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Original Article

Standardizing *Cordia sinensis* Lam. Stems: Microscopic Analysis and TLC Fingerprinting of Phenolics and Flavonoids

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ABSTRACT

Introduction: *Cordia sinensis* Lam., also known synonymously as *Cordia rothii*, is a small tree from the Boraginaceae family, widely recognized for its therapeutic value in traditional medicine. Although parts like the fruit, bark, leaves, and seeds have been extensively studied; comprehensive pharmacognostic investigations of the stem remain largely unexplored. This study aimed to conduct a detailed microscopic evaluation of *Cordia sinensis* Lam. stems, establish quality parameters, and perform Thin Layer Chromatography (TLC) profiling to identify flavonoids and phenolics. **Methods:** Authenticated stem samples of *Cordia sinensis* Lam. underwent thorough pharmacognostic, physicochemical, and phytochemical analyses following established protocols. Additionally, TLC was performed using both ethanolic and aqueous extracts of the stem to detect flavonoids and phenolics. **Results:** Key microscopic features identified included well-differentiated cork, phellogen, xylem fibers, medullary rays, calcium oxalate crystals, and various wood elements. Phytochemical screening confirmed the presence of flavonoids, phenolics, tannins, and steroids. The TLC profile further revealed distinct bands, indicating the presence of flavonoids and phenolics, with clear visualization under visible light, UV light at 254 nm, and 366 nm. **Conclusion:** These findings contribute valuable data for the standardization and quality control of *Cordia sinensis* Lam. stems in herbal medicine applications.

Keywords: *Cordia sinensis* Lam., Pharmacognostical Evaluation, Physicochemical Parameters, Phytochemistry, TLC Analysis.

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INTRODUCTION

Traditional herbal medicines have been integral to global healthcare systems, employed by various cultures throughout history. The plant kingdom, rich in secondary metabolites, serves as the primary source of traditional medicines. In India, the practice of herbal medicine is deeply rooted in ancient traditions, encompassing systems such as Ayurveda, Siddha, Unani, and Homeopathy, collectively offering a vast repository of therapeutic knowledge.[1-3] The genus *Cordia*, part of the Boraginaceae family, comprises over 250-300 species of trees and shrubs predominantly found in tropical and subtropical regions. These plants are renowned for their medicinal properties and have been utilized in traditional medicine for centuries. Phytochemical research on the *Cordia* genus has revealed a variety of compounds, including alkaloids, phenolics, flavonoids, quinones, triterpenoids, lignans, saponins, steroids, phenylpropanoids, polyphenols, porphyrins, and coumarins.[4-7]

Cordia sinensis Lam. (Syn.: *Cordia rothii* Roem. & Schult., *Cordia angustifolia* Roxb.), commonly known as grey-leaved saucer berry or grey-leaved cordia, is a notable member of this genus. Native to the arid and semi-arid regions of Africa, the Middle East, and parts of Asia, it thrives in dry savannas, bushlands, and along riverbanks.[8-9] Traditional healers have utilized various parts of this plant to treat a wide range of ailments, including headaches, coughs, fevers, chest pain, digestive issues, eye infections, joint pain, swelling, dental pain, parasitic infections, and as a diuretic, underscoring its significance in folk medicine.[10-11] A decoction made from the bark and roots of *Cordia sinensis* Lam. is employed to treat malaria, relieve stomachaches, and alleviate chest pain.[12] The bark decoction also possesses astringent properties and is used as a gargle.[13-14]

Despite its long-standing use in traditional medicine, *Cordia sinensis* Lam. has only recently garnered significant scientific interest. Initial phytochemical studies have identified bioactive compounds such as flavonoids, alkaloids, terpenoids, and phenolic substances, which are believed to contribute to its medicinal properties.[15-16] Pharmacological research has begun to substantiate traditional claims, uncovering the plant's potential in modern medical applications, including antibacterial, antioxidant, antimicrobial, and antidiabetic activities.[17-19]

Although *Cordia sinensis* Lam. is widely used in traditional medicine, the pharmacognostic properties of its stems remain underexplored. Comprehensive investigations into the stems' physicochemical properties and phytochemical composition are crucial for establishing a scientific basis for their medicinal applications. This study includes Thin Layer Chromatography (TLC) profiling to detect flavonoids and phenolic compounds within the stems, enhancing our understanding of their phytochemical makeup. We standardized the stems of *Cordia sinensis* Lam. by ensuring correct identification and assessing their safety and efficacy through detailed microscopic examination, physicochemical characterization, and phytochemical profiling. By bridging traditional knowledge with scientific validation, this research lays the groundwork for the safe and effective integration of *Cordia sinensis* stems into contemporary healthcare.

