

Pharmacognostical Standardization of Leaf and Stem Bark of *Elaeodendron glaucum* (Rottb.) Pers.: An Important Ethno-Medicinal Plant

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ABSTRACT

Background: *Elaeodendron glaucum* (Rottb.) Pers. is an essential ethnomedicinal plant belonging to Celastraceae used in the treatment of various ailments like antidotes for snake bites, dog bites, wounds and cracks, dysuria, swellings, burning sensations, hyperacidity, dysentery, headaches, fever, etc. **Aim:** The Present study aimed to establish the pharmacognostical profile of *Elaeodendron glaucum* to generate data on distinguishing characteristics from other *Elaeodendron* species. **Materials and Methods:** Pharmacognostical studies were conducted with the scope of organoleptic, macroscopic, microscopic, preliminary phytochemical evaluation, physicochemical evaluation, fluorescence and HPTLC studies. **Results:** Macroscopic observations of the stem bark revealed horizontal and transverse striations, transverse fissures with vertical cracks, exfoliating in irregular scales, numerous lenticels, and constrictions. Microscopic observation of the same reveals a wide zone of cork and cortex, forming a rhytidoma. Macroscopic observations of the leaf show an entire margin, an ovate to ovate-elliptic shape, sub-coriaceous texture, and crenate margins. Microscopic observation of the same revealed the presence of prismatic crystals on both the upper and lower epidermis; mesophyll tissue embedded with vascular strands. The organoleptic characteristics of the stem bark powder are Rough in touch, with a bitter taste. The leaf powder is smooth to the touch, with a slightly bitter taste. The preliminary phytochemical screening revealed the presence of Glycosides, Alkaloids, Flavonoids etc. The physicochemical studies for leaf and stem bark were assessed for Loss on Drying, Total ash, Acid-insoluble ash etc. The HPTLC analysis revealed the presence of various phyto-constituents, as evidenced by diverse R_f values. **Conclusion:** This study will serve as a standard reference for future research and applications on *Elaeodendron glaucum*.

Keywords: *Elaeodendron glaucum*, Fluorescence analysis, Medicinal plants, Microscopic observations, Pharmacognosy, Phytochemical screening.

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Received: 22-05-2026;

Revised: 03-07-2026;

Accepted: 18-08-2026.

INTRODUCTION

The Indian subcontinent boasts a rich heritage of traditional medicine, with various health care systems flourishing for centuries. It mainly consists of three major systems of medicine: Ayurveda, Siddha, and Unani (Wadnerwar *et al.*, 2023; Pratap *et al.*, 2026). The medicinal importance of approximately 20,000 plants has been documented in India, but traditional practitioners

use only 7,000-7,500 of them to treat various diseases. Every traditional system has its own flora: Ayurveda about 2500 plants, Siddha about 1350 plants, Unani about 350, Homoeopathy about 850, Tibetan about 550, Modern about 250, and folk about 4500 (Hussain & Hussain, 2010). In every system of medicine, quality control has been considered a significant task to verify the authenticity of drugs from the time of inspection onward, to prevent adulteration and its harmful effects. The plants from Ayurveda, Unani, and Siddha have their own pharmacopoeias with standardization methods. But plants of ethnobotanical importance lack standardized methods for authenticating drugs (Pratap *et al.*, 2026). In such cases, pharmacognostical standardization is needed to authenticate the drug. With this view, a study was undertaken to produce a record documenting the



DOI: 10.5530/pres.20270070

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microscopical, macroscopical, phytochemical and HPTLC profile of *Elaeodendron glaucum* (Rottb.) Pers. is an ethno-medicinally important plant (Pratap et al., 2021). *Elaeodendron glaucum* is known for its sternutatory action and is employed to relieve severe headache and skin infections (Malpani et al., 2019). It is also very effective in controlling hysteria. The bark is employed to treat Dysuria, wounds, burning sensations, and hyperacidity (Johnson et al., 2015), and the leaves are an effective remedy for cough with phlegm, swellings, and menstrual disorders (Bharath & Suryanarayana, 2008; Jagtap et al., 2009). There has already been significant research on the pharmacological and ethnobotanical properties of *Elaeodendron glaucum*, but identifying it in dried form from other *Elaeodendron* species remains a challenge. A review of the existing literature on this plant highlights a lack of standardized studies. Therefore, there is an urgent need for such standardization. To fill this gap, the present study was conducted (Pratap et al., 2012).

MATERIALS AND METHODS

The plant material was collected from Yenugu Mallamma konda, Annamayya district, Andhra Pradesh, in August 2022. The collected specimen was authenticated by Gudivada Sudharshanam, Professor of Botany, using local floras, and the specimen was deposited in the herbarium of the Department of Botany, Sri Venkateswara University, and assigned herbarium reference no. SVUH-1271 for future reference (Gamble, 2005; Jain & Rao, 1976; Pratap et al., 2009, 2010; Pullaiah, 2018; Sekhar et al., 2011).

Evaluation of Macroscopic and organoleptic characters

The collected raw drugs were observed with the naked eye, and their macroscopic characters were noted. The organoleptic characteristics were documented by using standard procedures as follows (Jyothi et al., 2015, 2020; Kumari et al., 2019, 2021, 2020, 2022; Pratap et al., 2012, 2016, 2021, 2024, 2026; World Health Organization, 2000).

Evaluation of Microscopic characters

The crude or fresh material of the leaf and stem bark was subjected to free-hand sections, and microscopic observations were performed using standard procedures as follows (Jyothi et al., 2015, 2020; Kumari et al., 2019, 2021, 2020, 2022; Pratap et al., 2012, 2016, 2021, 2024, 2026; World Health Organization, 2000).

Evaluation of powder microscopic characters

The leaf and stem bark powders were treated with different reagents and subjected to microscopical observations using following standard procedures (Jyothi et al., 2015, 2020; Kumari et al., 2019, 2021, 2020, 2022; Pratap et al., 2012, 2016, 2021, 2024, 2026, World Health Organization, 2000).

Physicochemical analysis

The plant material was subjected to various physicochemical parameters according to standard procedures (Anonymous, 1996; Evans, 2002; Jyothi et al., 2015, 2020, 2024; Khandelwal, 2008; Kumari et al., 2019, 2020, 2021, 2022; Pratap et al., 2012, 2016, 2021, 2024, 2026; WHO., 2000).

Phytochemical investigation

The leaf and stem bark powders were extracted with water, methanol, chloroform, ethanol, and hexane. The extracts were subjected to preliminary phytochemical screening according to standard procedures, as follows (Brain & Turner, 1975; Jyothi et al., 2015, 2020; Khandelwal, 2008; Kumari et al., 2019, 2021, 2022; Pratap et al., 2012, 2014, 2016, 2021, 2024, 2026; WHO., 2000).

Fluorescence analysis

The powdered plant material was subjected to different chemical reagents, and the resulting colour reactions were observed under visible as well as Ultraviolet (UV) light (Anonymous, 1996; Evans, 2002; Kumari et al., 2019, 2021; Pratap et al., 2012, 2020, 2021, 2024, 2026; WHO., 2000).

