International Journal of Pharmacy and Analytical Research (IJPAR)

IJPAR |Vol.13 | Issue 4 | Oct - Dec -2024

www.ijpar.com

ISSN: 2320-2831

DOI : https://doi.org/10.61096/ijpar.v13.iss4.2024.888-901

Research

A Holistic Approach to the Pharmacognostic and Proximate Analysis of *Cleome rutidosperma* DC Leaves: Insights for Taxonomic Refinement

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Published on: 31 Dec 2024

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Abstract

The Hydro alcoholic extract of *Cleome rutidosperma* (HAECR) leaf was analyzed by gas chromatography-mass spectrometry (GC-MS). The anatomical, physicochemical, and pharmacognostic properties of the extract were analyzed. The results showed that the leaves of HAECR exhibit organoleptic and macroscopical characteristics, showing distinctive features that enhance its taxonomic profile. The powder microscopy of the leaf reveals key microscopic features, including epidermal cell density, stomatal density, palisade ratio, and vein structure. The fluorescence analysis of the powder under visible, short-wave, and long-wave UV light revealed distinct color reactions with various reagents. The physiochemical constants of leaf powder reveals several important quality parameters. The Thin Layer Chromatography (TLC) analysis revealed a diverse composition of bioactive compounds, evident from distinct retention factor (Rf) values, spot colors, and varied mobile phases. These findings establish a foundation for further exploration of phytochemicals in medicinal plants, which are known for their therapeutic potential, reinforcing the value of TLC as a tool for the identification and characterizing bioactive constituents.

Keywords: rutidosperma, Taxonomic Refinement

INTRODUCTION

Cleome rutidosperma, commonly known as Fringed Spider Flower, belongs to the *Cleomaceae* family. This family includes a diverse range of herbaceous plants and small shrubs, with around 300 species distributed across tropical and subtropical regions worldwide [1]. These plants are typically found in open, sunny locations, often thriving in disturbed soils and grasslands [2]. *Cleome rutidosperma* is native to West Africa but has naturalized widely throughout Southeast Asia, including countries such as India, Malaysia, and the Philippines [3]. It is a common weed in gardens, agricultural fields, and disturbed areas, where it can grow easily in warm, moist climates. Medicinally, *Cleome rutidosperma* is widely used in traditional medicine for its anti-inflammatory, analgesic, and antimicrobial properties. The plant has applications in treating conditions such as fever, earaches, diarrhea, rheumatism, and eye infections, and it is often used as a natural remedy for various

respiratory and digestive issues [4].

MATERIALS AND METHODS

Plant collection

The leaves of *Cleome rutidosperma* belongs to ***cleomaceae*** family were collected from a location of Ezhudesam village, Kanyakumari district in Tamil Nadu, India during the month of February 2024.

Authentication

Taxonomist

The plant sample was authenticated by Dr. D. Stephen, Department of Botany, The American College, Madurai.

Numerical Taxonomy

The genus Cleome from the family Cleomaceae includes tropical trees and shrubs native to regions like Southeast Asia, as well as countries in West Africa, including Nigeria, Ghana, and Cameroon. The two most well-known species, Cleome gynandra and Cleome viscosa, are cultivated worldwide for their edible fruits. Other, lesser-known species, such as Cleome houtteana, are primarily found in the tropical rainforests of Southeast Asia. The following are the four species of Cleome was selected for numerical taxonomy

- *Cleome rutidosperma*
- *Cleome gynandra*
- *Cleome viscosa*
- *Cleome houtteana*

Chart Of Similarity

The organoleptic and morphological typescripts of aerial parts of various species in genus

Cleome

Table 1: Chart of similarity for four species of *Cleome*

SPECIES CHARACTERS	*Cleome rutidosperma*	*Cleome gynandra*	*Cleome viscosa*	*Cleome houtteana*
Leaf arrangement	**1**	**1**	**1**	**1**
Petiole	**1**	**1**	**2**	**1**
Leaflets	**1**	**2**	**2**	**2**
Shape	**1**	**2**	**2**	**1**
Lamina ratio	**1**	**1**	**1**	**2**
Apex	**1**	**2**	**1**	**1**
Base	**1**	**2**	**2**	**2**
Margin type	**1**	**2**	**2**	**1**
Sinus type	**1**	**1**	**1**	**2**
Tooth shape	**1**	**1**	**1**	**1**

Leaf arrangement: alternate-1, not alternate-2
Petiole: swollen-1, not swollen-2
Leaflets:3 leaflets -1, not 3 leaflets-2
Shape: elliptical-1, not elliptical-2
Lamina ratio: 2:1-1, not 2:1-2
Apex: acute-1, not acute-2
Base: acute convex-1, not convex-2
Margin type: serrate-1, not serrate -2
Sinus type: round-1, not round -2
Tooth shape: absent-1