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MATERIALS AND METHODS

Collection and authentication of plant material

The stems of *Cordia sinensis* Lam. were collected from the local area of Mandwaria (Sirohi, Rajasthan). The plant material was identified and authenticated by Dr. V. Rama Rao, Research Officer (Botany) at the Central Ayurveda Research Institute in Bengaluru, India. A voucher specimen (RRCBI-mus470) has been deposited in the institute's herbarium for future documentation and reference. After collection, the stems were shade-dried under ambient conditions, powdered, and stored in a sealed container for subsequent studies.

Pharmacognostic Standardization

Morphology and Microscopy

In the study, the organoleptic characteristics of the stems of *Cordia sinensis* Lam. were evaluated, including shape, size, color, odor, taste, and fracture.

Microscopic studies involved preparing thin hand sections of the stems, which were washed with ethanol and stained using a Phloroglucinol-hydrochloric acid solution (1:1) to enhance the visibility of lignified cell components. These sections were then mounted in glycerine for microscopic examination.[20-21] Additionally, powder microscopy and histochemical studies were conducted to analyze the inclusions and detailed anatomical features of the material.[22-23]

Physicochemical Parameters

The physicochemical properties, including total ash, acid-insoluble ash, water-soluble ash, and alcohol-and water-soluble extractive values, were determined according to the procedures outlined in the WHO methods for quality control of medicinal plant materials (1998).[24]

Extraction Method

The stem powder of *Cordia sinensis* Lam. was sequentially extracted using a Soxhlet apparatus with solvents of increasing polarity. The extraction process began with petroleum ether, followed by chloroform, ethanol, and, finally, water (decoction). Each resulting extract was concentrated and dried using a water bath to obtain the respective extracts. Subsequently, the color, consistency, and extractive value of the extracts were evaluated.[25-27] These extracts were stored in airtight containers for further analysis.

Preliminary Phytochemical Investigation

The various extracts of the *Cordia sinensis* Lam. were analyzed for the detection of phytochemicals by using the standard procedures described by Khandelwal and Sethi (2015).[28]

Thin Layer Chromatography (TLC) Analysis of *Cordia sinensis* extracts

Ethanolic and aqueous extracts of *Cordia sinensis* stems were utilized for Thin Layer Chromatography (TLC) analysis to detect flavonoids and phenolic compounds. The ethanolic extract was prepared by adding ethanol in a 1:5 ratio (w/v) to the powdered stems and extracting for 5 hours at 50°C, followed by filtration and evaporation to dryness. Similarly, the aqueous extract was obtained using water in a 1:5 ratio (w/v), with extraction at 65^0 C for 5 hours, and subsequent filtration and drying. For TLC analysis, 10 mg/mL solutions of both extracts were prepared by dissolving 1 mg of the dried extract in 100 µL of methanol. The TLC plates (3 cm × 5 cm) containing a fluorescent indicator (F254) were used, and the mobile phase comprised chloroform, methanol, and formic acid in a 4.4:0.35:0.25 ratio. After spotting 5 µL of each extract, the plates were developed until the solvent front reached 90% of the plate height. The spots were visualized under visible light and UV light (254 nm and 366 nm) before and after spraying with 1% ethanolic $AlCl_3$ solution. Rf values for each compound were calculated using the standard formula, providing insights into the presence and movement of individual flavonoid and phenolic compounds in the extract.[29]

RESULTS AND DISCUSSION

Macroscopical evaluation

The outer bark of *Cordia sinensis* Lam. stems is brown, contrasting with the pale creamy-brown inner bark. The outer bark exhibits minor striations and scars, while the inner bark has fine striations. The stem's surface is smooth, yet hard and brittle. Upon drying, the stem bark channels and forms a single quill with fibrous fractures. It is odourless and has a slightly bitter taste (Figure 1).

