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Original Article

Pharmacognostic Standardization and Chromatographic Profiling of Indian Folklore Medicinal Plant Kesaraj-*Wedelia chinensis*

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ABSTRACT

Background: *Wedelia chinensis* (Asteraceae) is a traditionally used medicinal herb. Despite its extensive ethnomedicinal use, comprehensive Pharmacognostic and Phytochemical standards are required for proper identification, authentication, and quality control of the crude drug. **Objectives:** The present study was undertaken to establish phytochemical and pharmacognostic standards for Wedelolactone from leaves and stems of *Wedelia chinensis* with supportive chromatographic fingerprinting for quality evaluation and prevention of adulteration. **Materials and Methods:** The authenticated plant material underwent macroscopic, microscopic, and powder analysis. Physicochemical parameters were determined according to pharmacopeial standards Soxhlet extraction of ethanolic extracts of stems and leaves was done and phytochemical screening was carried out. Fluorescence Analysis, Thin Layer Chromatography, Gas Chromatography-Mass Spectrometry and High-Performance Thin Layer Chromatography, analysis was employed to produce fingerprint profiles of the plant extract. **Results:** The diagnostic macroscopic and microscopic characters of stem and leaves were also defined. The Physicochemical test showed fixed ash values, extractive value, and moisture content, which are within acceptable limits and showed purity and quality of the drug. Phytochemical test showed presence of alkaloids, glycosides, flavonoids, carbohydrates, proteins, amino acids, and steroids. Fluorescence showed fixed colors under visible and ultraviolet light. TLC and HPTLC showed bands which fixed point appeared in R_f value of about 0.55 which corresponds to Wedelolactone in *Wedelia chinensis.* The GC-MS test showed major components which are Stigmasterol, n-hexadecanoic acid, neophytadiene, and Long-chain alcohol derivatives. **Conclusion:** The study provides reliable pharmacognostic, physicochemical, and chromatographic standards for *Wedelia chinensis.* The established standards support authentication and quality control of *Wedelia chinensis*

Keywords Kesaraj, Pharmacognostic standardization, Phytochemical screening, Stigmasterol, *Wedelia chinensis*, Wedelolactone.

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INTRODUCTION

Wedelia chinensis it is a perennial herb belonging to the family Asteraceae. It is generally known in Chinese as *Wedelia, Bhringraja* in Hindi, and *Manjal Kirisalanganni* in Tamil (Indian Pharmacopoeia Commission, 2022). This herb is found in many parts of the Indian terrain, in the states of Assam, Uttar Pradesh, Andhra Pradesh, and in the coast of the country, besides being cultivated in Japan and China (Nomani *et al*., 2013). It is a weak, hairy, spreading herb, with opposite, simple, short-stemmed, subsessile leaves that have dense short white hairs. The herb also displays yellow axillary flower heads, while the fruits are oval with a hairy surface (Pharmacopoeia Commission for Indian Medicine and Homeopathy, 2016). The leaf is used in the traditional treatment of grey hair, while the juice, pounded root, and iron salts are used in the production of the ink or the black pigment (Bano *et al*., 2017). Because of the ample number of phenolic compounds present, the wound-healing properties of *Wedelia chinensis* have already been examined. The leaf paste of

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Wedelia chinensis is used in rural areas as a topical application for wounds, whereas the fresh leaf juice is employed as such for the treatment of acne, dermatitis, eczema, and skin disorders (Meena *et al.*, 2011). The plant is also naturally used for these medical conditions such as coughs, headache, baldness, gastrointestinal problems, rheumatic conditions, and gynaecological complaints including uterine bleeding (Koul *et al.*, 2012). Phytochemical analysis indicates the presence of Phytoconstituents that belong to the class of Flavonoids, Terpenoids, Steroids, and Coumestans like Wedelolactone in *Wedelia chinensis* (Ha *et al.,* 2023). Thus, the standardization of *Wedelia chinensis* involves the microscopic, physicochemical, and chromato analytical study including Thin Layer Chromatography, HPTLC, and GC-MS analysis, it plays an important role in recognizing, assessing the quality, and identifying the active components of the medicine that have pharmacological effects (Rehana *et al.*, 2013; Banu & Nagarajan, 2014).

MATERIALS AND METHODS

Plant Materials and Authentication

The leaves and stem of *Wedelia chinensis* was collected from Chennai, Tamil Nadu, India, and it was botanically identified and authenticated (Certificate No: S08072401C) by Dr. K.N. Sunil Kumar, Research officer and HOD Pharmacognosy, Central Council for Research in Siddha, Ministry of AYUSH, Government of India, Arumbakkam, Chennai-600106.

Macroscopic Characters

Freshly collected and shade-dried stems and leaves of *Wedelia chinensis* were subjected to macroscopic test. The external morphological features such as size, shape, color, surface characteristics, texture, fracture, odour, and taste of the stem and leaves were examined with the naked eye and using a simple hand lens. The diagnostic macroscopic characteristic was documented to ensure accurate identification and verification of the plant material (S. *et al.*, 2012).

Microscopic Characters

Fresh-hand transverse sections of the stem and leaf, including the midrib and lamina, of *Wedelia chinensis* were prepared by using a sharp blade. The sections were treated with chloral hydrate solution, stained with phloroglucinol-hydrochloric acid, mounted in glycerin, and observed under a compound microscope (Singh Bora & Pant, 2017). Anatomical features such as epidermis, hypodermis, cortex, mesophyll tissue, vascular bundles, xylem, phloem, pith, stomata, and trichomes were observed. For powder microscopy, the dried stem and leaf materials were ground into powder separately. They were then cleared using chloral hydrate, stained as required, mounted in glycerine, and observed under a microscope. Diagnostic characters such as stomata, trichomes, fibres, vessels, starch grains, and epidermal cells were noted (Langhi *et al.*, 2020).