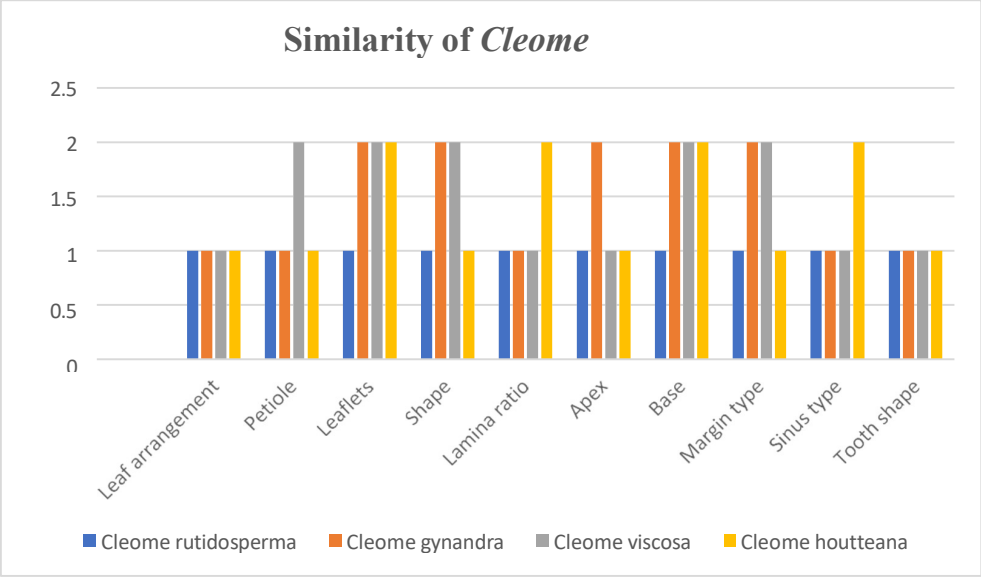


Fig 1: Chart Of Similarity Of *Cleome* Species

Chart Of Dissimilarity

Table 2: Chart of dissimilarity for four species of *Cleome*

Species	*Cleome rutidosperma*	*Cleome gynandra*	*Cleome viscosa*	*Cleome houtteana*
Cleome rutidosperma	1	0.65	0.65	0.65
Cleome gynandra	0.65	1	0.65	0.65
Cleome viscosa	0.65	0.65	1	0.65
Cleome houtteana	0.65	0.65	0.65	1

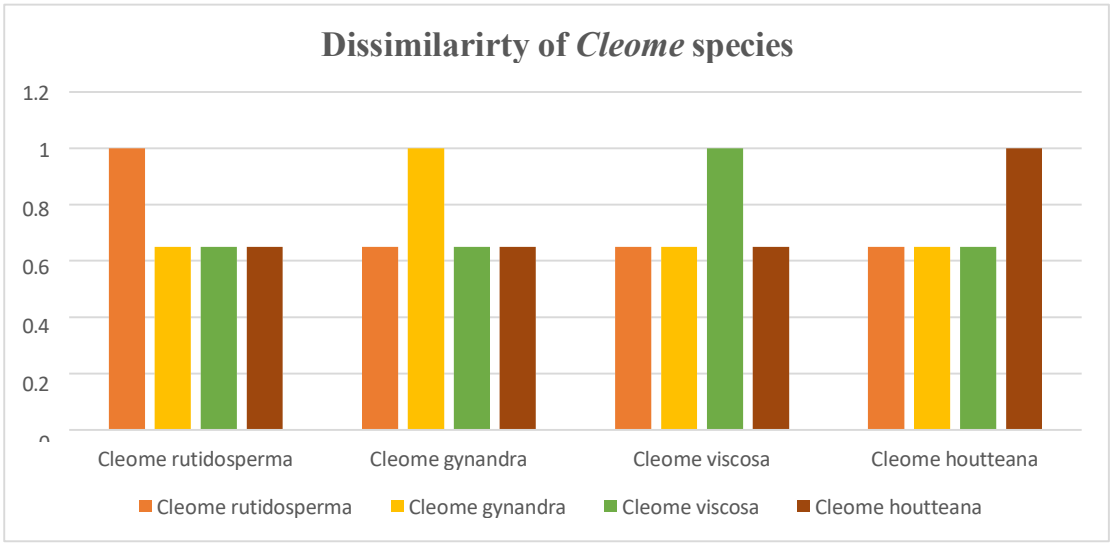


Fig 2: Dissimilarirty of *Cleome* species

SAMPLE MATCHING
COEFFICIENT SSM = NS / NS + ND X 100
=26/26+14 X 100
= 0.65 X 100

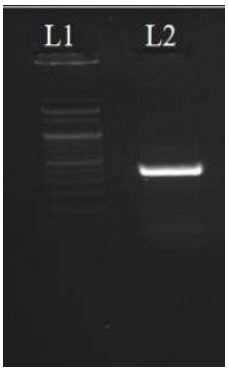
SSM= 65%
NS – Number of similar characters, ND- Number of dissimilar characters
The matching co-efficient of *Cleome rutidosperma* with respect to other species was found to be 65%

DNA Barcoding

The genomic DNA was isolated from the authenticated sample and its quality was assessed spectrophotometrically followed by agarose gel electrophoresis (Table 2). The genomic DNA concentration was 992.8 ng/µl. An absorbance (A260/A280) ratio of 2.09 indicated insignificant levels of contaminating proteins and polysaccharides. The PCR amplified product after electrophoresis was subjected to gel documentation together with a 100 bp DNA ladder (Fig.6).The sequence was obtained using ITS as the marker, the sequence was converted to barcode (Table 7) and the sequences were submitted to GenBank (Table 3).

Table 3: Quality check and quantification of DNA

Sample	Concentration in ng/µl	A260/280	A230/260
Cleome rutidosperma	992.8	2.09	1.85



L1: Ladder, L2: CR

Fig 3: Gel image of PCR amplified product

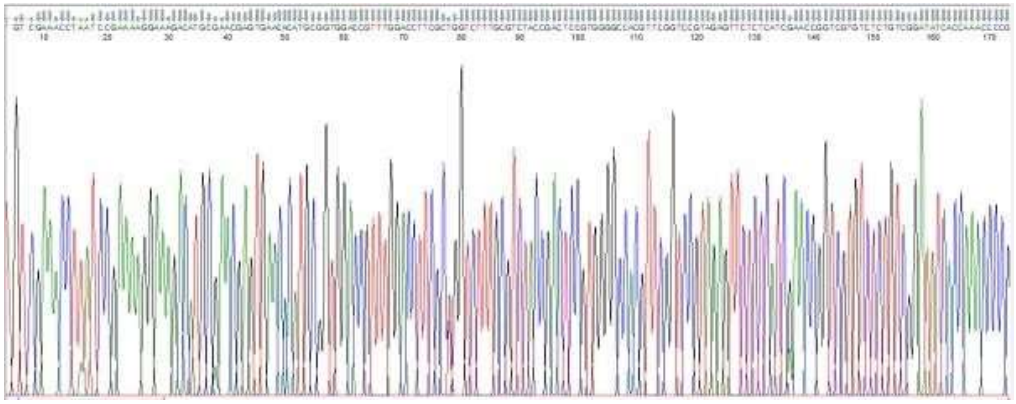
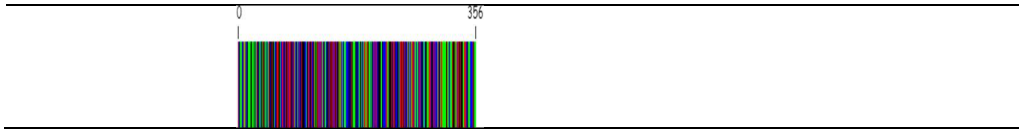