Microscopy Evaluation: Transverse Section

The mature bark of *Cordia sinensis* Lam. exhibits 8 to 15 layers of cork composed of tangentially elongated storied cells and dead tissues of rhytidome. The cells in both the outer and inner cork vary in size and shape, containing yellow-brown contents. The cortical cells are thin-walled and occasionally contain stone cells and prismatic crystals of calcium oxalate. The phloem fibers are thick-walled, highly lignified bast fibers with pointed tips and companion cells. The medullary rays are multiseriate, wavy, and dilated towards the outer side, consisting of thin-walled, radially elongated cells. The vascular bundles are bicollateral, showing cambium presence with peripheral metaxylem and protoxylem towards the pith region, and are surrounded by highly lignified xylem fibers that provide mechanical strength to the stem. Additionally, abundant, highly lignified pericyclic fibers are observed in the cortical region of the stem (Figure 2).

Microscopy Evaluation: Powder Characteristics

Microscopic examination revealed cork cells containing yellow and brown substances on the surface. Cortical parenchyma cells were found to contain tannins and storage materials. Fragments of lignified phloem fibers exhibited reticulate thickening, while xylem vessels displayed reticulate patterns and bordered pitting. Wood elements, including tracheids and tracheae, were also identified. Stone cells were infrequently observed. Additionally, starch grains and prismatic crystals of calcium oxalate were distributed throughout the powder (Figure 3).

Physicochemical parameters

The physicochemical analysis of *Cordia sinensis* Lam. stems revealed essential parameters, including total ash, acid-insoluble ash, and water-soluble ash, along with ethanol and water extractive values. The results of these analyses are detailed in Table 1, which provides a concise overview of the inorganic matter and extractive contents.

Figure 1: *Cordia sinensis* Lam.: A - *Cordia sinensis* Lam. tree, B - *Cordia sinensis* Lam. stems.

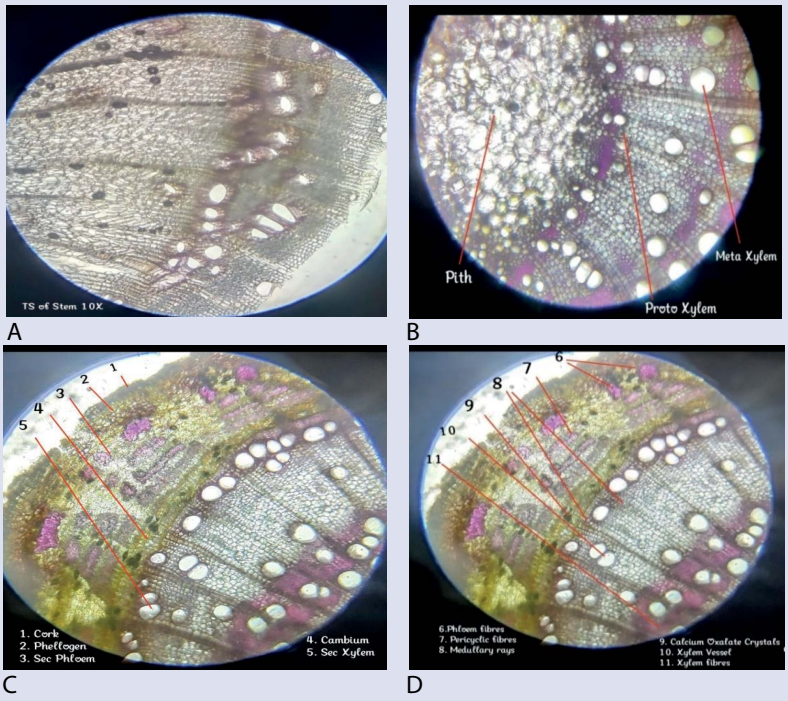

Figure 2: T S of *Cordia sinensis* Lam. stems: A - T S of stem under 10X, B - Pith Region, C - Cork and Cortex Region (1-5) and D - Cork and Cortex Region (6-11).