High-Pressure Thin-Layer Chromatography of Leaf in Ethanol Extract

The leaf extract was filtered and concentrated to approximately 5 mL, after which it was analyzed using Thin-Layer Chromatography (TLC). About 7 µL of the ethanol extract was carefully applied onto the TLC plate. The plate was developed using a mobile phase consisting of toluene, ethyl acetate, and methanol in the ratio of 7:2:1 (v/v/v).

After chromatographic development, the plate was air-dried and observed under UV light at 254 nm and 366 nm. Subsequently, the plate was sprayed with vanillin-sulphuric acid reagent and heated at 110°C for approximately 5 min. The developed plate was then examined under visible light. Several distinct spots were detected on the chromatogram, and the plate was photographed for documentation as shown in Figure 1.

HPTLC fingerprint of the Stem bark in ethanol extract

The applied methodology was the same as HPTLC of leaf, but the mobile phase was changed to Chloroform: Methanol (6.5:2, v/v) (Kumari et al., 2020, 2022; Pratap et al., 2021, 2024, 2026).

RESULTS

Taxonomy of the plant (Macroscopical characters)

Elaeodendron glaucum is a small tree species. The leaves are sub-opposite, with margins that are entire or slightly crenate. The petiole is up to 2 cm long. The leaf blade is ovate to ovate-elliptic, somewhat coriaceous, with crenate margins, measuring up to 12

× 6.5 cm. The cymes are dichotomously branched and generally shorter than the leaves. Pedicels are about 0.2 cm long. The petals are white and glabrous, while the anthers are yellow. The disc is green and obovoid. The fruit is a drupe, less than 1 cm long, usually containing a single seed (Table 1).

Organoleptic properties

They were assessed by the sensory organs. The results were depicted in the Table 1.

Microscopy

Leaf

Midrib

TS of midrib shows a single layer of upper and lower epidermal cells with thick cuticle; both epidermis embedded with prismatic and cluster of calcium oxalate, upper epidermis followed by single layer of hypodermis, a patch of collenchymatous tissue below the hypodermis present in the central region, but continuous as a band above the lower epidermis, embedded with brownish content; centrally located one well developed large vascular bundles in shape of are beneath the two smaller vascular bundles, upper two vascular bundles surrounded by continuous ring of sclerenchymatous fibres, while the lower vascular bundles is surrounded by discontinuous ring of sclerenchymatous fibres, phloem containing cluster crystal of calcium oxalate (Figures 1-3).

Lamina

Dorsiventral, single layer of upper and lower epidermal cells with thick cuticle, a single layer of hypodermis present just below the upper epidermis; two rows of palisade cells beneath the hypodermis and spongy parenchyma; both epidermis embedded with calcium oxalate-prismatic cluster crystals; mesophyll tissue embedded with vascular strands (Figures 4 and 5).

Petiole

Transfer section of petiole is irregularly circular in outline, with a single layer of epidermis with very thin cuticle, several layers of parenchymatous tissue beneath the epidermis; parenchymatous tissue of the cortical region contains cluster and prismatic calcium oxalate crystals. Bicolateral vascular bundles are centrally located and capped fully by a ring of grouped pericyclic fibres. In the centre of the petiole, parenchymatous pith is noticed with intercellular spaces (Figure 6).

Powder microscopy of Leaf

The fragments of upper and lower epidermis are arranged with straight anticlinal walls. Anomocytic stomata present on lower epidermis in surface view; both epidermis embedded with cluster, rosette and prismatic crystals of calcium oxalate. There

are fragments of fibres with thick and thin lumen, forked, pitted, sharp and blunt ends. Vessels exhibit spiral thickenings and few parenchyma with crystals embedded, a few parenchyma cells contain embedded crystals. Additionally, some parenchymatous cells display reddish-brown contents. The starch grains are few and consist of a single component, measuring up to 30 µm. The stomatal index ranges from 9.99 to 12.46 on the lower surface, with a stomatal number between 141 and 187 (Figure 7).

Stem bark

Detailed TS of stem bark shows a wide zone of cork and cortex, forming a rhytidoma, cork consist of tangentially elongated rectangular thin-walled cells with reddish brown content, group of stone cells in patches in outer and inner cortex, inner cortex consists of thin-walled parenchymatous cells embedded with group of stone cells and reddish content: followed by wider zone of phloem tissue (Figures 8 and 9).

Powder microscopy of the stem bark revealed characteristic pharmacognostic features including polygonal cork cells, cork cells with embedded stone cells, blunt-ended fibres, thick-walled and pitted stone cells, stout sclereids, simple and compound starch grains, and prismatic calcium oxalate crystals under polarized light (Figure 10A-10H). These diagnostic microscopic characters are valuable for the identification, authentication, and quality control of the stem bark .

Physicochemical

The results for the physicochemical analysis, fluorescence analysis, and preliminary phytochemical screening for both leaf and stem bark are summarized in Tables 2-4, respectively

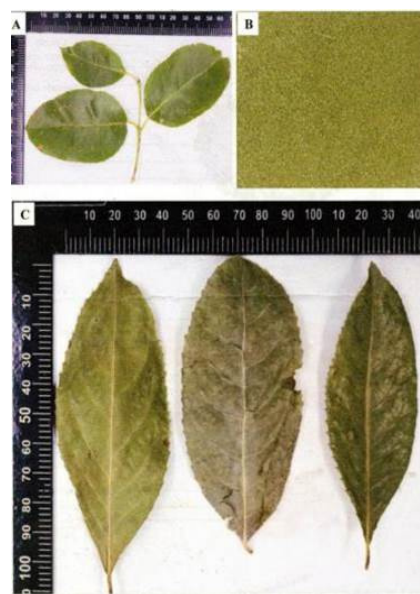


Figure 1: A. Fresh leaves, B. Leaf powder, C. Dried leaves.

Table 1: Macroscopic characters.

Sl. No.	Macroscopic Parameter	Leaf	Stem bark
1.	Appearance	Simple, coriaceous, glabrous, shriveled, petiolate, shows crenate-serrate margin, reticulate venation prominent on lower surface.	Dried rectangular thick pieces of varying sizes, with horizontal and transverse striations, transversely fissured with vertical cracks and exfoliating in irregular scales numerous lenticels and constrictions present.
2.	Size & shape	8-10 cm long and 3-4 cm width in ovate-oblong, apex acute-acuminate, Petiole 1.5-2 cm, woody.	Up to 1.5 cm in thick, irregularly rectangular pieces.
3.	Colour	Upper light green, lower surface greyish green.	External surface reddish-brown colour, Internal surface cream coloured.
4.	Texture	Smooth.	Rough with longitudinal striations, fracture short.
5.	Oduor	Characteristic.	Characteristic.
6.	Taste	Prominently bitter, slightly astringent.	A Stringent and bitter.