Fig 4: Chromatograms of *Sieruela rutidosperma*

Table 4: Sequence obtained

Sample code	Sequence with Barcode and BLAST Hit
>SCRIPCOGC R33	tcgaaacctaatccgaaaaggaaagacatgcgaacgagtgaacacatgcggtggaccgtttggaccttcg ctggtcttgcgtctaccgactccgtggggccacgttcggtccgtagagttctctcatcgaaccggtcgtgtct ctgtcggatatcaccaaaccccggcgtgaaaagcgccaaggaccattaattgaaacagctctctccgggg aagccccttaaggggtgcttgcccggacttgttgctgccatcactattctaacaactctcggcaacggaaa tttcggctctcccatcgattaaaaaagtaaccaaatgggatacttggtgggaatttccgaaacccctgaaa



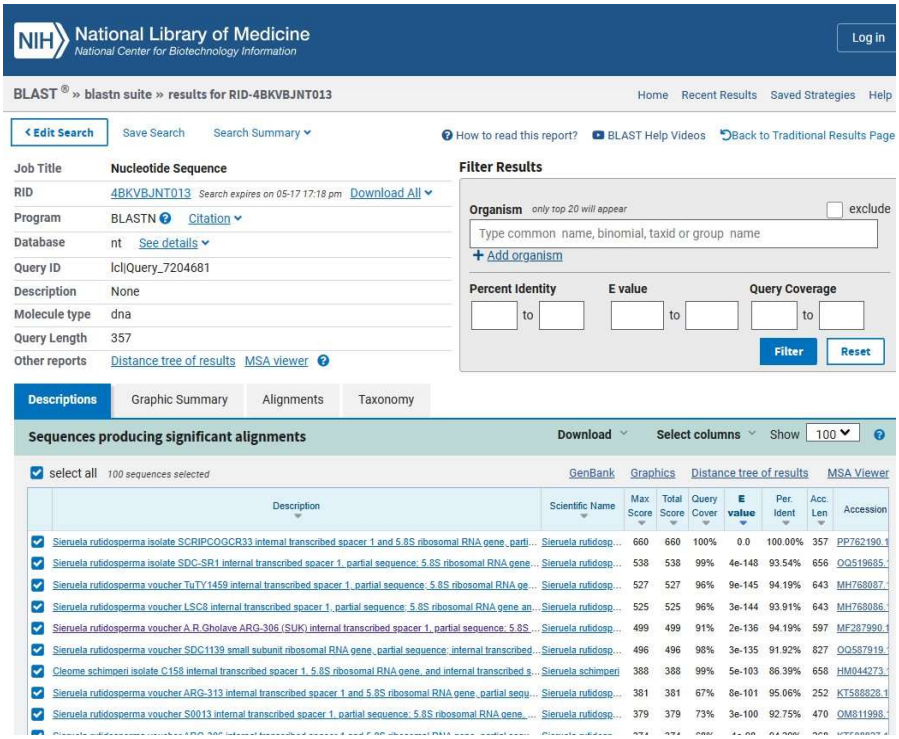


Fig 5. National library of medicine -CR

Table 5: Gene Bank submission details

Voucher no.	Specimen name	Submission Id	Accession no	Gene Bank Link
SCRIPCOGCR33	*Sieruela rutidosperma*	SUB14406323	PP762190	Under processing

