc-glands 5. Capsule depressed, 3-lobed, stellate-tomentose, 0.8 cm across; seeds globose (Figure – 1:1-5). Distributed in plains from the coast, in wastelands in India and Myanmar, Thailand, Andaman Islands and Malesia [1].
C. rottleri is acrid, poisonous, emetic, cathartic, drastic corrosive and traditionally used for the treatment of various diseases. In Sudan, people use stems or whole plant as powder and apply it to wounds to improve healing. The plant is also used in Saudi Arabia and India to treat Jaundice and purifying blood. An infusion of seeds and leaves is taken as a laxative in Ethiopia and Senegal. The fruits yield a purplish blue dye, which is used to dye mats in East Africa. The fruit juice is given in cases of cough and cold in Nepal. The leaves of *C. rottleri* are very much beneficial in treatment of skin diseases and are also used as depurative agent [2]. Aqueous extract of leaf has a significant anti-helminthic property against *Pheritima posthuma* (Indian Earth worm) [3] and possess phytotoxic activity on rice, wheat and mustard. Suparna and Tapaswi [4] reported that the leaf extracts of *C. rottleri* exhibited higher inhibition of shoot,

root and radial elongation than the stem and root. Juice of the fruit is given in cases of cough and cold in countries like Nepal and leaf is used as purifying agent and seed is used as laxative [2, 5]. The seeds are used as cathartic and have purgative properties [6].

The major phytochemicals of *C. rottleri* include alkaloids, carbohydrate, glycosides, tannins, steroids, flavonoids and saponins, rutin, acacetin 7- orutinoside and apigenin 7-o-b-d-[6-(3,4- dihydroxybenzoyl)] –glucopyranoside [7]. The oil from the seed of *C. rottleri* was reported to be rich in linoleate, while the leaves and root contain xanthone glycosides and chromone glycoside. Maharaj and Prabhakaran [8] and Mothana *et al.,* [9] reported that *C. rottleri* had adverse allelopathic effects on the germination and growth of rice seedlings. Hydroalcoholic, methanolic, ethyl acetate and hexane extracts of *C. rottleri* were found to possess concentration dependent scavenging activity on DPPH radical, hydroxyl radicals and superoxide generated by photoreduction of riboflavin. Methanolic extract of *C. rottleri* showed the maximum inhibition Gram positive and Gram negative bacteria [10].

Materials and methods

Anatomical studies

Fresh whole plant (40 - 60 cm height) (Figure – 1:1) was collected from Redhills, near Chennai and authenticated using regional flora [1, 11]. Root, stem, petiole and leaf samples were cut in to small pieces and fixed immediately in Formalin-Acetic-Alcohol for 24 h. After fixation they were washed thoroughly in distilled water, dehydrated, embedded in paraffin wax after infiltration and sectioned using rotary microtome to the thickness of 8-12 µm [12]. Sections were stained with Toluidine blue and photographed. Leaf epidermal appendages and stomata were stained with Saffranin and photographed.

Phytochemical analysis

For the phytochemical analysis, aerial part and root were separated and shade dried for a week and powdered. Powdered samples were subjected to physico-chemical analysis, such as the percentage of water soluble extractive, alcohol soluble extractive, total ash, acid insoluble ash [13] and extraction yield in different solvents using soxhlet apparatus.

Thin Layer Chromatographic (TLC) Analysis

Five grams of powdered sample was refluxed with 50 mL of methanol in a water-bath at 60 °C for 30 min, consecutively 3 times, and then concentrated and dried. The final extract was re-dissolved in methanol and used for the TLC analysis. Precoated Silica Gel F^{254} (Merck) plate was used for stationary phase and Toluene: Ethylacetate (9.3:0.7) as mobile phase. After development the plate was dipped in 1% vanillin sulphuric acid and heated at 105 °C in hot air oven for 5 min. to develop the colour and the spots were recorded.

Observations

Root anatomy (Figure – 2:1-4)

A cross section of the root about 6 to 7 mm diameter of thickness shows outside a clear periderm with 6 to 8 compact layers with spongy and rectangular shaped cells in a compressed manner. Following to the periderm, cortex region present with dilated phloem rays which shows flame like structure. Phloem fibres present in the dilated region and phloem cells are compactly arranged. Druses types of calcium oxalate crystals present in the parenchymatous cells of cortex. Xylem region circular in shape composed of xylem elements. Vessel elements are circular in shape and arranged in long radials. Larger vessels toward inner and small vessels towards outer region. Xylem rays uniseriate.