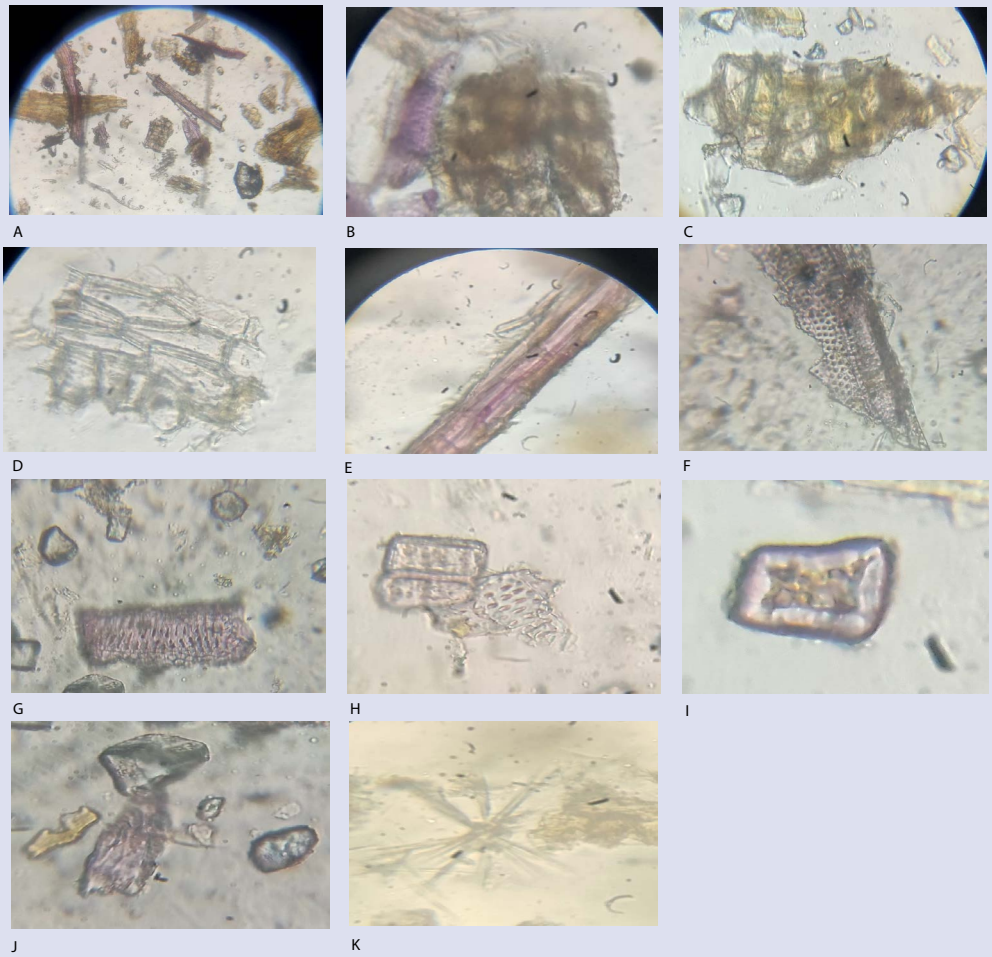

Figure 3: Powder Microscopy of *Cordia sinensis* Lam. stems: A - Under 10X, B - Cortex, C- Cork, D - Epidermis (Young stem), E - Phloem fibres lignified, F- Xylem border pitted, G -Xylem reticulate, H - Tracheids and trachea, I - Stone cells, J - Calcium oxalate crystals, K - Calcium sulphate crystals.

Table 1: Physicochemical Analysis of *Cordia sinensis* Lam. stems.

Parameter	% w/w
Total Ash	6.22
Acid Insoluble Ash	1.21
Water Soluble Ash	1.32
Alcohol soluble extractive value	2.9
Water soluble extractive value	6.8

Table 2: Histochemical reactions of *Cordia sinensis* Lam. stems.