Extraction

Soxhlet extraction method was utilized for extracting the phytoconstituents. Treated stem and leaves of *Wedelia chinensis* were coarsely powdered. The coarse powder of plant material was accurately weighed and measured and introduced into the thimble. Hexane was initially used to defat the material to remove fat and non-polar components. Further, extraction was carried out using ethanol as main solvent after defatting. The processes were repeated till siphon solvent became clear (Darah *et al.*, 2013).

Physicochemical investigation

The quality control parameters have been established through the quantitative physio-chemical analysis of the stem & leaves of *Wedelia chinensis*. In essence, all the parameters have been determined following the standard pharmacopeia procedure, & the experiments have been conducted thrice (Arunachalam *et al.*, 2021).

Determination of Ash Values

The weighed powder samples were accurately burnt in the silica crucible to obtain the total ash. The ash was boiled with distilled water, and thus the water-soluble ash was determined, and with the sample was treated with dilute hydrochloric acid, and the acid-insoluble ash was determined. The sulphated ash was prepared by moistening the ash with sulphuric acid and then heating it until it ignited. The respective weights were recorded after cooling (Rao & Xiang, 2009).

$$\text{Total ash}\left(\frac{w}{w}\%\right) = \frac{\text{weight of ash}}{\text{weight of sample}} \text{ x } 100$$

Determination of Extractive Values

Maceration of powdered drugs with alcohol and distilled water in the presence of shaking action for a specified time interval was utilized for determining alcohol-soluble and water-soluble extractive values. After filtering, evaporation, or drying, weighed calculations for determining the extractive value were performed (Hossen *et al.*, 2020).

$$\text{Extractive value (\%)} = \frac{(\text{Final weight-Initial weight})}{\text{weight of drug}} x100$$

Loss on Drying (LOD)

The loss on drying was determined by first precisely weighing a sample of the powdered drug and then placing it in a hot air oven maintained at a temperature of 105°C (Etkin, 2004).

$$\text{LOD\%} = \frac{\text{weight of sample before drying-weight of sample after drying}}{\text{initial weight of sample}} \text{x100}$$

Crude Fibre Content

The quantity of crude fibre was estimated by acid and alkali digestion of the defatted powder of the drug. The quantity of the crude fibre was measured by the weight of the dried residue after burning (Chandaka, 2025).

$$\text{Crude fibre \%} = \frac{\text{Loss in weight on ignition (W2-W3)}}{\text{Weight of sample(W1)}} \text{x100}$$

Where W_1=initial sample weight,

W_2=weight of crucible+ dried residue,

W_3=weight of crucible+ ash.

Foaming Index and Swelling Index

Foaming index was defined by making a solution with the powder drug and keeping it in water decoction and then shaking it to observe the continued formation of foam. Swelling index was determined by allowing the powder drug to swell in water and observing the volume of swelling (Singh *et al.*, 2014).

$$\text{Foaming index} = \frac{1000}{a}$$

Where a = The amount of decoction in millilitres used to make the dilution in the tube where foaming reaches a height of 1 cm.

Qualitative Investigation

Phytochemical analysis on the *Wedelia chinensis* employed the ethanol extracts of dried plant stems and leaves. In the initial procedure, the raw plant material had to be defatted with hexane solution. Following this, the plant material had to go through extraction using ethanol in the Soxhlet apparatus (Bhatnagar, 2016). After concentrating the extracted solution, phytochemical analysis followed. From standard tests, the major phytochemicals to be searched included alkaloids, carbohydrates, flavonoids, glycosides, tannins, phenols, amino acids, proteins, saponins, terpenoids, steroids, fixed oils, fats, and resin. In the analysis, the observations were recorded depending on the color reactions or precipitate formations, which were signs to confirm the presence or absence of the phytochemicals in question (Shaikh & Patil, 2020).

Fluorescence analysis

Fluorescence analysis was performed on powder of *Wedelia chinensis* using different chemical reagents. The samples were analyzed for fluorescence under visible light, short ultraviolet light at 254 nm, and long ultraviolet light at 366 nm. These aspects were recorded by with utmost attention (Vidya *et al.*, 2019).

Thin layer chromatography

Thin Layer Chromatography was carried out using the ethanolic extract obtained from the dried stems and leaves of *Wedelia chinensis*. The stationary phase in this process was a pre-coated silica gel plate. The solvent system used was Toluene, Acetone, and Formic acid in a ratio of 11:6:1, respectively. The air-dried chromatographic plates after development were observed under a visible light, UV at 254 nm, and UV at 366 nm. The R_f values of resolved spots were calculated (Kowalska & Sajewicz, 2022).

HPTLC

To develop the fingerprint profile, the ethanolic extract of the leaves and the dried stem of *Wedelia chinensis* was analyzed using High Performance Thin-Layer Chromatography. The stationary phase employed in this analysis was pre-coated silica gel 60 F254 aluminium plates. A tool called the CAMAG Linomat was used to apply the samples in the form of a band. The mobile phase used was a mixture of Toluene, Acetone, and Formic acid in the ratio of 11:6:1 by volume. was pre-saturated in two troughs prior to the development of dishes. Finally, when the dishes are allowed to dry by airing, observation of dishes was done using a CAMAG TLC Scanner at UV vapors of 254, 366, and 520 nm. The color and R_f values of developed samples were recorded (Selvaraj *et al.*, 2019; Balabhaskar & Vijayalakshmi, 2018).

GC-MS

GC-MS analysis was performed on the mixture of compounds obtained from the hydrolysis of the excipients. The GC-MS procedure was conducted on the mixture of compounds using a gas chromatography machine coupled to a mass selective detector operating on electron ionization mode at 70eV. Separation of the mixture from the mixture was achieved on HP-5MS capillary column of length 30 meter, I.D. of 0.25 mm, film thickness of 0.25 micron, at the following conditions: oven temperature: from 60° to 280° at the increments of 10° every minute, injection volume:1microliter, injection mode:split mode (10:1), injection port temperature:250°, head pressure:1.0ml/min, film temperature:230°, quadrupole temperature:150°, ion source temperature:230°, scanning of the ions:m/Z of 40 to 600. The compounds were dissolved in HPLC-grade methanol following sonication of the compounds to ensure the complete dissolvability of the reaction samples. Following sonication, the reaction samples were allowed to cool to room temperature (Joseph & Thangavel, 2023; Teoh *et al.*, 2023).