Stem anatomy (Figure – 3:1-5)

A cross section of stem about 5 to 6 mm diameter of thickness shows circular in outline with single layer of epidermis consisting of small cubical cells. Following to the epidermis, 8 to 9 layers of collenchymatous cells arranged in the cortex. Phloem region consist of phloem elements and phloem fibres. Xylem region is broad, vessel elements mostly polygonal shape in outline and arranged in long radial multiples. Xylem fibres uniseriate. Ground tissue consists of circular or oval shaped parenchyma cells. Druses types of calcium oxalate crystals present in both cortex and ground tissues.

Leaf anatomy (Figure – 4:1-6; Figure – 5:1-3)

Petiole (Figure – 4: 5-6)

The transverse section of the petiole shows almost circular in outline and a small grove in the adaxial side. The epidermis is single layer, small cubical shaped cells with abundant stellate trichomes, followed by 3-4 layers of collenchymatous cells. A large, single vascular bundle present in the middle and in griddle shape. Phloem covered the xylem. The center region of vascular bundle is filled with ground tissue made up of large parenchymatous cells. Druses types of calcium oxalate crystals present in both cortex and ground tissues (Figure 4:6).

Lamina (Figure – 4: 1-4; Figure – 5: 1-3)

The midrib has small convex in adaxial surface, a single layer epidermis with cuboid shape cells followed by 3-4 layers of collenchyma cells (Figure 4:1-2). A bowl shaped large vascular bundle is in dorsal side and small circular shaped vascular bundle in ventral side consisting of xylem and phloem. The centre ground tissue is made up of parenchymatous cells. In lamina, the upper epidermis has large, rectangle shaped cells and the lower epidermis has small, cuboid shaped cells. The upper epidermis has cuticle. Next to the upper epidermis, narrowly cylindrical palisade parenchyma with air space and spherical spongy parenchyma cells are compactly arranged (Figure 4:3-4). Vascular bundles are small and of different sizes. The stomata anamocytic type and amphistomatic, distribution is lesser in upper epidermis (Figure 5:3). Stellate trichomes with 8-12 branchlets abundantly covered the entire leaf (Figure 5:1-2). Druses types of calcium oxalate crystals present in both midrib and lamina (Figure 4:2, 4).

Phytochemical analysis

The results of physico-chemical analysis, extraction yield in methanol, Chloroform, Hexane and TLC fingerprint profiles are presented in Table – 1 & 2.

Table 1: Results of physico-chemical analysis and extraction yield

Sample	Water soluble extractives (%)	Alcohol soluble extractives (%)	Total Ash (%)	Acid Insoluble Ash (%)	Extraction yield (%)		
					Methanol	Chloroform	Hexane
Aerial part	13.8 ± 0.10	4.79 ± 0.28	9.76 ± 0.36	1.58 ± 0.15	8.10 ± 0.25	2.77 ± 0.10	1.54 ± 0.05
Root	6.74 ± 0.30	1.79 ± 0.16	5.47 ± 0.50	1.30 ± 0.42	4.53 ± 0.13	0.91 ± 0.01	0.52 ± 0.11

Values from triplicate (Mean ± SD)

Table 2: Thin Layer Chromatographic (TLC) analysis (Figure – 6)

Visible Light (After spray)	
Rf – values	**Colour of the spot**
0.12	Violet
0.20	Violet
0.28	Violet
0.40	Light green
0.41	Violet
0.43	Dark green
0.48	Violet
0.52	Violet
0.62	Violet
0.64	Yellow
0.82	Violet
0.89	Violet

Fig 1: Morphology of *Chrozophora rottleri*
Fig 1:1: Habit
Fig 1:2: Root
Fig 1:3: Leaf adaxial side
Fig 1:4: Leaf abaxial side
Fig 1:5: Inflorescence with male & female flowers