Components	Reagents	Color	Histological Zone	Degree of Intensity
Cellulose	Iodine solution + Sulphuric acid	Bright blue	Cortex	*
Lignin	Phloroglucinol + conc.HCl (1:1)	Pink	Xylem Sclerenchyma	***
Starch	Iodine solution	Blue	Cortex, Pith	**
Mucilage	Methylene blue	Deep blue	Cortex, Pith	*
Resins	Sudan red III	Stains red	Cortex	*
Proteins	Millon's reagent	Brick red	Spongy Parenchyma	*
Fixed oils and Fats	Sudan red III	Red	Cortex, Pith	*
Volatile oils	Sudan red III	Red	Cortex, Pith	*
Tannins / Phenolics	Dilute ferric chloride solution	Greenish black	Cork, Cortex, Pith	***
Calcium oxalate	Sulphuric acid	Colorless needles	Cortex , Pith	*
Calcium carbonate	Sulphuric acid	Colorless needles	Cortex , Pith	*
Steroids / Triterpenoids	Antimony trichloride	Reddish pink	Cortex, Pith	**

***High, ** Moderate, *Slight

Table 3: Preliminary phytoprofile of stems of *Cordia sinensis* Lam.

	Petroleum ether	Chloroform	Ethanol	Water
Colour	Dark greenish yellow	Brown	Dark brown	Brownish black
Consistency	Sticky	Sticky	Sticky	Non sticky
Extractive Value % Yield w/w	0.73 %	0.96 %	3.16%	7.76 %

Table 4: Preliminary phytochemical investigation of *Cordia sinensis* Lam. stems.

Phytochemical Constituent	Tests	Petroleum ether Extract	Chloroform Extract	Ethanol Extract	Aqueous Extract
Carbohydrates	Molisch's test	-	-	+	+
	Fehling's test	-	-	+	+
	Benedict's test	-	-	+	+
Alkaloids	Mayer's test	-	+	-	-
	Dragendorff's test	-	+	-	-
	Wagner's test	-	+	-	-
	Hager's test	-	+	-	-
Proteins and free amino acids	Biuret test	-	-	+	+
	Millon's test	-	-	+	+
	Xanthoprotein test	-	-	+	+
	Ninhydrin test	-	-	+	+
Phytosterols	Salkowski's test	++	+	++	-
	Liebermann-Burchard's test	++	+	++	-
Saponins	Foam test	-	-	-	+
Glycosides	Modified Borntrager's test	-	-	+	+
	Legal's test	-	-	-	-
Tannins	Alkaline reagent test	-	-	+++	+++
	Bromine water test	-	-	+++	+++
	Potassium dichromate test	-	-	+++	+++
Phenolics	Iodine test	-	-	+++	+++
	Ferric chloride test	-	-	+++	+++
	Silver nitrate test	-	-	++	++
	Lead acetate test	-	-	+++	+++
Flavonoids	Ferric chloride test	-	-	+++	+++
	Shinoda test	-	-	+++	+++

+++ High, ++ Moderate, + Slight, - Absent

Table 5: TLC characteristics for Flavanoids and Phenols in ethanolic extract before spraying with 1% ethanolic $AlCl_3$.

Sample	Rf value	TLC profile characteristics		
		Visible light	254nm	366nm
CS-EE	0.05	Light orange	Violet	-
	0.27	-	Violet	-
	0.32	Green	Greenish brown	Fluorescent orange
	0.37	Green	Brown	Fluorescent orange
	0.42	Pale yellow	Light brown	-
	0.77	-	-	Fluorescent blue
	0.85	-	Light brown	-
	0.9	Pale green	-	-
CS-AE	0.05	Light orange	Violet	-
	0.27	-	Violet	-
	0.32	Green	Greenish brown	Fluorescent orange
	0.37	Green	Light brown	Fluorescent orange
	0.42	-	Light brown	-
	0.47	-	Light purple	-
	0.9	Pale green	-	-
	0.95	-	Light purple	-

CS-EE = *Cordia sinensis* ethanolic extract; CS-AE = *Cordia sinensis* aqueous extract

Table 6: TLC characteristics for Flavanoids in ethanolic extract after spraying with 1% ethanolic $AlCl_3$.