RESULTS

Pharmacognostic Characterization of *Wedelia chinensis*

Macroscopic Characters

The leaves of *Wedelia chinensis* were found to be opposite, subsessile, linear oblong to spathulate oblanceolate with entire margins. Figure 1 shows Macroscopic stages of plant as fresh material, dried stem and leaves, dried stem-leaf powder. The leaves measured up to 5 cm in length and 1.5 cm in width, with a fibrous fracture and were scabrous due to the presence of short white hairs. The upper surface was dark green to brownish green,

Table 1: **Physicochemical constants of *Wedelia chinensis*.**

Sl. No.	Parameters	*Wedelia chinensis*
I	Ash Values	
1	Total ash	18.93±0.24
2	Water soluble ash	11.21±0.21
3	Acid insoluble ash	5.83±0.27
4	Sulphated ash	21.76±0.34
II	Extractive Values	
1.	Alcohol soluble extractive	14.85± 0.28
2	Water soluble extractive	25.71±0.53
III	Loss on drying	12.11±0.02
IV	Swelling index	NIL
V	Foaming index	NIL
VI	Crude fibre content	19.6±0.21

while the lower surface appeared pale green to pale brown. On pressing the fresh leaves with water, the fingers turned black. The stem was cylindrical, yellowish brown to pale brownish green in colour, with an internodal distance of 6.5-7 cm. The stem exhibited a pubescent surface and fibrous fracture. Both stem and leaves had a slightly astringent and mucilaginous taste, with the leaf showing a characteristic odour.

Microscopic Characters

Stem

The transverse section of the stem had an almost circular shape the skin had a thin cuticle, and the epidermis consisted of irregularly shaped cells. some of which contained yellowish material. Underneath the epidermis, there were 2 to 4 layers of collenchymatous hypodermis. The cortex was aerenchymatous and contained parenchymatous cells with large spaces between them. The endodermis and pericycle were clearly defined, with the pericycle forming a sclerenchymatous cap over the vascular bundles. The vascular bundles were collateral, with xylem the

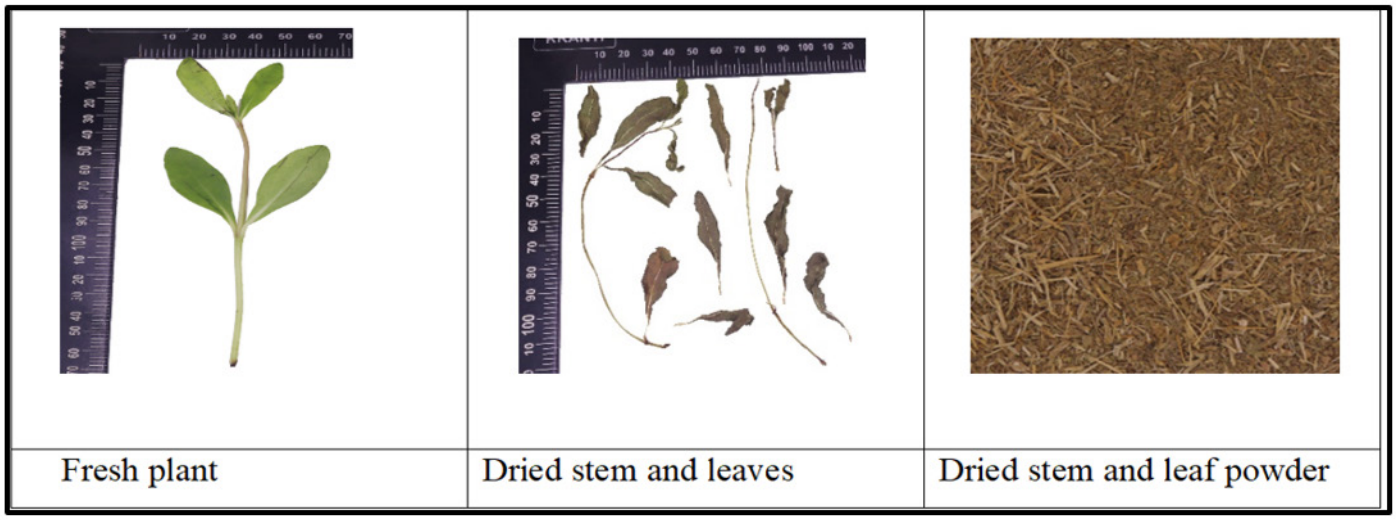


Figure 1: Macroscopic stages of plant.

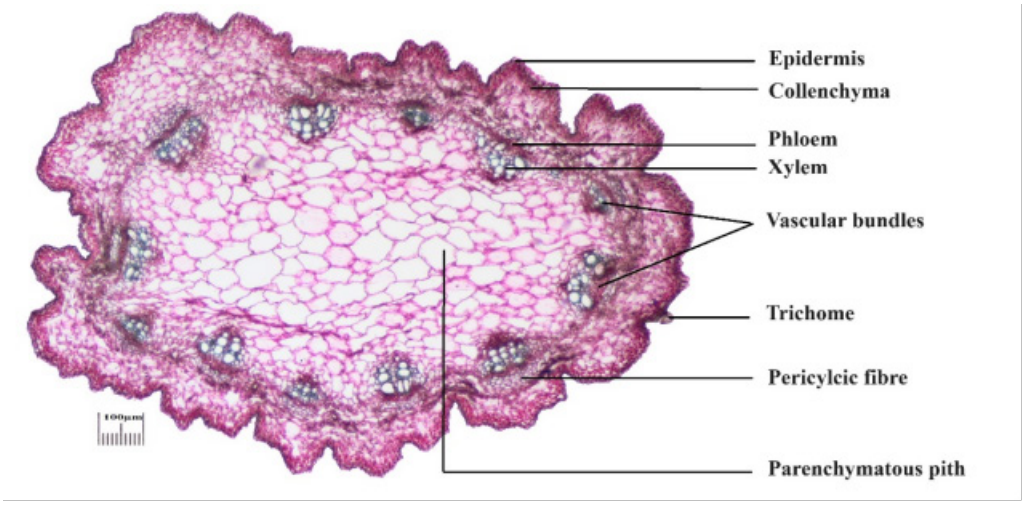


Figure 2: Detailed TS of *Wedelia chinensis* stem.