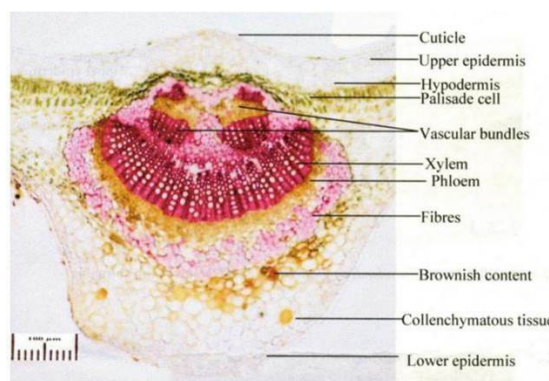


Figure 2: Detailed TS of midrib stained with phloroglucinol + Conc. HCl.

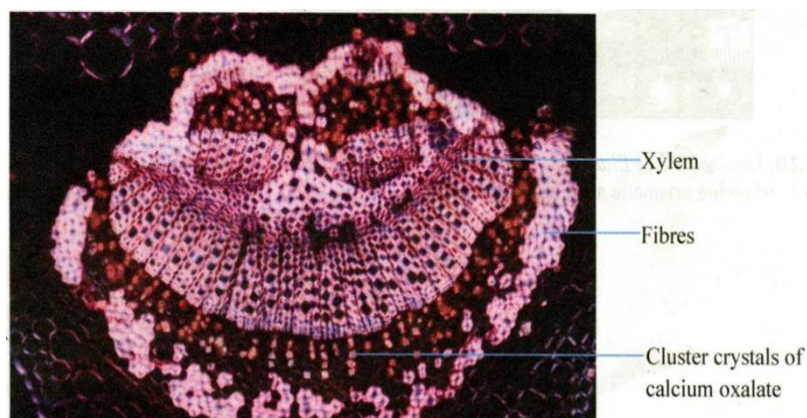


Figure 3: Detailed TS of midrib under polarized light.

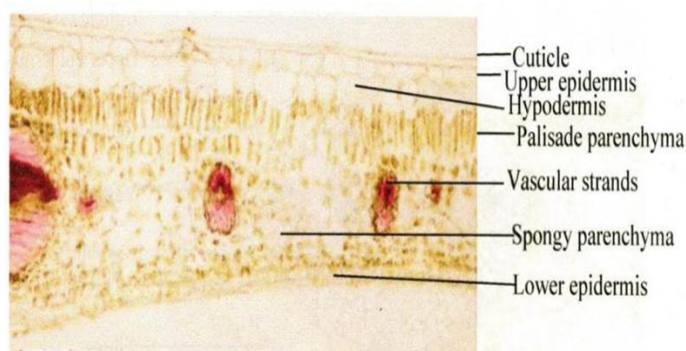


Figure 4: Detailed TS of lamina with saffranine.

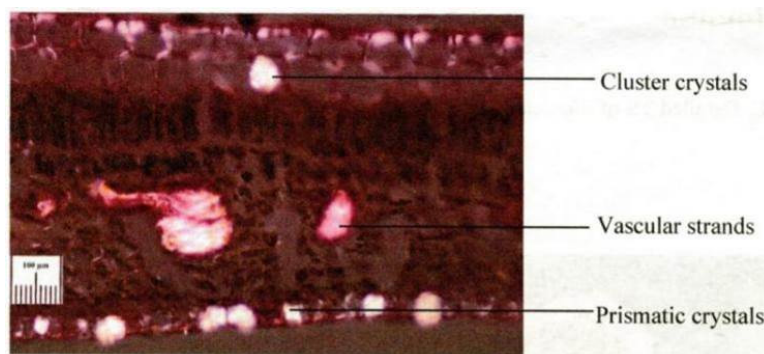


Figure 5: Detailed TS of lamina under polarized light showing prismatic calcium oxalate crystals.

Table 2: Physicochemical analysis.

Sl. No.	Parameters	Leaf (%) w/w	Stem bark (%) w/w
1.	Loss on Drying	0.88	9.17
2.	Total ash	8.34	3.25
3.	Acid insoluble ash	0.86	0.19
4.	Water soluble extractive value	39.33	14.32
5.	Alcohol soluble extractive value	20.35	16.65
6.	Ph (10 % aqueous solution)	6.52	6.3

HPTLC Screening

Retention factor (R_f) values and corresponding spot characteristics were recorded and are presented in the respective tables (Tables 5-10). Densitometric chromatograms (densitogram) were generated to support qualitative and quantitative evaluation of the phytochemical profile. For the leaf ethanol extract, HPTLC analysis revealed the presence of multiple phytoconstituents (Figure 11). Under wavelength of $\lambda_{\max}$ 366 nm UV light, nine distinct bands were observed with R_f values of 0.01, 0.09, 0.15, 0.25, 0.31, 0.53, 0.81, 0.93, and 0.98, indicating the presence of several fluorescent compounds (Figure 12). The bands exhibited variable fluorescence intensity, suggesting differences in concentration of the constituents. At wavelength of $\lambda_{\max}$ 254 nm, four spots were detected at R_f values 0.01, 0.09, 0.31, and 0.98, (Figure 13) appearing as light-absorbing zones, confirming the presence of UV-active compounds. After derivatization with

vanillin-sulphuric acid reagent and scanning at wavelength of $\lambda_{\max}$ 576 nm, five coloured spots appeared at R_f values 0.01, 0.10, 0.28, 0.72, and 0.81, indicating the presence of terpenoids, phenolics, or related secondary metabolites (Figures 14 and 15). The densitogram showed major peaks at R_f 0.01 and 0.10, confirming them as dominant constituents in the leaf extract HPTLC profiling of the stem bark (Figure 16) with ethanol extract, demonstrated a comparatively distinct chemical pattern. When scanned at wavelength of $\lambda_{\max}$ 366 nm, four major fluorescent spots were observed at R_f values 0.004, 0.280, 0.509, and 0.941 (Figure 17). Under wavelength of $\lambda_{\max}$ 254 nm, four absorption bands were detected at R_f 0.01, 0.31, 0.58, and 0.95, (Figure 18) indicating the presence of UV-absorbing phytochemicals. Following derivatization and scanning at wavelength of $\lambda_{\max}$ 576 nm, six well-resolved coloured spots appeared at R_f values 0.01, 0.16, 0.23, 0.34, 0.57, and 0.93, suggesting the presence of

Table 3: Fluorescence analysis.