Sample	Rf value	TLC profile characteristics		
		Visible light	254nm	366nm
CS-EE	0.05	Light orange	Deep brown	Intense yellow-green
	0.17	-	-	Intense light blue
	0.27	-	Violet	-
	0.3	Green	Greenish brown	Light orange
	0.35	Green	Brown	Light orange
	0.42	Pale yellow	Light brown	-
	0.77	-	-	Fluorescent blue
	0.85	-	Light brown	-
	0.9	Pale green	-	-
CS-AE	0.05	Light orange	Violet	Green-yellow
	0.17	-		Intense light blue
	0.27	-	Violet	Fluorescent blue
	0.3	Green	Greenish brown	Fluorescent orange
	0.35	Green	Light brown	Fluorescent orange
	0.42	-	Light brown	-
	0.47	-	Light purple	-
	0.9	Pale green	-	-
	0.95	-	Light purple	-

CS-EE = *Cordia sinensis* ethanolic extract; CS-AE = *Cordia sinensis* aqueous extract

Histochemical color reactions

Histological reaction studies in plants are performed to quickly identify and localize important metabolites, within plant tissues. These studies serve as a preliminary screening tool to detect bioactive compounds before conducting more detailed phytochemical research.[30] In the context of *Cordia sinensis* stems, these reactions are crucial for standardizing plant materials and ensuring the consistent quality and efficacy of herbal medicines derived from them. Histochemical color reactions on the stems of *Cordia sinensis* Lam. are presented in Table 2.

Extraction of *Cordia sinensis* Lam. stems

The ethanol and water extracts demonstrated higher extractive values, measuring 3.16% and 7.76% respectively, whereas the petroleum ether and chloroform extracts exhibited lower extractive values, at 0.73% and 0.96% respectively. Further observations are detailed in Table 3.

Preliminary Phytochemical Investigation

Phytochemical analysis of various extracts from *Cordia sinensis* Lam. stems revealed the presence of several secondary metabolites, including alkaloids, tannins, saponins, flavonoids, and phenolics. These compounds were identified through qualitative screening techniques, and their concentrations across different extracts are detailed in Table 4, demonstrating the plant's rich phytochemical profile, which contributes to its medicinal potential and value in traditional herbal medicine.

Rf values and TLC profile characteristics

The results of the Thin Layer Chromatography (TLC) analysis indicated the presence of several distinct spots with varying retention factor (Rf) values when visualized under different light conditions, including visible light, UV light at 254 nm and 366 nm, both prior

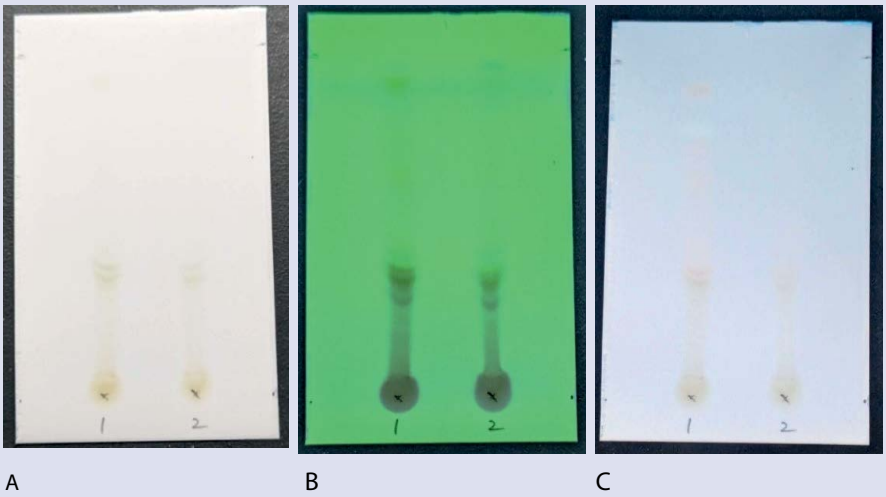

Figure 4: TLC images of the ethanolic (1) and aqueous (2) extracts of *Cordia sinensis* stems, visualized under (A) visible light, (B) short-wave UV light (254 nm), and (C) long-wave UV light (366 nm) before spraying with 1% ethanolic $AlCl_3$ solution.