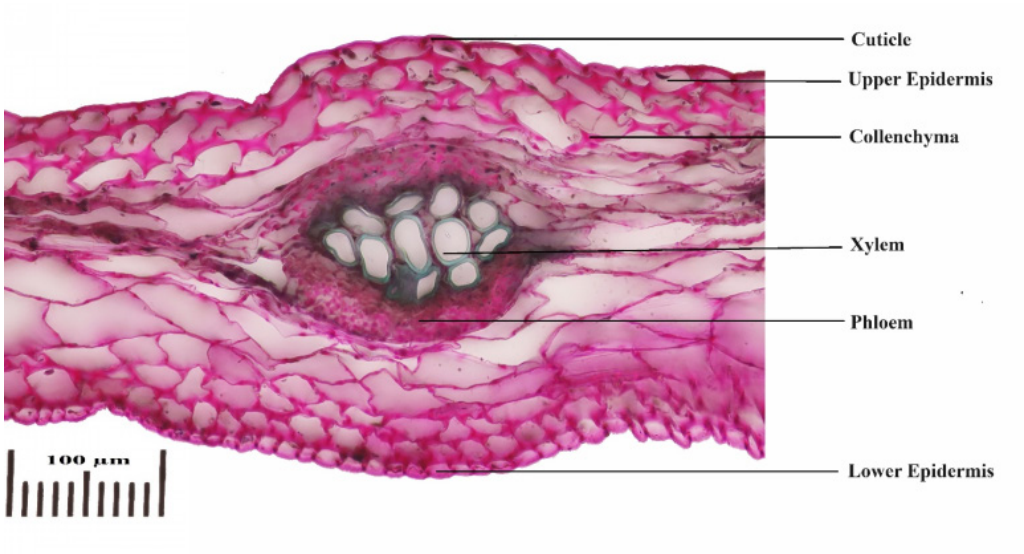


Figure 3: Detailed TS of midrib.

Table 2: Extraction method and its characters.

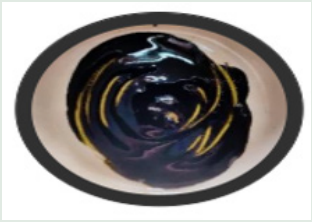
Plant name	*Wedelia chinensis*
Solvent	Ethanol
Method of Extraction	Soxhlet extraction
Physical nature	Semi-solid
Colour	Greenish yellow
Yield	8.45%
Appearance	

Table 3: Qualitative Phytochemical Analysis.

Sl. No.	Phytochemical test	*Wedelia chinensis*
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Glycosides	+
4.	Flavonoids	+
5.	Tannins and phenols	-
6.	Protein and amino acids	+
7.	Saponins	-
8.	Terpenoids	-
9.	Steroids	+
10.	Fixed oils and fats	-

Note: + indicates presence, - indicates absence.

phloem formed a continuous ring, consisting of sieve tubes and companion cells. The pith was centrally located, large, and parenchymatous. Figure 2 shows the detailed TS of *Wedelia chinensis* stem.

Leaf

Midrib

The transverse section of the midrib was slightly convex on the adaxial side, the structure is less convex, whereas the abaxial side is more convex. Both the upper and lower epidermis are covered with a thin cuticle. Collenchymatous tissue was present adjacent to both epidermal layers. The vascular bundle was centrally located and bicollateral, with normal xylem and phloem elements. Mesophyll tissue consisted of parenchymatous cells. Figure 3 shows a detailed transverse section of *Wedelia chinensis*-midrib.

Lamina

The lamina was dorsiventral in nature. Both the upper and lower epidermis were composed of a single layer and were covered with a cuticle. The surface view revealed the presence of anomocytic and anisocytic stomata. Two types of trichomes were observed:

(i) Long, single-celled trichomes that have a warty appearance, with 9 to 12 epidermal cells radiating from the base and,

(ii) Small, 3-5 celled trichomes with undifferentiated basal cells were present. The upper epidermis was succeeded by a single layer of palisade parenchyma, followed by the spongy parenchyma consisted of 6-8 loosely arranged layers. The mesophyll tissue was traversed by numerous vascular bundles. Figure 4 shows the detailed Transverse Section of *Wedelia chinensis*-lamina.

Powder Microscopy

Powder microscopy of *Wedelia chinensis* showed the presence of anomocytic and anisocytic stomata in both epidermal

surfaces. Thin-walled pitted fibres with blunt and sharp ends were observed. Multicellular covering warty trichomes and multicellular glandular trichomes were present. Simple, round to oval starch grains were observed, each having a central hilum and measuring between 20 and 25 µm. The vessels showed scalariform pitting and spiral thickening. Polygonal epidermal cells of the stem were seen in surface view. Few brownish ergastic contents were also observed. Figure 5 shows the powder microscopy of *Wedelia chinensis.*

Physicochemical Studies of *Wedelia chinensis*

Physicochemical Parameters

The physicochemical parameters such as moisture content, ash values (total ash, water soluble, acid insoluble ash and sulphated ash) and extractive values using various solvents were established for the stem and leaves. Physicochemical constants of *Wedelia chinensis* is summarized in Table 1.