Macroscopy Of *Cleome rutidosperma DC* Leaves

Plate 1: *Cleome rutidosperma* plant

Plate 2: Dorsal View Of Various Sized Leaves

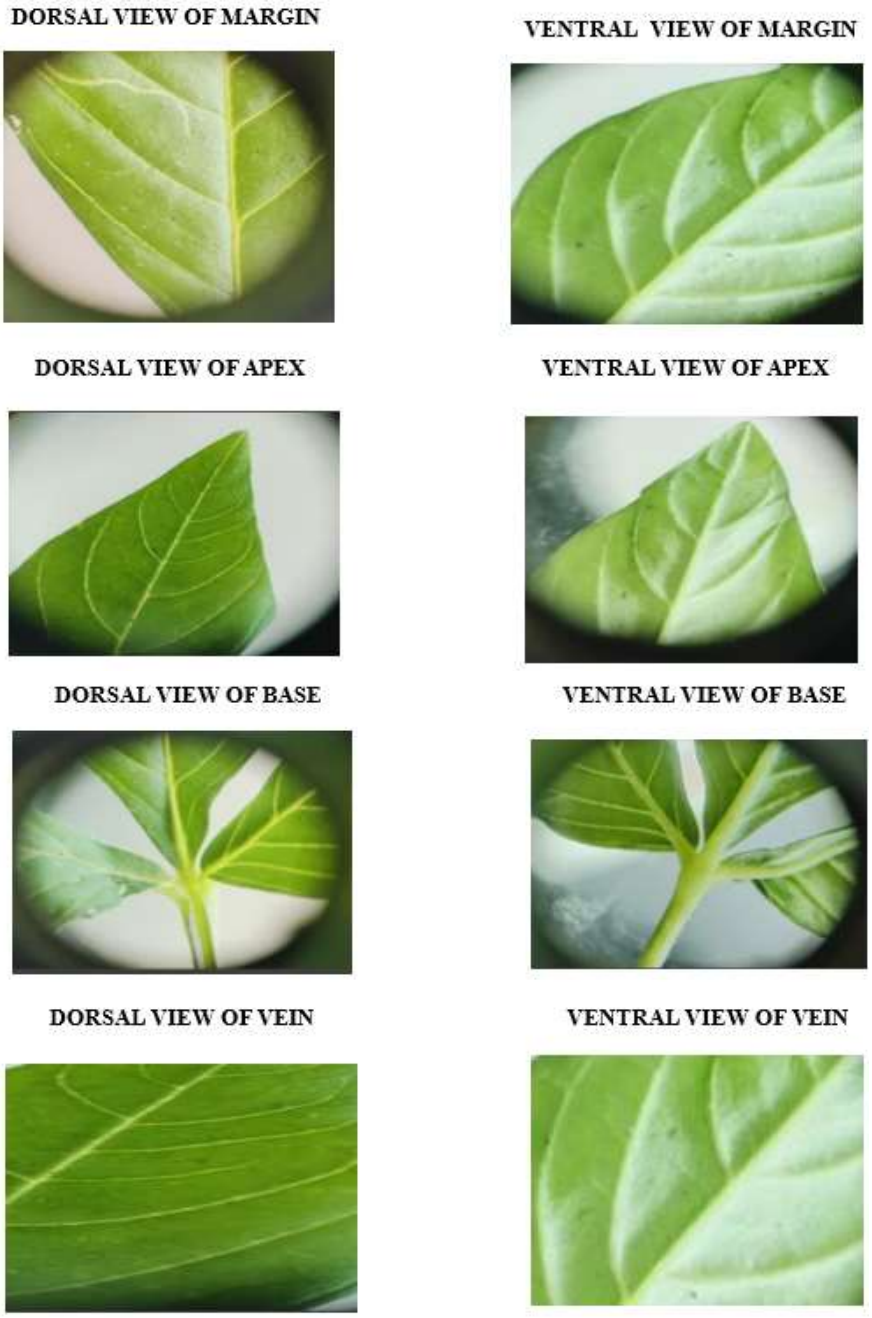


Plate 3: Dorsal & Ventral View Of Margin, Apex, Base And Vein

Table 6: Organoleptic and Macroscopical features of leaves of *Cleome rutidosperma*

S.No	Morphological Features	*Cleoma rutidosperma*
1	Condition	Fresh
2	Colour	Green
3	Type	Compound withTrifoliate
4	Arrangement	Alternate
5	Shape	terminal leaf rhomboidal in shape lateral two are ovate to oblong or obovate
6	Leaf blade	leafblade access rarely with minute prickles
6	Apex	Acute with convex
7	Base	Narrow And Webbed
8	Margin	entirely serrulate with minute conical straight hairs,
9	Rachis	Present
10	Petiole	long quadrangular petiole is green when young, while purplish green in matured with fairly minute hair
11	Surface	Smooth And Slightly Gloosy
12	Stipulate	Present at the leaf base
13	Taste	Bitter
14		
15	Number Of Leaflets	5-7
16	Length And Width	3 – 7 Cm and 1 – 3cm

RESULTS

The leaves of ***Cleome rutidosperma*** were observed for their organoleptic and macroscopical characteristics, showing distinctive traits that are valuable for species identification. The leaves were fresh with a green color and exhibited a trifoliate, compound structure with alternately arranged. Trifoliation comprises terminal leaflets one is larger than lateral two. Morphologically, the leaf shape was noted as terminal leaf is rhomboidal in shape, whereas lateral two are ovate to oblong or obovate, with an acute apex with straight and a narrowly cuneate and convex, webbed base. Midrib of leafblade access rarely with minute prickles: The leaf margin is entirely serrulate with minute conical straight hairs, and a long quadrangular petiole is green when young, while purplish green in matured with fairly minute hair, bsae is concurrently bulbous: The leaf surface appeared glaberous and slightly glossy: Stipules observed at the leaf base. Consequently, the pattern of venation observed as pinnate. Veins prominently depressed above and elevated below.

In terms of taste, the leaves were bitter, a typical characteristic of many medicinal plants. Each compound leaf contained 5 to 7 leaflets, measuring between 3–7 cm in length and 1– 3 cm in width. These defining morphological features support the identification of *Cleome rutidosperma* and provide essential information for recognizing this plant in medicinal and taxonomic studies.

T.S OF *Cleome rutidosperma* PETIOLE

The transverse section (TS) of the petiole is nearly heart-shaped. The upper epidermis is a single layer of small, circular to rounded cells, which are covered by a cuticle. Beneath the epidermis, there are 3 to 4 layers of chlorenchymatous cortex followed by a broad parenchymatous ground tissue. There are five vascular bundles in the petiole. The parenchyma around the vascular bundles is loosely arranged and takes on a synovate (or synovial) shape. Three of these vascular bundles are located centrally; while two are positioned on a layer of pericyclic fibers surrounds the lateral sides. Each vascular bundle. The xylem towards the inner side of the vascular bundle, while the phloem is located above the xylem elements. The cambium is present between the xylem and phloem in the vascular bundle. A brownish pigment is present in the chlorenchyma. Pericyclic fibres are also present in the phloem, providing structural support to the vascular bundles.