Sl. No.	Treatment	Leaf		Stem bark	
		Visible light	UV light	Visible light	UV light
1.	Powder as such	Green	Green	Reddish brown	Brown
2.	Powder+50% HNO ₃	Yellow	Green	Yellowish brown	Light brown
3.	Powder+ 1 N HCl	Pale yellow	Yellowish green	Reddish brown	Reddish brown
4.	Powder+50% H ₂ SO ₄	Yellowish green	Green	Reddish brown	Reddish brown
5.	Powder+5% Ferric chloride	Green	Green	Deep brown	Black
6.	Powder+ H ₂ O	Green	Green	Brown	Brown

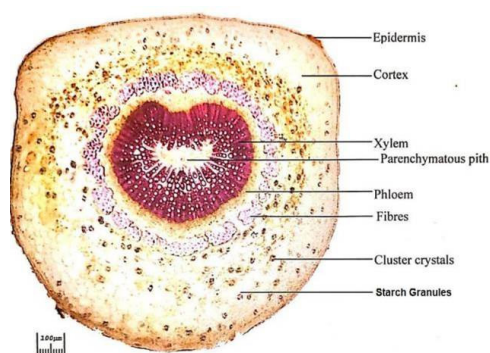
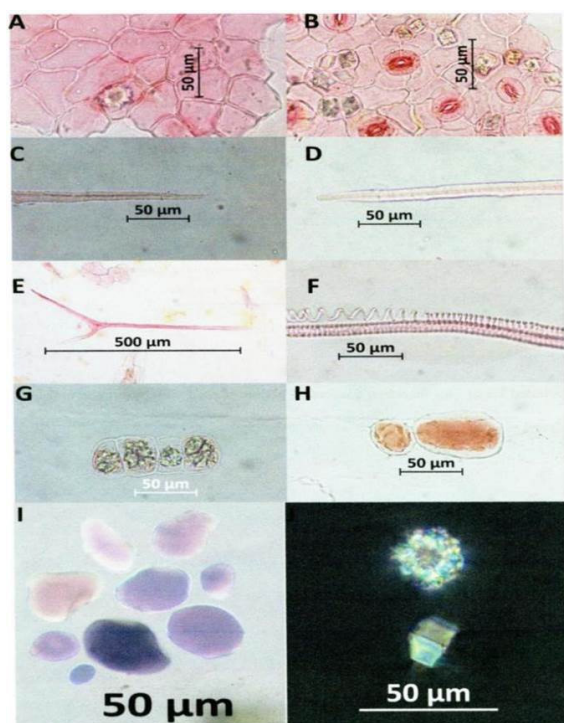

Figure 6: Detailed TS of petiole stained with phloroglucinol-HCl

Figure 7: Powder microscopy of Leaf. A. Upper epidermis with crystals in surface view; B. Lower epidermis with anomocytic stomata, prismatic and cluster crystals and slightly wavy anticlinal walls in surface view; C. Thick walled fibers; D. Blunt end fibers; E. Forked fibres; F. Spiral vessels; G. Cluster crystals; H. Reddish brown content; I. Starch grains; J. Rosette and prismatic crystals (under polarized light).

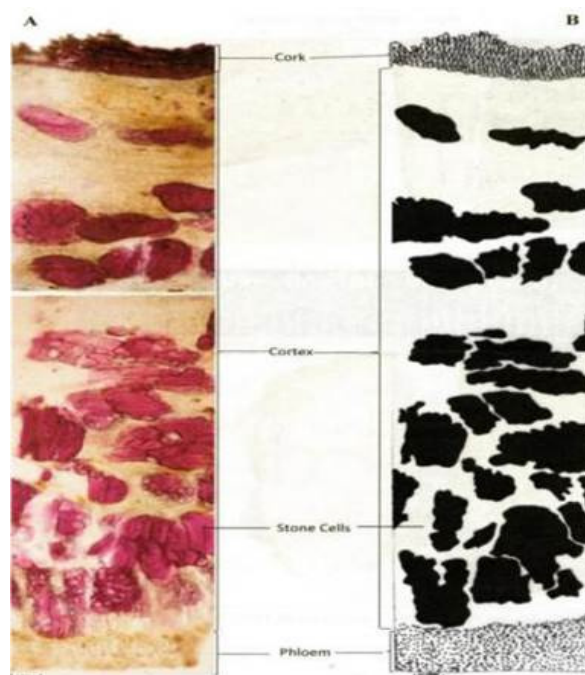
Figure 8: A. Fresh stem bark; B. Dried stem bark powder; C. Dried stem bark.

Figure 9: A. Detailed Transverse Section of stem bark with safranin, B. Diagrammatic Transverse Section of stem bark.

Table 4: Phytochemical screening.

Sl. No.	Phytoconstituents	Chemical tests	Plant Part	Water extract	Methanol extract	Chloroform extract	Ethanol extract	Hexane extract
1.	Alkaloids	Dragendorff's test	Leaf	--	++	++	++	++
			Stem bark	--	++	++	++	++
		Mayer's test	Leaf	--	++	++	++	++
			Stem bark	++	++	++	++	++
2.	Glycosides	Borntrager's test	Leaf	++	++	--	++	--
			Stem bark	++	++	--	++	--
3.	Flavonoids	Test with Mg ribbon and Conc. HCl	Leaf	--	++	++	++	--
			Stem bark	++	++	--	++	--
4.	Protein	Millon's test	Leaf	++	--	--	--	--
			Stem bark	++	--	--	--	--
5.	Tannins	Test with Ferric chloride	Leaf	++	++	--	++	--
			Stem bark	++	++	--	++	--
6.	Phenols	Test with FeCl ₃	Leaf	--	++	++	++	--
			Stem bark	++	++	++	++	--
7.	Carbohydrates	Molisch's test	Leaf	++	--	--	--	--
			Stem bark	--	--	--	--	--
8.	Reducing Sugars	Fehling's test	Leaf	++	++	--	--	--
			Stem bark	++	--	--	--	--
9.	Saponins	Froth test	Leaf	++	++	--	++	--
			Stem bark	++	++	--	++	--

diverse classes of secondary metabolites (Figures 19 and 20). The densitometric scan revealed major peaks at R_f 0.57 and 0.93, representing predominant compounds in the stem bark extract. Overall, the HPTLC fingerprint profiles of both leaf and stem bark ethanol extracts demonstrated chemical complexity and distinct phytochemical screenings, which can serve as a reliable reference for identification, quality control, and standardization of the plant material.

DISCUSSION

The present study aimed to establish pharmacognostic standards for the leaf and stem bark of *Elaeodendron glaucum*, an important ethnomedical plant. Herbal drugs are often collected from natural habitats and used in their crude form, making proper identification and standardization essential to ensure their quality, safety, and therapeutic value. Macroscopic