Extraction Method

The dried stem and leaves of plants *Wedelia chinensis* were defatted using hexane and extracted with ethanol in accordance with the procedures described in the Ayurvedic Pharmacopoeia of India (API). Table 2 provides description of Extraction method and its characters of *Wedelia chinensis.*

Qualitative Phytochemical Tests

The qualitative identification of phytoconstituents from ethanolic extract of dried stem and leaves of plants *Wedelia chinensis was* performed and reported. Table 3 summarizes the Qualitative Phytochemical Analysis of *Wedelia chinensis.*

Fluorescence Analysis

Fluorescence analysis the powder was examined using visible light and ultraviolet light at wavelengths of 254 nm and 366 nm. Table 4 summarizes Fluorescence analysis of powder *Wedelia chinensis.*

Thin Layer Chromatography (TLC)

The TLC studies on ethanolic extract of dried stem and leaves of plants *Wedelia chinensis was* performed and tabulated. Table 5 shows TLC of *Wedelia chinensis* and it's R_f value and Figure 6 shows TLC of ethanolic extracts of stems and leaves of *Wedelia chinensis.*

HPTLC

The High Performance Thin Layer Chromatography (HPTLC) analysis of the ethanolic extract from the stems and leaves of *Wedelia chinensis* (Sample SD 4034) was performed using silica gel 60 F254 as the stationary phase and a mobile phase consisting of toluene, acetone, and formic acid in a ratio of 11:6:1 by volume. Table 6 shows Thin-layer chromatography analysis showing R_f values and spot colors of resolved components in three different sample. Figure 7 shows HPTLC fingerprint of *Wedelia chinensis* extract showing multiple resolved bands with characteristics fluorescence. The chromatographic profile showed well-resolved and distinct bands when scanned at 254 nm, 366 nm, and 520 nm, indicating the presence of multiple phytoconstituents. Figure 8 shows HPTLC chromatogram of *Wedelia chinensis* at 254 nm shows multiple peaks, indicating various phytochemical constituents**.** At 254 nm, several green-coloured bands were observed with R_f values around 0.14, 0.38, 0.46, 0.69, and 0.78, indicating UV-absorbing compounds. At 366 nm, blue, purple, pink, and red, fluorescent bands appeared with R_f values approximately 0.07, 0.30, 0.39, 0.46, 0.68, 0.73, and 0.79, suggesting the presence of fluorescent phytoconstituents. Figure 9 shows HPTLC chromatogram of *Wedelia chinensis* at 520 nm shows multiple peaks, indicating various phytochemical constituents. At 520 nm, violet, yellow, and green-coloured bands were observed with Rf values around 0.22, 0.39, 0.44, 0.47, 0.65, 0.81, and 0.89. The presence of a prominent band near $R_f \approx 0.55$ corresponds to Wedelolactone, a characteristic marker compound of *Wedelia chinensis*. The well-defined and reproducible HPTLC fingerprint confirms the chemical complexity of the extract and

Table 4: Fluorescence analysis of powder *Wedelia chinensis*.

Sl. No.	Solvent	S 1:9 Powder	Short UV (254 nm) Powder	Long UV (366 nm) Powder
1.	Distilled water	Puff coloured	Puff coloured	Brown
2.	1 N HCl	Puff coloured	Puff coloured	Black
3.	1N NaOH (ethanolic)	Brown	Fluorescence green	Dark blue
4.	1N NaOH	Brown	Fluorescence green	Dark blue
5.	50% HNO_3	yellowish green	Fluorescence green	Blackish Green
6.	50% H_2SO_4	Light yellowish green	Green	Light brown
7.	Acetic acid	Puff coloured	Dark brown	Black
8.	H_2S_4	Bright pink	Dark green	Black
9.	Iodine	Dark brown	Dark green	Indigo
10.	KOH 50%	Fluorescence Yellow	Fluorescence green	Brown

can be effectively used for identification, authentication and quality control of *Wedelia chinensis.*

GC-MS

The GC-MS chromatogram showed good resolution of the compounds from the *Wedelia chinensis* plant. There were several compounds from the *Wedelia chinensis* plant recognized in the chromatogram. Table 7 summarizes GC-MS identified compounds of *Wedelia chinensis* with their retention times and relative peak areas. Major constituents include stigmasterol, n-hexadecanoic acid, neophytadiene, and related compounds. Figure 10 shows GC-MS total ion chromatogram of *Wedelia chinensis* extract showing well-resolved peaks of major phytoconstituents. Compounds were identified by mass spectral matching with the NIST library. Five compounds were recognized from the chromatogram using retention times and matching of

Table 5: TLC OF *Wedelia chinensis* and it's R_f value.

Ethanolic extract	Solvent system	No. of spots	R_f Value
Wedelia chinensis	Toluene: Acetone: Formic acid (11:6:1)	10	0.39
			0.44
			0.476
			0.49
			0.52
			0.55 (Wedelolactone)
			0.63
			0.76
			0.82
			0.85

Table 6: Thin-layer chromatography analysis showing R_f values and spot colors of resolved components in three different samples.

R_f value	color	R_f value	color	R_f value	color
0.14	Green	0.07	Blue	0.22	Violet
0.38	Green	0.3	Purple	0.39	Yellow
0.46	Green	0.39	Pink	0.44	Violet
0.53	Green	0.46	Pink	0.47	Yellow
0.69	Green	0.55	Pink	0.65	Violet
0.78	Green	0.68	Red	0.72	Green
		0.73	Blue	0.81	Green
		0.79	Red	0.89	Violet