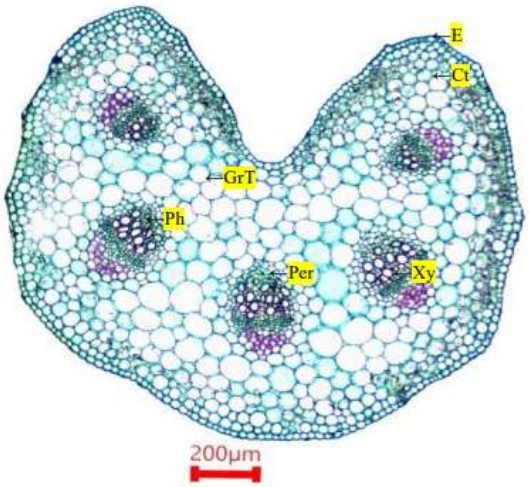


Plate 4: TS of petiole

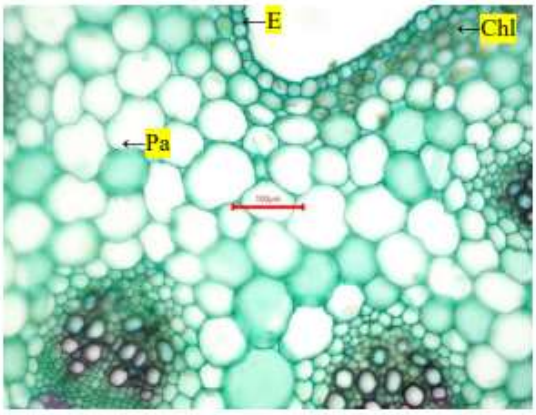


Plate 5: Upper portion enlarged view

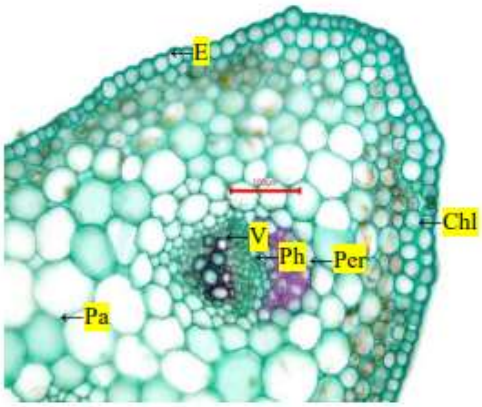
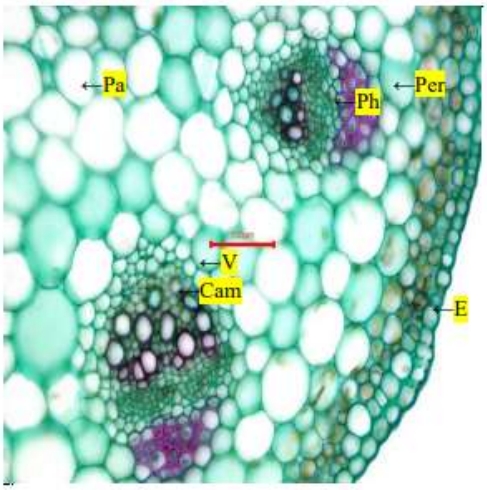


Plate 6: Wing portion enlarged



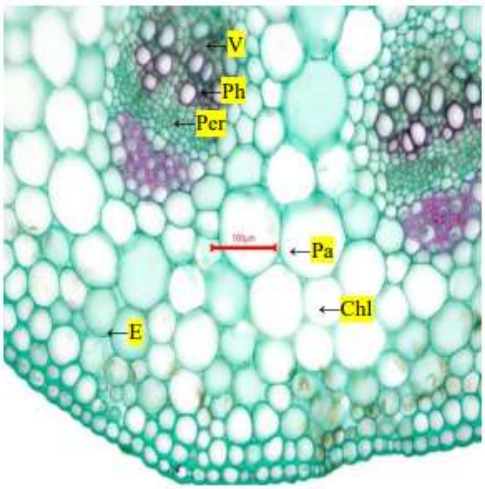


Plate 7: Enlarged view of lower portion

Cam - cambium; **Chl** - chlorenchyma; **Ct** - cortex; **E** - epidermis; **GrT** - ground tissue; **Pa**- parenchyma; **Per** - pericycle; **Ph** - phloem; **V** - vessel; **Xy** - xylem

T.s of cleome rutidosperma Leaflet

TS of leaflet shows slightly grooved upper surface and U shaped convex lower surface with lateral laminar extensions.

T.s cleome rutidosperma Midrib

The midrib is U-shaped on the adaxial (upper) side, and it contains four vascular bundles. The upper epidermis is a single layer of cells, covered by a cuticle. The lower epidermis is also a single layer of cells. Beneath the upper epidermis, there are 3 to 4 layers of parenchyma, while 4 to 7 layers of parenchyma are present beneath the lower epidermis. Glandular trichomes (hair-like structures) are found on the upper epidermis. The cells of the lower epidermis are irregular and smaller in size compared to those in the upper epidermis. The vascular tissue consists of xylem, phloem, and cambium. The xylem faces the upper side of the petiole, while the phloem faces the lower side. Xylem vessels are also present within the xylem tissue.