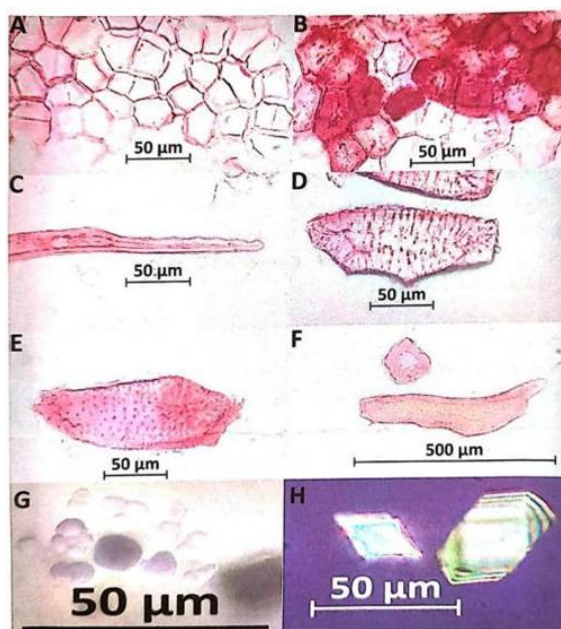
evaluation of the leaf and stem bark revealed distinct taxonomic characteristics, including morphology, colour, texture, taste, and structural markings. These external features are crucial for the preliminary identification of the crude drug and can help distinguish it from substitutes or adulterants, as well as from related species. Microscopic examination identified key diagnostic features, including a dorsiventral lamina, well-developed vascular bundles, sclerenchymatous fibres, stone cells, and calcium oxalate crystals in various forms. Additionally, powder microscopy revealed characteristic elements, including fibres, vessels, stone cells, crystals, starch grains, and epidermal fragments. These micro-morphological features of the powdered material serve as the basis for identifying the drug in powdered form. Physicochemical parameters, including ash content, loss on drying, extractive values, and pH, were assessed to evaluate the purity and quality of the plant material. A low moisture content

Table 5: HPTLC R_f values and Peak list of leaf in ethanol extract obtained at 254 nm in Absorbance mode.

Sl. No.	Max R_f	Max pos. (mm)	Height	Height %	Area	Area %
1.	0.01	10.40	0.33	72.90	0.0028	41.93
2.	0.09	16.50	0.03	5.99	0.0005	7.29
3.	0.31	31.80	0.02	5.49	0.0008	11.62
4.	0.98	78.60	0.07	15.63	0.0026	39.16

Table 6: R_f values and Peak list of leaf ethanol extract obtained at 366 nm in Fluorescence mode.

Sl. No.	Max R_f	Max pos. (mm)	Height	Height %	Area	Area %
1.	0.01	10.40	0.36	43.33	0.0028	17.01
2.	0.09	16.40	0.01	1.65	0.0003	1.74
3.	0.15	20.30	0.07	8.96	0.0015	9.24
4.	0.25	27.40	0.04	4.30	0.0008	5.06
5.	0.31	31.90	0.09	10.90	0.0026	15.37
6.	0.53	47.10	0.03	3.29	0.0010	5.97
7.	0.81	66.50	0.08	9.42	0.0033	19.69
8.	0.93	74.80	0.09	10.64	0.0029	17.14
9.	0.98	78.60	0.06	7.52	0.0015	8.77


Figure 10: Powder microscopy of stem bark A. Cork cells in surface view; B. Cork cells in surface view with embedded stone cells; C. Blunt end fibres; D. Stone cells with walls; E. Stone cells with showing pits; F. Stout stone cells; G. Starch grains single and compound; H. Prismatic crystals under polarized light.

indicates a reduced risk of microbial growth and a prolonged shelf life. The ash values provide information about inorganic matter and potential contamination, while extractive values indicate the nature and quantity of active constituents soluble in different solvents. Higher extractive values in polar solvents suggest the presence of polar phytoconstituents. Fluorescence analysis showed characteristic colour changes in the powdered drug under visible and ultraviolet light after treatment with various reagents. This technique is a quick and reliable method for detecting adulteration and confirming the identity of the

crude drug. Preliminary phytochemical screening revealed the presence of several bioactive compounds, including in both leaf and stem bark extracts. HPTLC fingerprint profiles of leaf and stem bark showed well-developed bands and various R_f values under 254 nm and 366 nm in fluorescence mode of UV light. The differences in chromatographic patterns between the leaf and stem bark indicate variations in chemical composition. The obtained HPTLC data can serve as a perfect chemical profiling tool for quality control and authentication.

Table 7: R_f values and Peak list of leaf ethanol extract obtained at 520 nm after derivatized with vanillin sulphuric acid reagent.

Sl. No.	Max R_f	Max pos. (mm)	Height	Height %	Area	Area %
1.	0.01	10.70	0.46	55.29	0.0057	35.70
2.	0.10	16.90	0.25	29.49	0.0049	30.62
3.	0.28	29.60	0.06	6.64	0.0023	14.26
4.	0.72	60.10	0.03	3.55	0.0012	7.23
5.	0.81	66.90	0.04	5.03	0.0019	12.18

Table 8: R_f values and Peak list of stem bark in ethanol extract obtained at 254 nm in Absorbance mode.

Sl. No.	Max R_f	Max pos. (mm)	Height	Height %	Area	Area %
1.	0.01	10.7	0.2	43.9	0.0034	17.0
2.	0.31	31.9	0.1	17.4	0.0038	18.7
3.	0.58	50.3	0.1	13.8	0.0034	16.8
4.	0.95	76.2	0.1	25.0	0.0096	47.5

Table 9: R_f values and Peak list of stem bark in ethanol extract obtained at 366 nm in Fluorescence mode.

Sl. No.	Max R_f	Max pos. (mm)	Height	Height %	Area	Area %
1.	0.004	10.30	0.25	63.30	0.0016	21.17
2.	0.280	29.60	0.04	10.37	0.0016	20.34
3.	0.509	45.60	0.03	6.88	0.0013	16.74
4.	0.941	75.90	0.08	19.45	0.0032	41.75

Table 10: R_f values and Peak list of stem bark in ethanol extract obtained at 576 nm after derivatized with vanillin sulphuric acid reagent.

Sl. No.	Max R_f	Max pos. (mm)	Height	Height %	Area	Area %
1.	0.01	10.60	0.10	8.74	0.0019	3.05
2.	0.16	21.00	0.07	6.70	0.0018	2.91
3.	0.23	26.40	0.03	3.07	0.0009	1.39
4.	0.34	33.70	0.28	24.90	0.0120	19.33
5.	0.57	50.10	0.38	34.54	0.0251	40.31
6.	0.93	75.30	0.24	22.05	0.0206	33.02

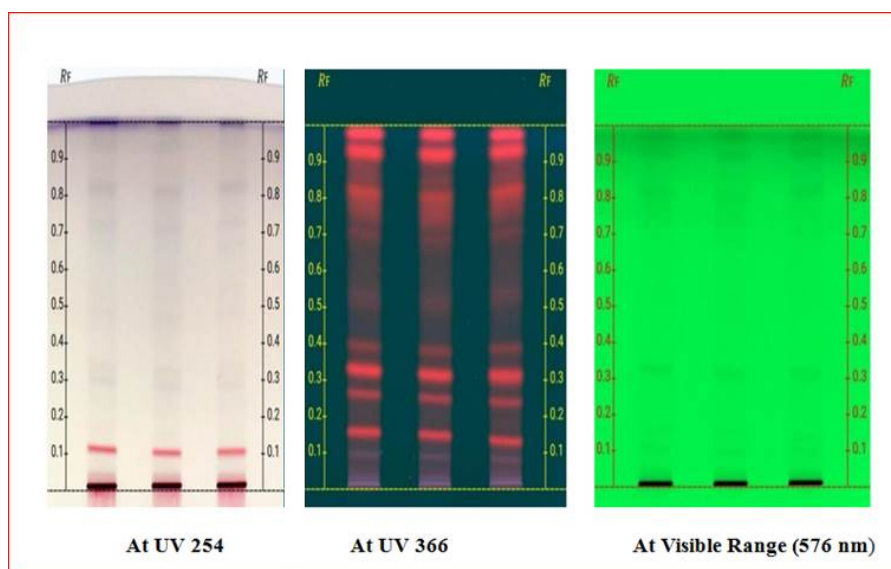


Figure 11: TLC plate photograph of Leaf Sample in ethanol extract.