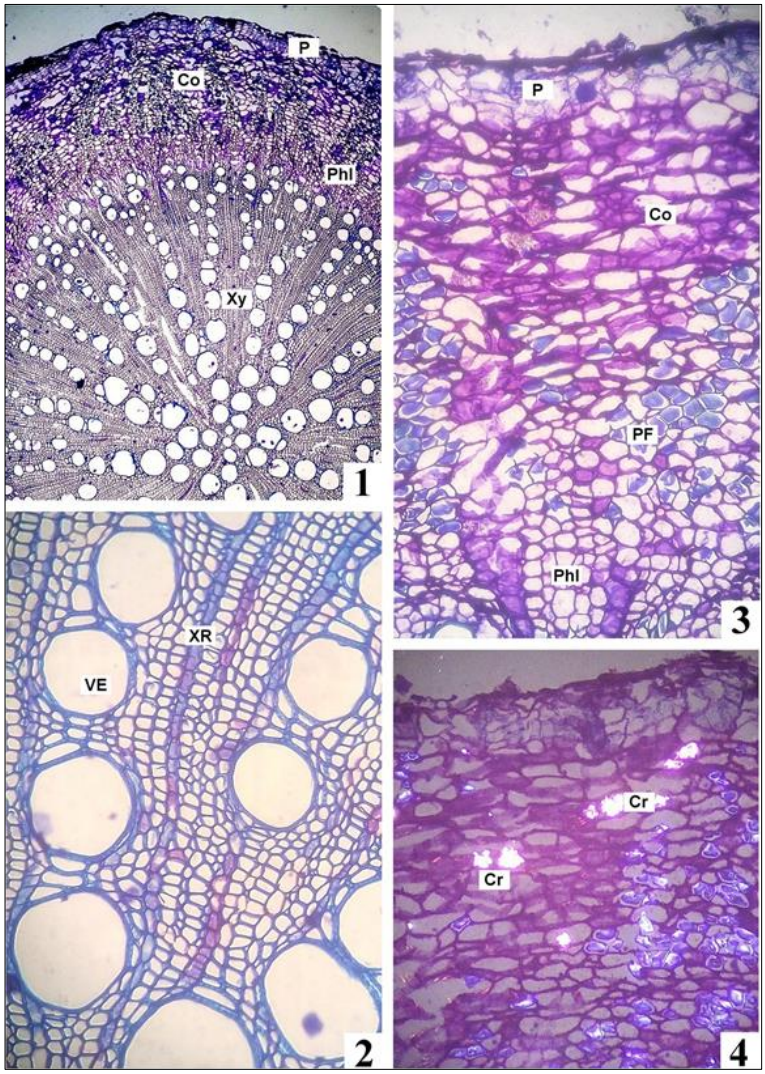


Fig 2: Root anatomy of *Chrozophora rottleri*
Fig 2:1: Cross section of root
Fig 2:2: Xylem region
Fig 2:3: Cortex & secondary phloem region
Fig 2:4: Cortex & secondary phloem region (under polarized light) (Co – Cortex; Cr – Crystals; P – Periderm; Phl – Phloem; PF – Phloem fibres; XR – Xylem ray; Xy – Xylem; VE – Vessel elements)