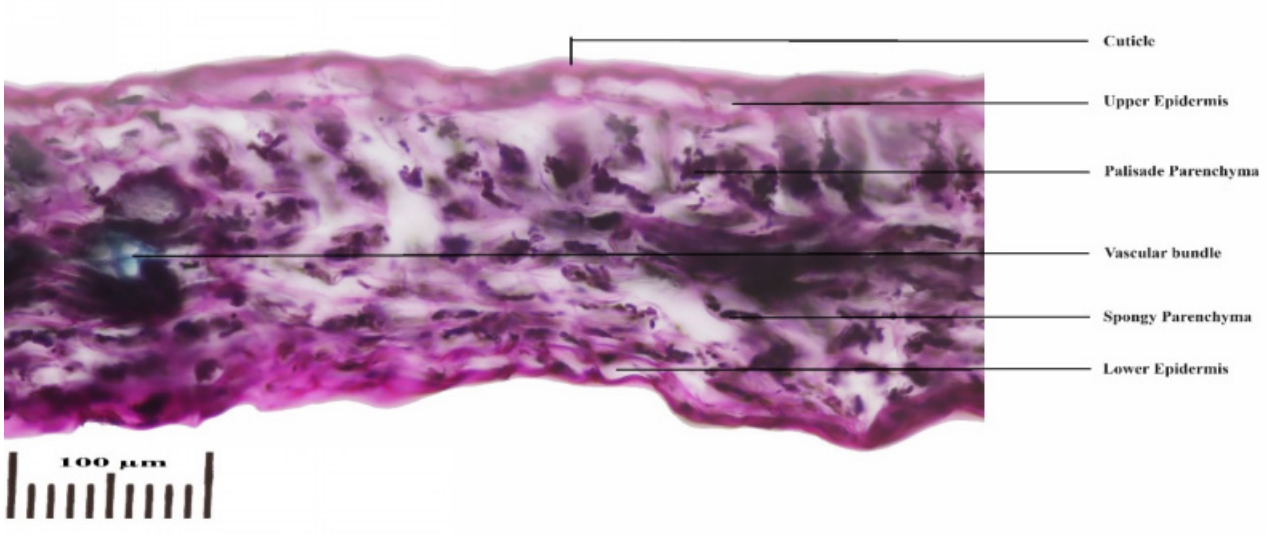


Figure 4: Detailed TS of lamina.

Table 7: GC-MS identified compounds of *Wedelia chinensis* with their retention times and relative peak areas.

Peak	R. Time	Area	Area%	Name	Activities
1	17.236	361294	5.5	Neophytadiene	Antioxidant, Anti-inflammatory, Anti-cancer
2	19.129	400618	6.1	Dibutyl phthalate	Nephrotoxicity, Anti-microbial, anti-malarial
3	19.205	630517	9.59	n-Hexadecanoic acid	Antioxidant, Anti-inflammatory, Anti-cancer, Anti-fungal
4	28.707	2013830	30.64	2,6,10,15,19,23-Pentamethyl-2,6,18,22-tetracosatetraen-10,15-diol	Antioxidant, Anti-inflammatory, Antifungal, cytoprotective
5	29.365	3166264	48.17	Stigmasterol	Antioxidant, Anti-inflammatory, Anti-cancer Anti-diabetic

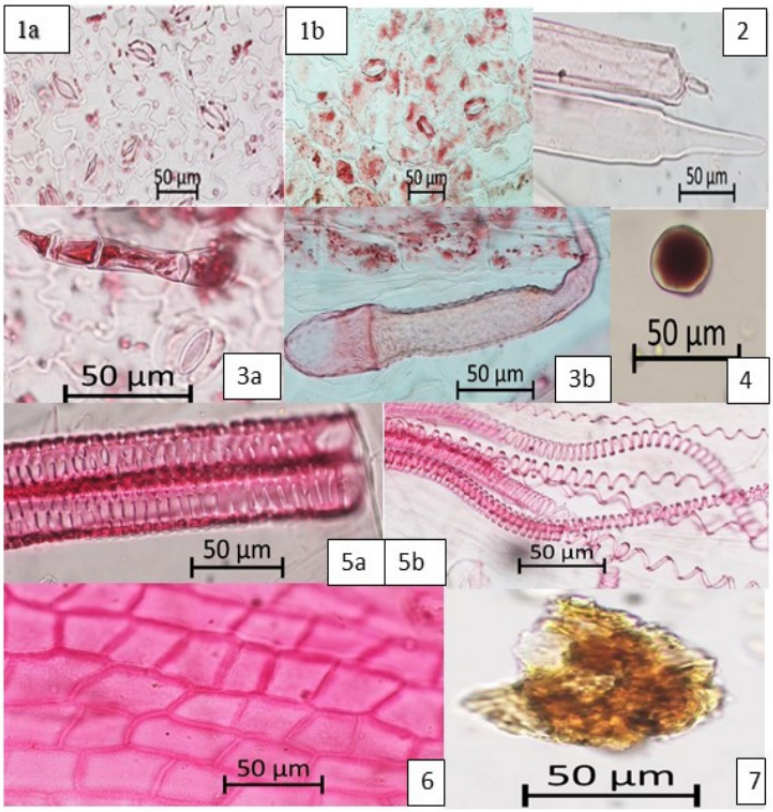


Figure 5: 1a-1b: Epidermal fragments showing anomocytic and anisocytic stomata. 2: Thin-walled, pitted fibre with blunt and sharp ends. 3b: Multicellular covering warty trichome. 3a: Multicellular glandular trichome. 4: Simple, round to oval starch grain with central hilum. 5a-5b: Xylem vessels showing scalariform pitting and spiral thickening. 6: Polygonal epidermal cells of stem in surface view. 7: Brownish ergastic contents.

mass spectral data. The dominant substance recognized from the *Wedelia chinensis* plant was stigmasterol, occupying 48.17% of the total peak area of compounds recognized. The retention time of stigmasterol corresponding to the dominant peak recognized on the chromatogram is 29.365 min. The second peak recognized on the chromatogram corresponding to the *Wedelia chinensis* plant belongs to 2,6,10,15,19,23-pentamethyl-2,6,18,22-tetracosatetraen-10,15-diol, occupying 30.64% of the total peak area of compounds. Other compounds recognized from the *Wedelia chinensis* plant were n-Hexadecanoic acid, dibutyl phthalate, and neophytadiene.