At UV 254, At UV 366, At Visible Range (576 nm)

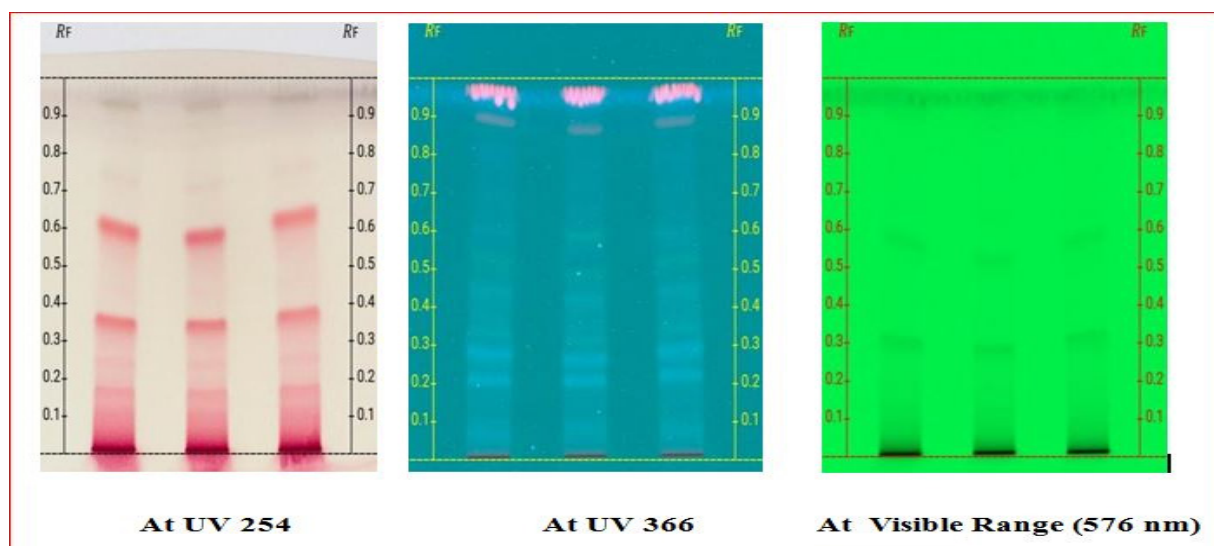


Figure 12: HPTLC finger print densitogram of leaf with ethanol extract scanned at 366 nm in Fluorescence mode.

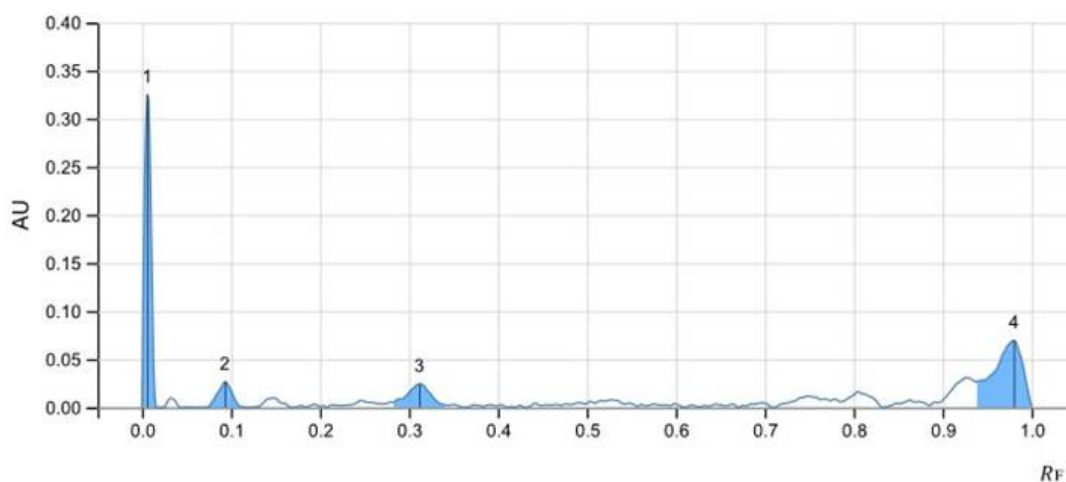


Figure 13: HPTLC finger print densitogram of leaf in ethanol extract scanned at 254 nm in Absorbance mode.

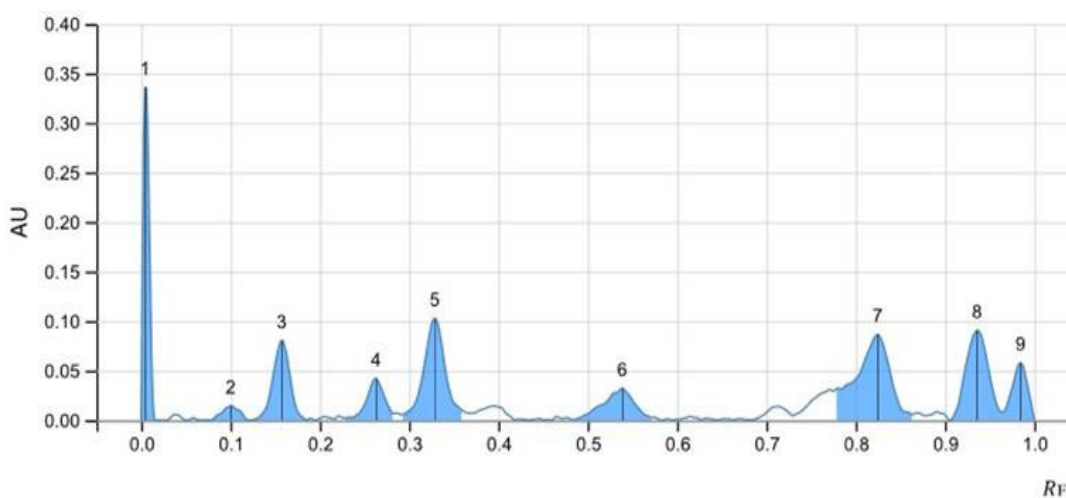


Figure 14: HPTLC finger print densitogram of leaf ethanol extract scanned at 576 nm after derivatized with vanillin sulphuric acid reagent.