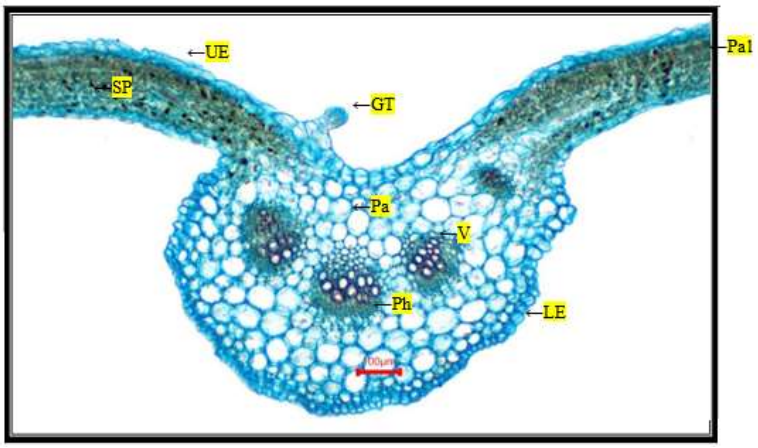


Plate 8: TS of midrib

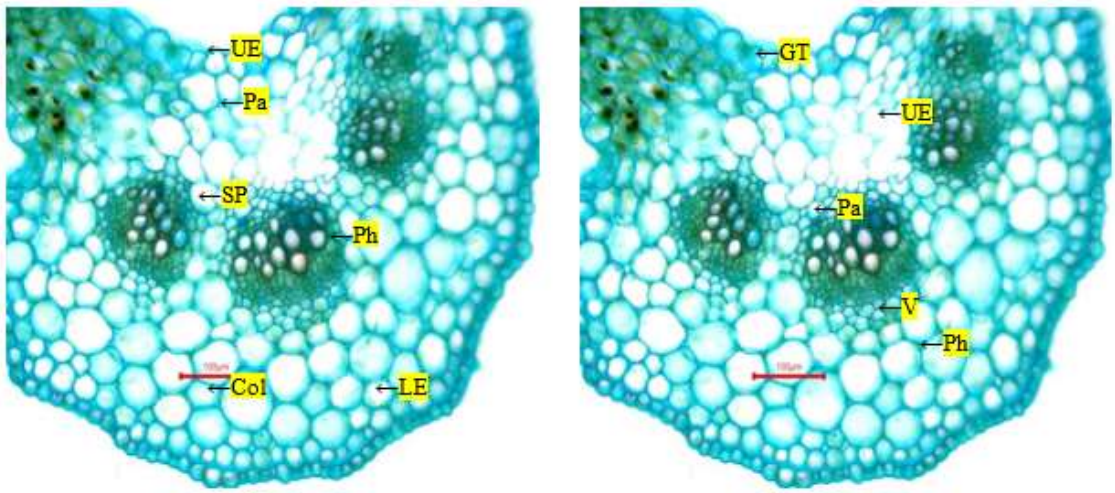


Plate 9: Midrib enlarged view

Fig 9: T.S OF MIDRIB

T.S OF *Cleome rutidosperma* LAMINA

The upper epidermis consists of thick-walled cells covered by a cuticle. The cells are oval to rectangular in shape, arranged in a single layer (uniseriate), and have straight walls. The lower epidermis is made up of rectangular, straight-walled cells, also with a thin cuticle, and papillae are present on the surface. The mesophyll tissue is differentiated into two distinct layers: the upper part contains a double layer of palisade cells, and the lower part consists of 3 to 4 layers of isodiametric spongy parenchyma cells, with intercellular spaces that facilitate gas exchange. Veins (vascular bundles) run through the mesophyll tissue, providing structural support and transport of water, nutrients, and sugars. The palisade cells are short and wide, loosely arranged in two layers.

Additionally, a thick- walled, oval-shaped security cavity is present, which may function in the plant's defense or as an air space

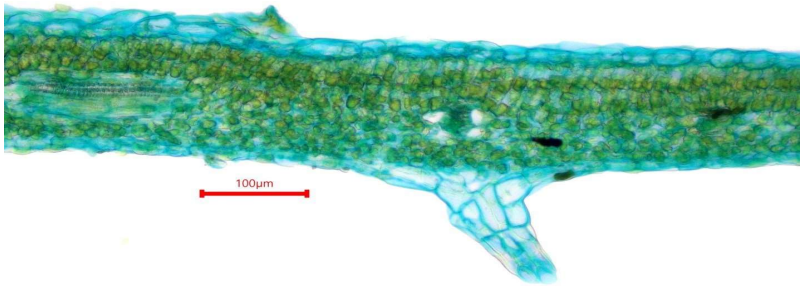


Plate 10.TS of *cleome rutidosperma* leaflet passing through midrib

Col - collenchyma; **GT** - glandular trichome; **LE** - lower epidermis; **Pa** - parenchyma; **Pal**- palisade parenchyma; **Ph** - phloem; **SP** - spongy parenchyma; **T** - trichome; **UE** - upper epidermis; **V** - vessel; **Ve** – vein

Table 7: Quantitative microscopy of *Cleome rutidosperma* leaf

Parameters	**Upper epidermis (/mm^2)**	**epidermis (/mm^2)**
Epidermal number	190 – 200	200 - 235
Stomatal number	56 – 72	110 - 120
Stomatal index	22.7 - 26.4	33.8 - 35.4
Palisade ratio	2 - 3	
Vein islets number	12 - 14	
Vein termination number	15 - 16	

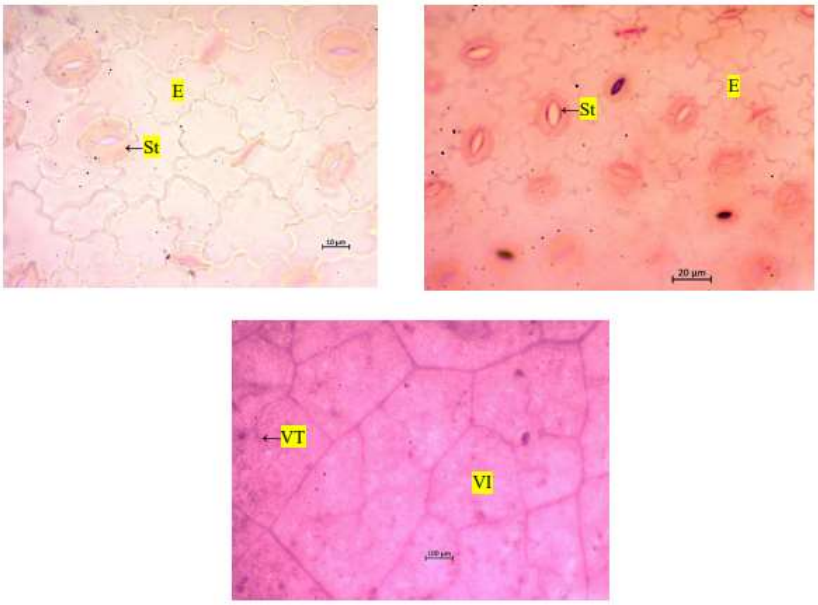
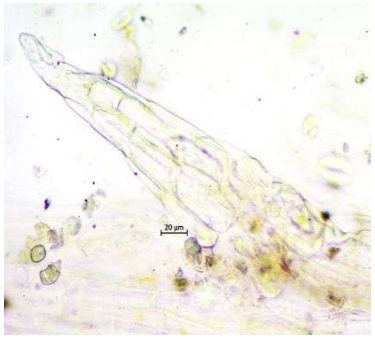


Plate 11. Quantitative microscopy of *cleome rutidosperma* leaf

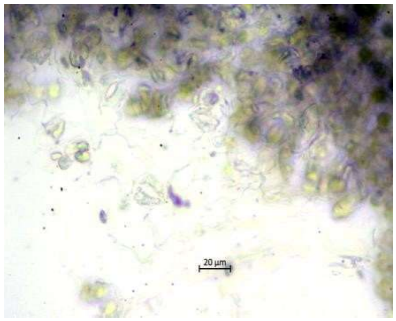
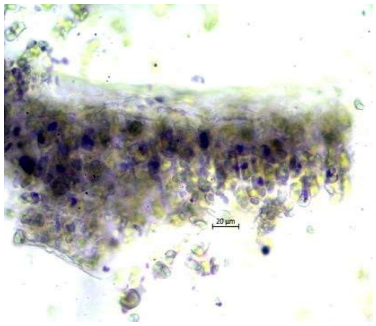
E - Epidermis; **St** - Stomata; **VI** - Vein Islet; **VT** - Vein Termination