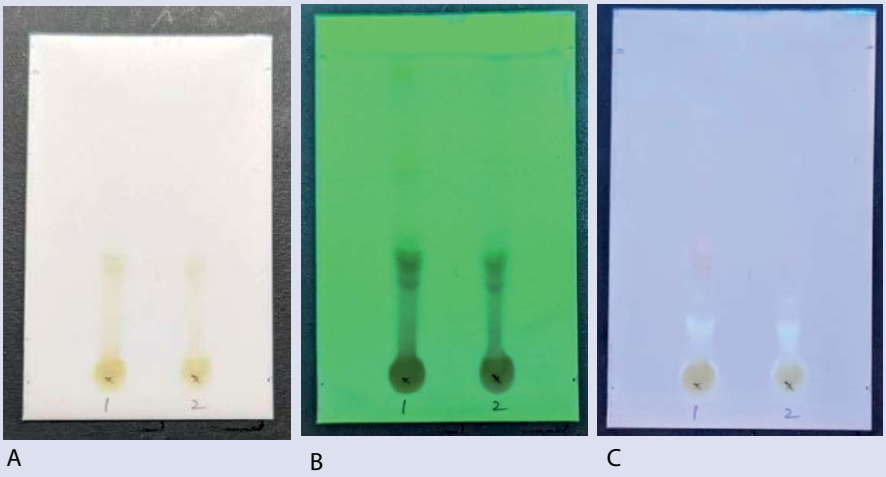

Figure 5: TLC images of the ethanolic (1) and aqueous (2) extracts of *Cordia sinensis* stems, visualized under (A) visible light, (B) short-wave UV light (254 nm), and (C) long-wave UV light (366 nm) after spraying with 1% ethanolic $AlCl_3$ solution.

to and following treatment with $AlCl_3$ solution. The findings for the ethanolic extract (CS-EE) and the aqueous extract (CS-AE) of *Cordia sinensis* stems are summarized in Tables 5 and 6, and illustrated in Figures 4 and 5.

Comparative Analysis: Both extracts exhibited comparable flavonoid profiles before and after the application of $AlCl_3$, with notable spots observed at Rf values of 0.32, 0.37, and 0.77 in the ethanolic extract, and at 0.32, 0.37, and 0.95 in the aqueous extract. Following the $AlCl_3$ treatment, additional fluorescent spots emerged at Rf 0.17 in both extracts, indicating the enhancement of flavonoid visibility under UV light due to the interaction with the reagent. Notably, the ethanolic extract exhibited a more pronounced fluorescent profile, particularly at Rf 0.05 and 0.77, suggesting a higher concentration of phenolic or semi-polar flavonoids that were more effectively extracted by the ethanol solvent. In contrast, the aqueous extract displayed unique non-fluorescent spots at Rf values of 0.47 and 0.95, which may indicate the presence of water-soluble phenolic compounds[31, 32].

CONCLUSION

The comprehensive investigation of *Cordia sinensis* Lam. stems has yielded significant insights through a combination of pharmacognostic, physicochemical, preliminary phytochemical analyses, and TLC fingerprinting. These investigations identified significant secondary metabolites, especially flavonoids and phenolics, recognized for their bioactive effects. Notably, the ethanolic extract displayed a wider variety of these compounds when visualized under UV light, indicating a greater extraction efficiency for certain phytochemicals compared to the aqueous extract.

This study supports the standardization of *Cordia sinensis* Lam. stems, providing essential data for their accurate identification, which is crucial for ensuring the safety and efficacy of herbal formulations. By establishing foundational information on the plant's pharmacognostic and phytochemical profile, this work also highlights its therapeutic potential. The findings underscore the need for further research

focused on isolating and characterizing specific bioactive compounds to deepen our understanding of its medicinal properties. Such studies could pave the way for developing new treatments based on the bioactive constituents of *Cordia sinensis*. Ultimately, this research lays an important groundwork for exploring the full therapeutic potential of this valuable species.

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ABBREVIATIONS

nm: Nanometer; **UV:** Ultraviolet; **WHO:** World Health Organisation

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