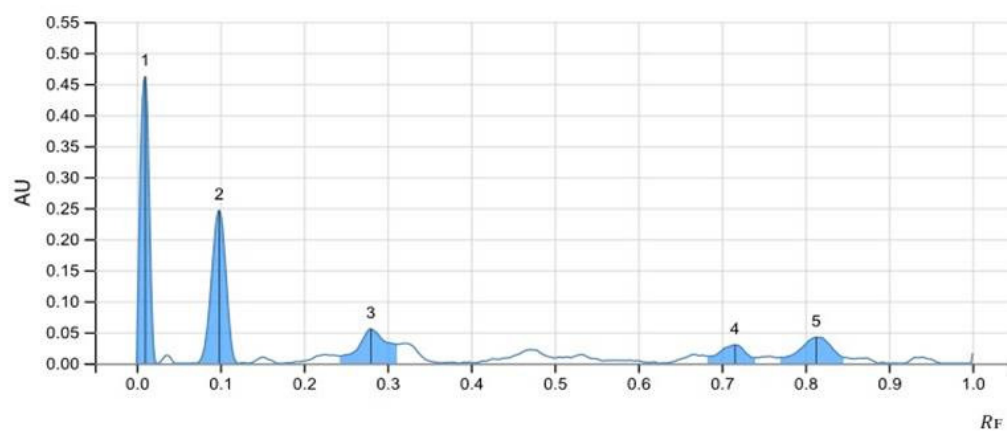


Figure 15: HPTLC finger print chromatogram of leaf with ethanol extract scanned at 366 nm in Fluorescence mode in Isometric view-sample preparation.

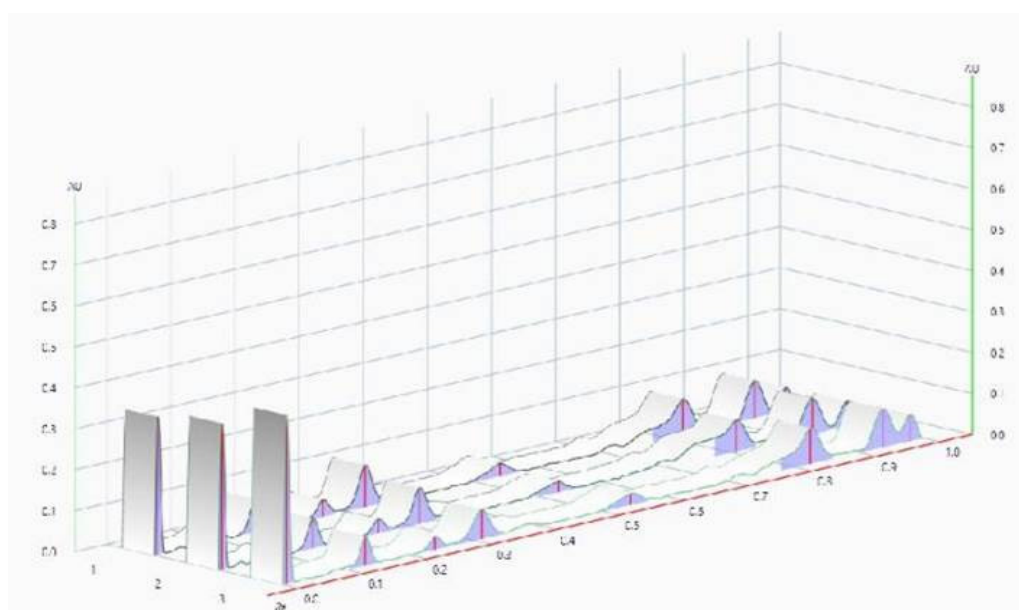


Figure 16: TLC plate photograph of Stem bark sample in ethanol extract.

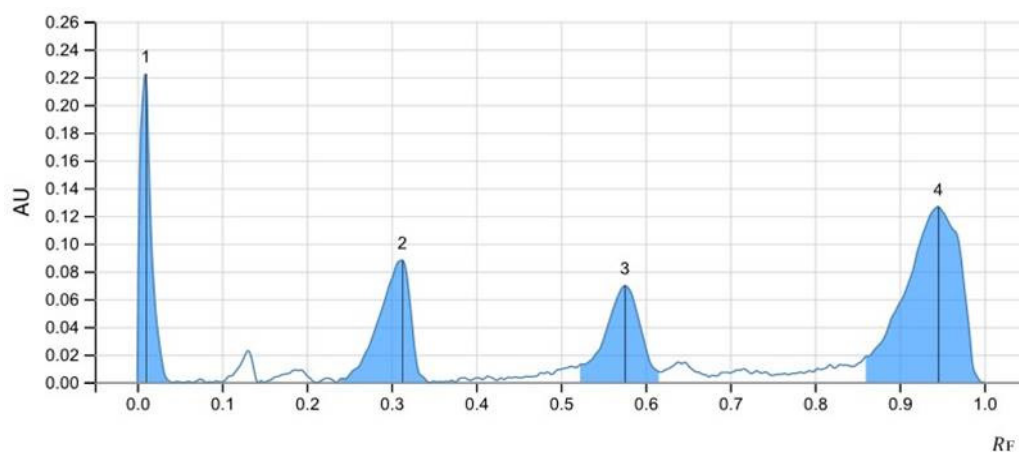


Figure 17: HPTLC fingerprint densitogram of stem bark with ethanol extract scanned at 254 nm in Absorbance mode.

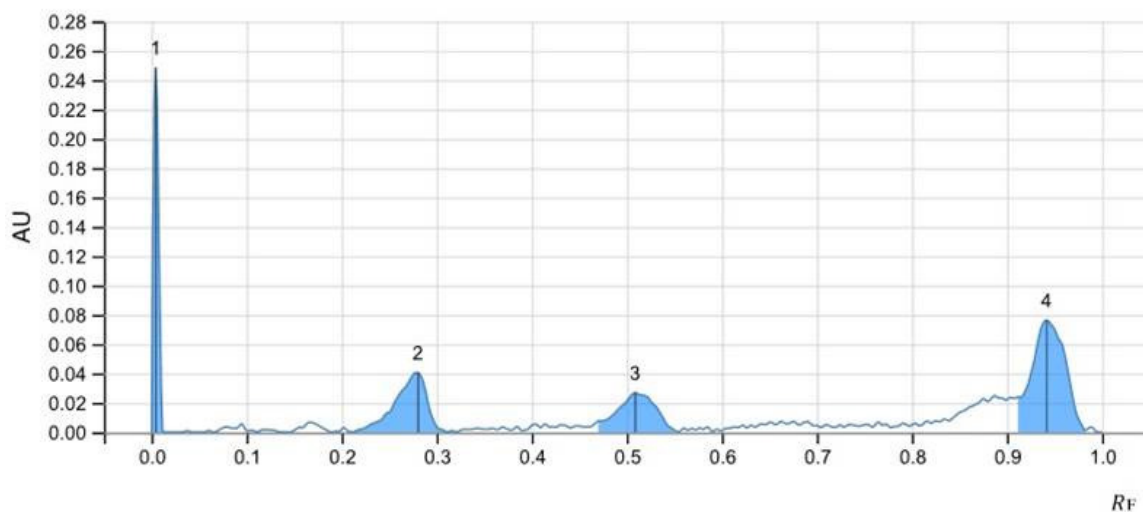


Figure 18: HPTLC fingerprint densitogram of stem bark in ethanol extract scanned at 366 nm in Fluorescence mode.