DISCUSSION

The current study has been able to set up some necessary pharmacognostic and physicochemical standards for the stem and leaves of the traditional Indian medicinal herb *Wedelia chinensis*. The pharmacognostic characters were found to be oppositely arranged, decussate, and subsessile with a scabrous surface and slightly astringent taste (Bano *et al.*, 2017). The microscopic characters included parenchymatous epidermis, bicollateral vascular bundles, a clear stem pith, dorsiventral leaves, multicellular trichomes, and anomocytic and anisocytic stomata. The physicochemical tests like ash values, extractive values, loss on drying, crude fibre content, foaming index, and swelling index helped to establish the purity, quality, and water stability of the crude drug, with higher water-soluble extractives establishing the traditional aqueous preparation (DSNBK, 2018). Phytochemical screening showed the presence of alkaloids, flavonoids, glycosides, steroids, proteins, and amino acids, which are linked to the established medicinal properties of the plant. Fluorescence analysis was an important supportive technique for plant authentication and standardization, as the powdered form of the drug and solvent extracts showed characteristic fluorescence properties under visible light and ultraviolet light of 254 nm and 366 nm. These properties are due to the presence of conjugated secondary metabolites, such as coumestans, flavonoids, and other phenolic compounds, which have natural fluorescence properties. The difference in fluorescence color and intensity under different wavelengths of light provided a rapid, sensitive, and non-destructive method for the detection

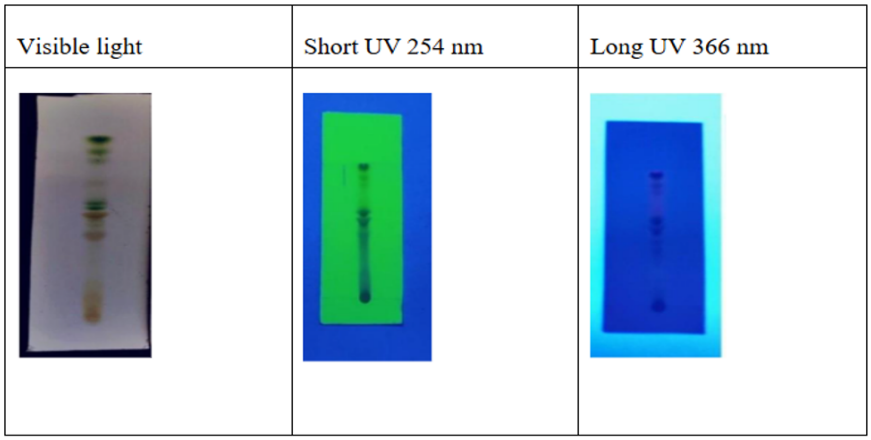


Figure 6: TLC of ethanolic extracts *Wedelia chinensis*.

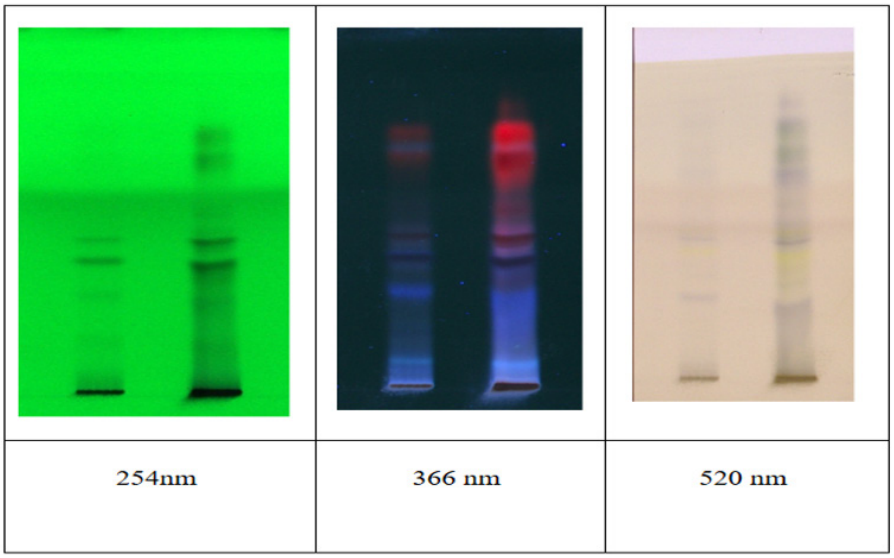
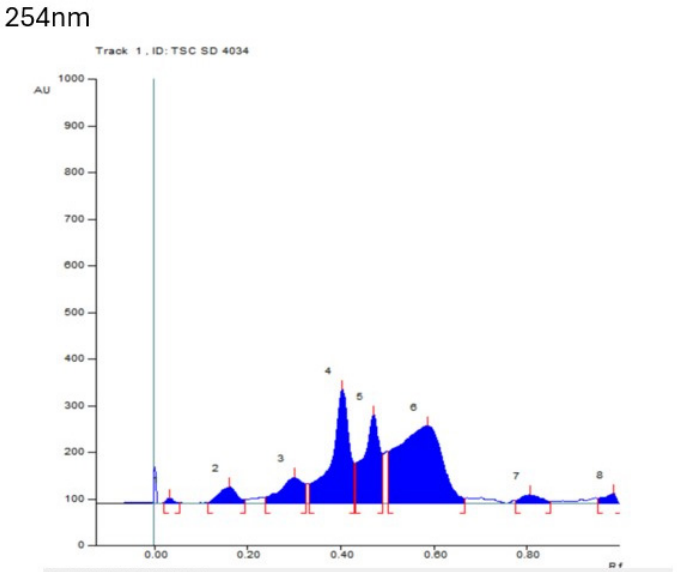


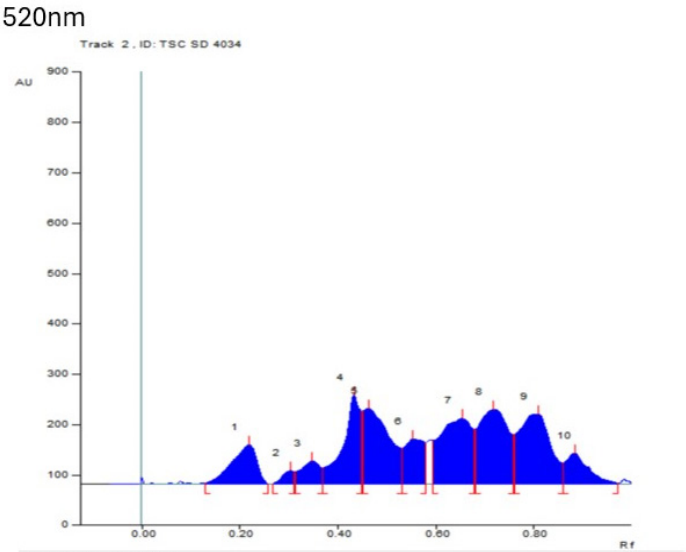
Figure 7: HPTLC fingerprint of *Wedelia chinensis* extract.