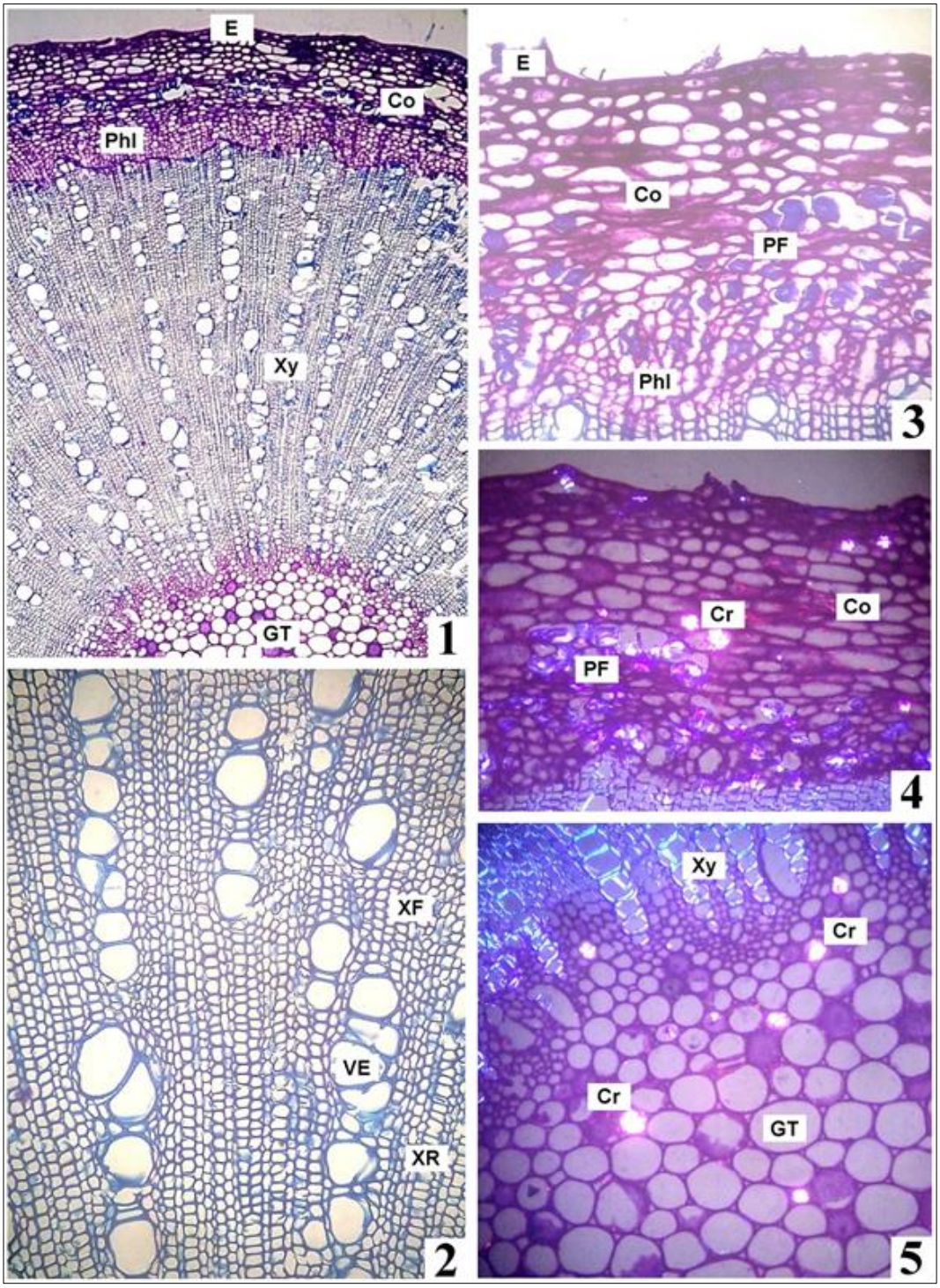


Fig 3: Stem anatomy of *Chrozophora rottleri*
Fig 3:1: Cross section of stem
Fig 3:2: Xylem region
Fig 3:3: Cortex & secondary phloem region
Fig 3:4: Cortex & secondary phloem region (under polarized light)
Fig 3:5: Pith region (under polarized light) (Co – Cortex; Cr – Crystals; GT – Ground tissue; P – Periderm; Phl – Phloem; PF – Phloem fibres; XF – Xylem fibre; XR – Xylem ray; Xy – Xylem; VE – Vessel elements)

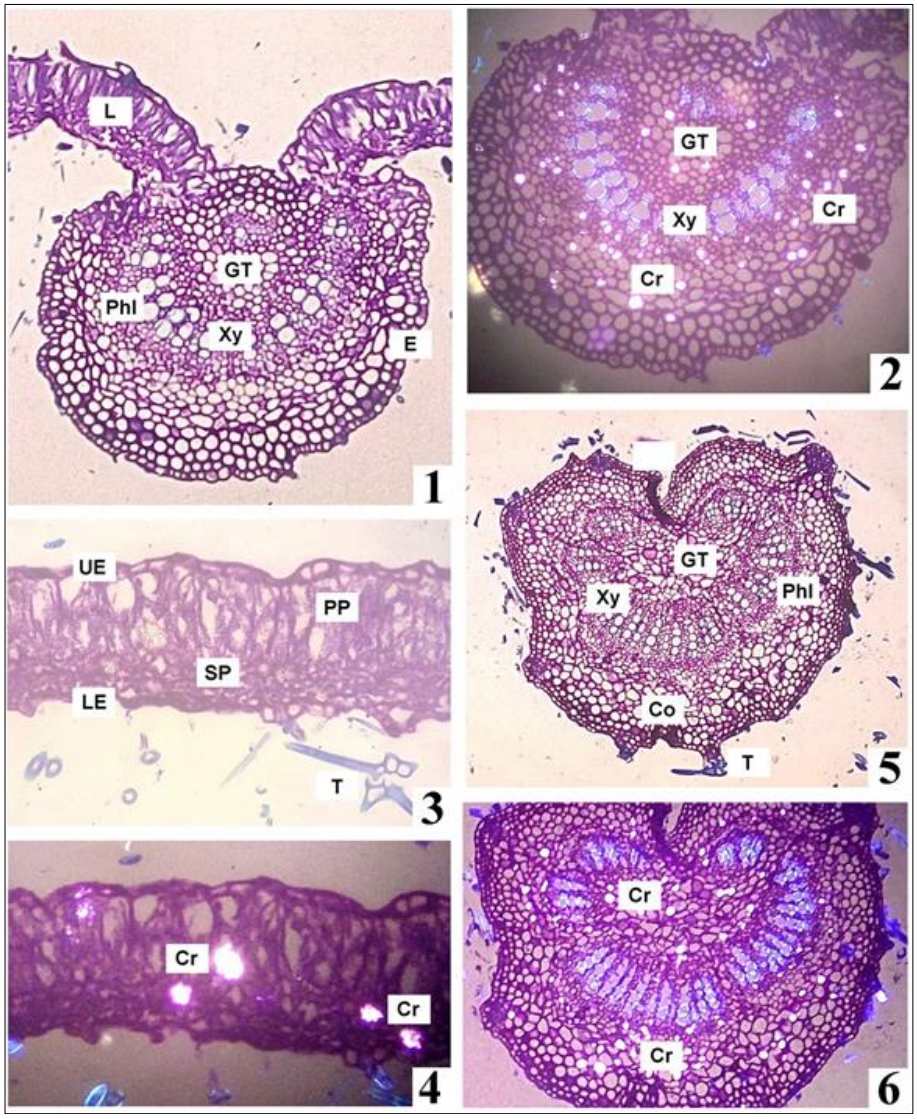
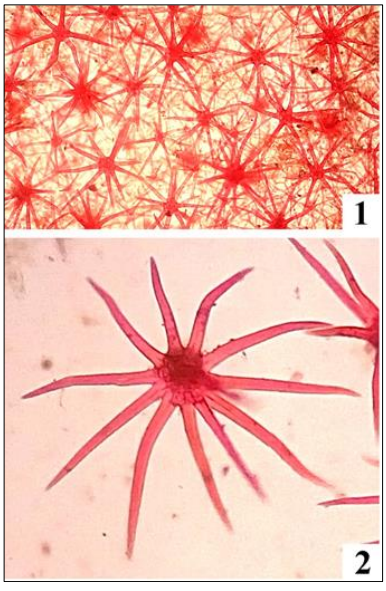