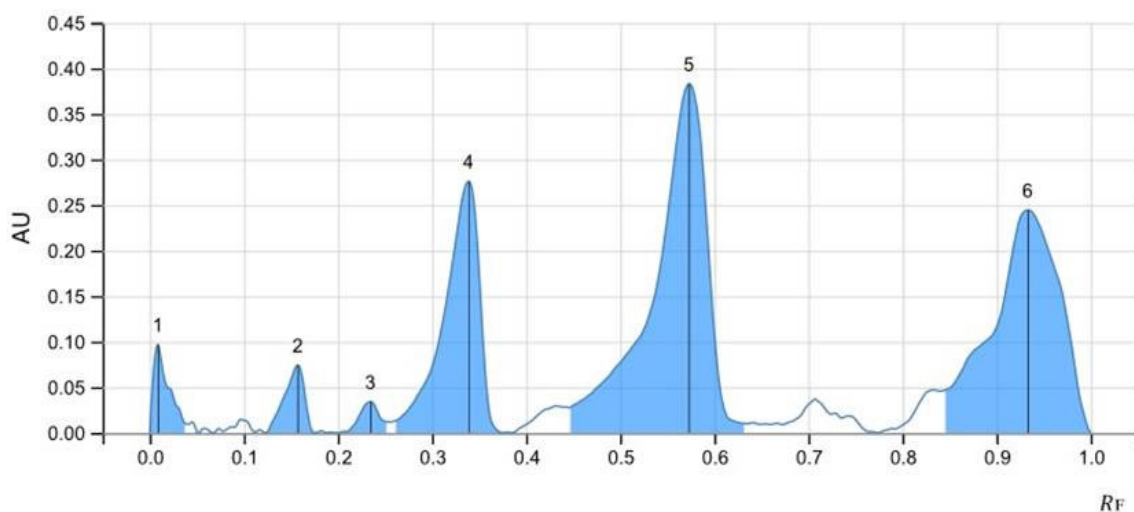


Figure 19: HPTLC fingerprint densitogram of stem bark in ethanol extract scanned at 576 nm after derivatized with vanillin sulphuric acid reagent.

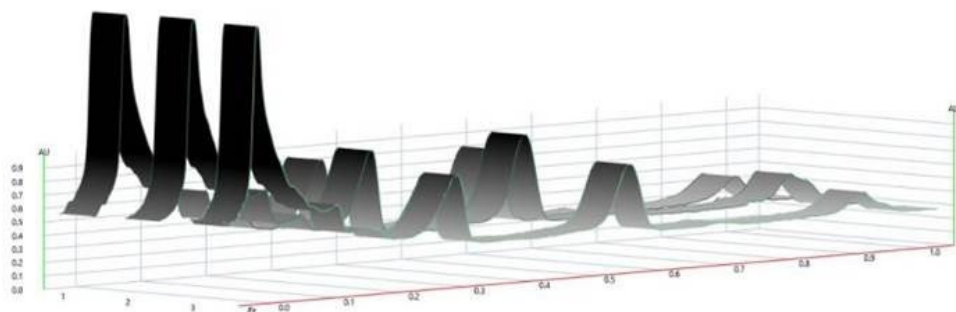


Figure 20: HPTLC finger print chromatogram of stem bark in ethanol extract scanned at 576 nm in Fluorescence mode in Isometric view.

CONCLUSION

This study provides a detailed pharmacognostical standard for the identification and authentication of *Elaeodendron glaucum* bark and leaves. These plant parts carry a significant ethnomedical value, and their identification in dried form is challenging. The findings from this research will serve as a valuable resource for future studies, including phytochemical analyses, formulation development, and quality assurance of herbal medicines derived from this plant.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh.

ABBREVIATIONS

HPTLC: High-Performance Thin-Layer Chromatography; **Rottb.:** Christen Friis Rottboell; **Pers.:** Christiaan Hendrik Persoon; **R_f:** Retention Factor; **TLC:** Thin-Layer Chromatography; **T.S.:** Transfer Section; **UV:** Ultraviolet.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Ramanjaneyulu Kanugala: Conceptualization, plant collection, experimental design, macroscopic and microscopic studies, data collection, and preparation of the original draft; **Goli Panchala Pratap:** Macroscopic and microscopic studies, data collection, and preparation of the original draft. **Mohd Kashif Husain:** Experimental design, macroscopic and microscopic studies, and critical revision of the original draft. **Ramanjaneyulu Kanugala, Goli Panchala Pratap, and Mohd Kashif Husain contributed equally to this work.** **Maramreddy Prathapa Reddy:** HPTLC analysis and physicochemical evaluation. **Gopal Sidama:** Preliminary phytochemical analysis and statistical analysis. **Mohammad Zakir:** Draft editing, manuscript review, and supervision. **Sudarsanam Gudivada:** Supervision, conceptualization, and experimental design.

SUMMARY

The present study was established to develop pharmacognostic standards for the leaf and stem bark of *Elaeodendron glaucum* (Rottb.) Pers., an important ethnomedicinal plant. Macroscopic and microscopic studies revealed the presence of specific diagnostic characters, including dorsiventral leaf structure, the presence of a single hypodermal layer, both epidermises embedded with prismatic calcium oxalate crystals, vascular tissues, fibres, and stone cells, which are essential for the authentication of the crude drug. Powder microscopy further confirmed the presence

of characteristic cellular components, including prismatic calcium oxalate crystals, stone cells and anomocytic stomata.

Physicochemical studies evaluated ash values, moisture content, extractive values, and pH, which indicate the quality and purity of the plant material. Fluorescence analysis exhibited distinct colour changes under visible and UV light, characteristics useful for drug identification and the detection of adulterants. Preliminary phytochemical screening evaluated the presence of important bioactive constituents from both leaf and stem bark.

The leaf and bark extracts were subjected to TLC analysis, and the plates showed various bands with different colours, characteristic of stem bark and leaves.

HPTLC fingerprinting studies provided distinct chromatographic profiles at 254 and 366 nm, with specific R_f values that differ between leaf and stem under UV light, indicating chemical variation between the two plant parts.

The study provides authentic pharmacognostical standards for the identification, quality control, and authentication of *Elaeodendron glaucum*.

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Cite this article: Kanugala R, Pratap GP, Husain MK, Reddy MP, Gopal S, et al. Pharmacognostical Standardization of Leaf and Stem Bark of *Elaeodendron glaucum* (Rottb.) Pers.: An Important Ethno-Medicinal Plant. *Pharmacog Res*. 2027;19(1):86-99.