Powder Microscopy

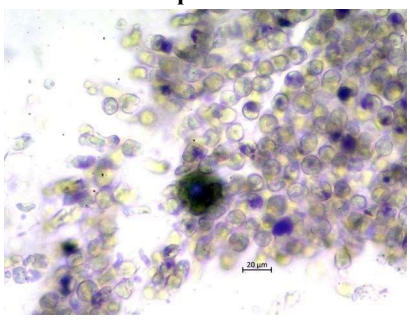
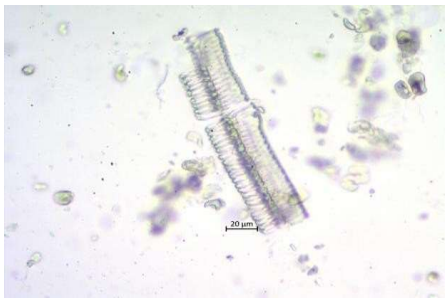
The powder is green coloured with characteristic odour and bitter taste and shows characters like multicellular covering trichomes,foliar epidermis with anomocytic stomata in surface view, sectional view of mesophyll tissue, spongy parenchymal cells, vessels with reticulate and spiral thickening, reddish brown contents, and sandy crystals.




Multicellular covering trichomes

Surface view of epidermis with stomata **Palisade cells in sectional view**

Spongy parenchyma cells **Spiral and reticulate vessels**

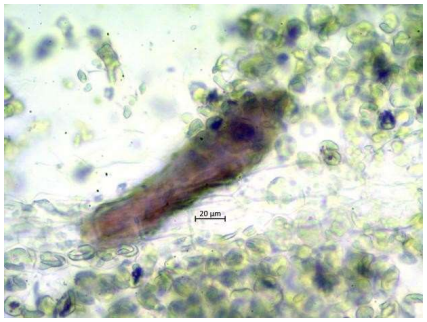
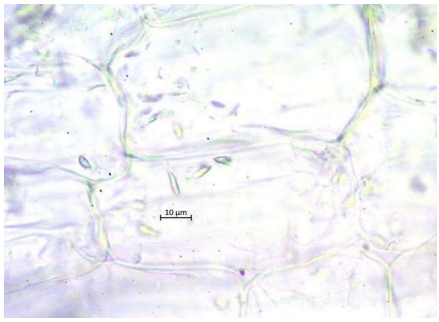



Plate 12: Powder microscopy of *cleome rutidosperma* leaf

Physiochemical Studies

Table 8: Organoleptic Parameter Of Leaf Powder Of *cleome rutidosperma*

S. No	Characters	Character of Cleome rutidosperma
1	Color	Green
2	Odor	Pungent
3	Taste	Bitter
4	Nature	Coarse powder

Table 9: Physiochemical constants of leaf powder of *cleome rutidosperma leaf*

S. No	Physiochemical Constants	Reports W/W%
1	Foreign Matter	Nill
2	Total Ash Value	5.7133
3	Water Soluble Ash Value	3.1%W/W
4	Acid Insoluble Ash Value	0.86%W/W
5	Sulphated ash value	6.81%W/W
6	Loss on Drying	9.933±0.0666%W/W
7	Petroleum ether Extract	0.1%W/W
8	Chloroform extract	0.1%W/W
9	Toluene extract	0%W/W
10	Ethyl acetate extract	0.7%W/W
11	Ethanol extract	10%W/W
12	50% Ethanol extract	0.5%W/W
13	60% Ethanol extract	0.4%W/W
14	70% Ethanol extract	0.9%W/W
15	Aqueous extract	0.3%W/W

Flow Properties Of The Powder		
1	Angle of repose	33.81
2	Carr's index	0.013

Plate 13: Quantitative Estimation

Table 10: Determination of inorganic and heavy metal analysis

S.NO	Inorganic elements	Observations
1	Aluminium	_
2	Arsenic	_
3	Borate	_
4	Calcium	+
5	Carbonate	_
6	Chloride	_
7	Copper	+
8	Iron	+
9	Lead	_
10	Mercury	_
11	Phosphate	_
12	Pottassium	+
13	Silver	_
14	Sulphate	_

DISCUSSIONS

The leaves of *Cleome rutidosperma* were observed for their organoleptic and macroscopical characteristics, showing distinctive traits that are valuable for species identification. The leaves were fresh with a green color and exhibited a trifoliate, compound structure with alternately arranged. Trifoliation comprises

terminal leaflets one is larger than lateral two. Morphologically, the leaf shape was noted as terminal leaf is rhomboidal in shape, whereas lateral two are ovate to oblong or obovate, with an acute apex with straight and a narrowly cuneate and convex, webbed base. Midrib of leafblade access rarely with minute prickles: The leaf margin is entirely serrulate with minute conical straight hairs, and a long quadrangular petiole is green when young, while purplish green in matured with fairly minute hair, bsae is concurrently bulbous : The leaf surface appeared sglaberous and slightly glossy: Stipules observed at the leaf base. Consequently, the pattern of venation observed as pinnate. Veins prominently depressed above and elevated below. In terms of taste, the leaves were bitter, a typical characteristic of many medicinal plants. Each compound leaf contained 5 to 7 leaflets, measuring between 3–7 cm in length and 1– 3 cm in width. These defining morphological features support the identification of *Cleome rutidosperma* and provide essential information for recognizing this plant in medicinal and taxonomic studies

DNA barcoding plays a crucial role in species identification and biodiversity documentation by providing a reliable method for differentiating species based on unique genetic markers. DNA barcoding *cleome rutidosperma* was performed to authenticate and identify the species. The genomic DNA was extracted with a concentration of 992.8 ng/µl and demonstrated high purity, indicated by A260/A280 and A260/A230 ratios. The ITS region was amplified and successfully visualized through agarose gel electrophoresis. The resulting sequence was submitted to GenBank, where it is currently under processing, with accession number PP762190. This molecular approach not only aids in accurate species identification but also enhances biodiversity documentation, contributing to the conservation of plant species. Furthermore, the obtained DNA barcode can facilitate future research on the plant's medicinal properties and ecological roles, supporting the sustainable management and utilization of herbal resources. The use of DNA barcoding is thus invaluable for advancing our understanding of plant biodiversity, ensuring accurate species authentication, and promoting conservation efforts.