Fig 4: Leaf anatomy of *Chrozophora rottleri*
Fig 4:1: Cross section of midrib
Fig 4:2: Cross section of midrib (under polarized light)
Fig 4:3: Cross section of lamina
Fig 4:4: Cross section of lamina (under polarized light)
Fig 4:5: Cross section of petiole
Fig 4:6: Cross section of petiole (under polarized light) (Co – Cortex; Cr – Crystals; E – Epidermis; GT – Ground tissue; L – Lamina; LE – Lower epidermis; Phl – Phloem; PP – Palisade parenchyma; SP – Spongy parenchyma; T – Trichome; UE – Upper epidermis; Xy – Xylem)



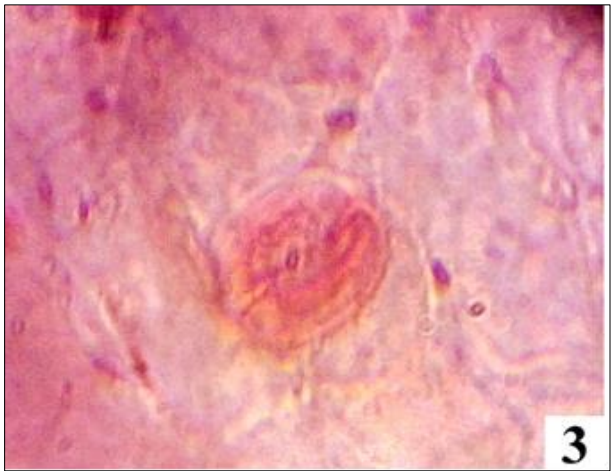


Fig 5: Leaf anatomy of *Chrozophora rottleri*
Fig 5:1: Stellate trichomes
Fig 5:2: Stellate trichome (enlarged)
Fig 5:3: Stomata

Fig 6: TLC profile of *Chrozophora rottleri*

Discussion and conclusion

Chrozophora rottleri, a medicinal weed found in the plains throughout India, used for various ailments in traditional systems of medicine. The plant reported to contain various phytochemicals such as alkaloids, carbohydrates, glycosides, tannins, steroids, flavonoids and saponins. However, there is no detailed pharmacognostical study on whole plant to help in the proper identification. Hence, the present study was undertaken with the aim to provide key diagnostic characters for identification. The following anatomical and phytochemical features are key diagnostic characters.

Whole plant: covered with stellate trichomes with 8-12 branchlets and druses type of calcium oxalate crystals distributed throughout the plant.

Root: vessel elements circular, arranged in long radials, larger vessels toward inner and small vessels towards outer region, uniseriate xylem rays.

Stem: polygonal shaped vessel elements, arranged in long radial multiples, xylem fibres uniseriate.

Leaf: stomata anamocytic and amphistomatic. TLC profile shows specific spots with Rf values.

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