The anatomical analysis of *Cleome rutidosperma* petiole, leaflet, midrib, and lamina shows both consistency with previous descriptions of *Cleome* species and distinctive features that enhance its taxonomic profile. The petiole has a heart-shaped cross-section with three central and two lateral vascular bundles within loosely arranged parenchymatous ground tissue surrounded by pericyclic fibers, with unique brownish pigmentation in the chlorenchyma [5]. In the leaflet, the grooved upper surface and U-shaped lower surface with lateral extensions, rarely described in *Cleome* studies, suggest adaptations specific to *C. rutidosperma* [6]. The midrib contains a single-layered cuticle with glandular trichomes on the upper epidermis and four vascular bundles with xylem facing inward, similar to some *Cleome* species; however, the presence of sandy crystals within the ground tissue, potentially serving as a defense and calcium-regulating mechanism, is less frequently documented across the genus [7]. The lamina is characterized by thick-walled, oval to rectangular cells in the upper epidermis, papillae on the lower epidermis, and an oval-shaped cavity within the mesophyll tissue, a rare feature that could serve as a taxonomic marker. The double-layered palisade structure and spongy parenchyma with intercellular spaces align with other *Cleome* species findings, yet the unique cavity sets *C. rutidosperma* apart, suggesting potential adaptations for its specific ecological niche.

The quantitative microscopy of *cleome rutidosperma* leaves reveals several key anatomical features, including epidermal cell density, stomatal density, palisade ratio, and vein structure, which provide diagnostic insights. Our findings show that the epidermal cell density ranges from 190 to 200 cells per mm² for the upper epidermis and 200 to 235 cells per mm² for the lower epidermis. These values are consistent with previous reports, such as those by [8]. who recorded 180 to 210 cells per mm² for the upper epidermis and 210 to 240 cells per mm² for the lower epidermis. Regarding stomatal characteristics, our data show 56 to 72 stomata per mm² on the upper epidermis and 110 to 120 stomata per mm² on the lower epidermis, which is in line with previous studies, particularly [9]. who found a stomatal density of 32 to 36 on the lower epidermis and a lower stomatal index on the upper epidermis, suggesting efficient gas exchange on the abaxial surface. The stomatal index in our study ranges from 22.7 to 26.4 on the upper epidermis and 33.8 to 35.4 on the lower epidermis, aligning with typical values for *Cleome* species. Furthermore, the palisade ratio observed in *C. rutidosperma* is between 2 and 3, consistent with findings from [10]. who reported a similar range in other sun-adapted *Cleome* species. Lastly, the vein islet numbers in our study range from 12 to 14, and vein termination numbers range from 15 to 16, which closely resemble the values reported by [11]. who found vein islets between 12 and 15 and vein terminations between 14 and 17. These anatomical parameters not only support the diagnostic value of these features for distinguishing *C. rutidosperma* within the *Cleome* genus but also contribute to the broader understanding of its taxonomic placement.

The powder microscopical evaluation of *Cleome rutidosperma* leaves shows the presence of multicellular covering trichomes, epidermis with stomata, palisade tissue, spongy parenchyma cells, spiral vessels, sandy crystals The *Cleome rutidosperma* leaves are said to ambihistomatic, since it contains only anomocytic stomata. The powder microscopy shows the presence of spongy paranchyma cells and sandy crystals, which is also confirmed by its presence in the spiral and reticulate vessels and reddish brown content of the leaves of *cleome rutidosperma*.

The physicochemical analysis of *Cleome rutidosperma* leaf powder reveals that the sample total ash value

(5.7133% w/w) aligns closely with typical values for other *Cleome* species, generally ranging between 5-7% w/w, indicating consistent inorganic content (Saini & Prakash, 2020). The acid-insoluble ash value of 0.86% w/w, lower than 1%, suggests minimal siliceous impurities, supporting previous findings on the purity of *Cleome rutidosperma* [12]. The sulphated ash value(6.81% w/w) also matches reports of significant mineral content for this species, as noted by Pathak et al. (2018). Additionally, the loss on drying (9.933 ± 0.0666% w/w) confirms low moisture content within acceptable limits, which is essential for stability and microbial resistance [13]**.** The ethanol extractive value (10% w/w) was highest among tested solvents, similar to [14]. who highlighted ethanol's efficiency in extracting a broad range of phytochemicals. Moderate yields in ethyl acetate (0.7% w/w) and 70% ethanol (0.9% w/w) are consistent with other medium- polar solvent extractions. while lower values in nonpolar solvents like petroleum ether and chloroform were also reported. The angle of repose (33.81°) suggests good flow properties [15]. and the Carr's index (0.013) and Hausner's ratio (1.42) confirm minimal powder cohesiveness, as reported by [16]. Together, these findings establish a quality benchmark for *Cleome rutidosperma* leaf powder for medicinal formulation consistency.

CONCLUSION

Cleome rutidosperma exhibits distinct morphological, anatomical, and physicochemical characteristics that facilitate its identification and medicinal potential. The organoleptic and macroscopic traits, such as its trifoliate leaves and bitter taste, are valuable for species recognition. Molecular DNA barcoding using the ITS region supports accurate species authentication. Anatomical features, such as the unique cavity in the lamina and sandy crystals, enhance its taxonomic profile. The physicochemical properties, including ash content and extractive values, provide essential quality benchmarks for its use in herbal formulations. These findings contribute to the understanding and conservation of Cleome rutidosperma as a valuable medicinal plant.

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