Track 2, ID: TSC SD 4034

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.08 Rf	0.8 AU	0.13 Rf	38.6 AU	3.29 %	0.18 Rf	0.0 AU	1415.9 AU	2.46 %
2	0.18 Rf	0.1 AU	0.29 Rf	89.9 AU	7.66 %	0.29 Rf	88.3 AU	3658.6 AU	6.36 %
3	0.29 Rf	88.4 AU	0.39 Rf	330.8 AU	28.16 %	0.41 Rf	92.3 AU	18169.9 AU	31.57 %
4	0.42 Rf	92.3 AU	0.46 Rf	326.2 AU	27.78 %	0.52 Rf	80.5 AU	12524.9 AU	21.76 %
5	0.52 Rf	80.6 AU	0.58 Rf	119.7 AU	10.19 %	0.65 Rf	16.3 AU	9217.5 AU	16.01 %
6	0.65 Rf	16.4 AU	0.72 Rf	118.4 AU	10.08 %	0.75 Rf	71.6 AU	5864.2 AU	10.19 %
7	0.75 Rf	71.7 AU	0.79 Rf	127.8 AU	10.88 %	0.85 Rf	0.7 AU	6276.5 AU	10.90 %
8	0.93 Rf	0.8 AU	0.98 Rf	23.1 AU	1.96 %	0.99 Rf	1.0 AU	435.9 AU	0.76 %

Figure 8: HPTLC chromatogram 254 nm.



Track 2, ID: TSC SD 4034

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.13 Rf	1.4 AU	0.22 Rf	78.0 AU	7.47 %	0.26 Rf	0.3 AU	3687.6 AU	7.48 %
2	0.27 Rf	0.1 AU	0.30 Rf	26.4 AU	2.53 %	0.31 Rf	23.6 AU	564.2 AU	1.14 %
3	0.31 Rf	23.8 AU	0.35 Rf	45.5 AU	4.35 %	0.37 Rf	32.4 AU	1642.3 AU	3.33 %
4	0.37 Rf	32.4 AU	0.44 Rf	176.7 AU	16.92 %	0.45 Rf	44.4 AU	5764.7 AU	11.70 %
5	0.45 Rf	144.6 AU	0.46 Rf	150.6 AU	14.42 %	0.53 Rf	70.7 AU	7505.2 AU	15.23 %
6	0.53 Rf	70.7 AU	0.56 Rf	88.7 AU	8.49 %	0.58 Rf	81.8 AU	3248.1 AU	6.59 %
7	0.60 Rf	86.2 AU	0.65 Rf	130.0 AU	12.45 %	0.68 Rf	08.4 AU	7822.8 AU	15.87 %
8	0.68 Rf	108.7 AU	0.72 Rf	148.0 AU	14.17 %	0.76 Rf	97.6 AU	8189.4 AU	16.62 %
9	0.76 Rf	97.9 AU	0.81 Rf	139.1 AU	13.32 %	0.86 Rf	42.0 AU	8261.1 AU	16.76 %
10	0.86 Rf	42.1 AU	0.88 Rf	61.3 AU	5.87 %	0.97 Rf	1.2 AU	2602.1 AU	5.28 %

Figure 9: HPTLC chromatogram at 520 nm.

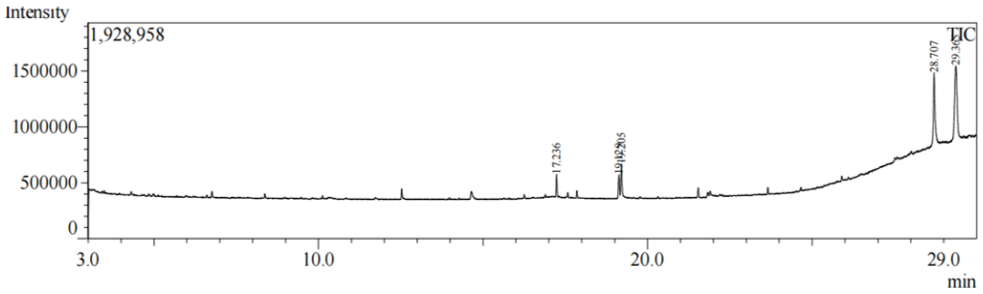


Figure 10: GC-MS total ion chromatogram.

of phytochemical consistency and plant identity (Sirsat *et al.*, 2025). Chromatographic analysis using TLC and HPTLC showed characteristic fluorescent bands with a prominent Rf value of 0.55 for Wedelolactone, while GC-MS analysis further supported the standardization of the plant by identifying the major phytoconstituents. The results obtained validate the quality, identity, and standardization parameters of *Wedelia chinensis* (Banu & Nagarajan, 2014).

CONCLUSION

The current study was able to provide detailed Pharmacognostic, physicochemical, and phytochemical standards for the stem and leaves of *Wedelia chinensis*. Their unique macroscopic and microscopic characteristics coupled with proven physicochemical parameters offer good standards of identification and authentication of the crude drug. The medicinal value of *Wedelia chinensis* is further confirmed by the presence of potential phytoconstituents with the aid of fluorescence analysis and TLC profiling. HPTLC fingerprinting enabled the identification of Wedelolactone as a key marker compound, thereby reinforcing the quality assessment and standardization of *Wedelia chinensis*. The data generated from this study can be effectively utilized for quality control, prevention of adulteration, and future standardization of *Wedelia chinensis* herbal extracts and related research. The HPTLC profile developed in this study can act as a standard fingerprint for regular quality control and identifying contamination. Additionally, the advanced analytical techniques such as GC-MS analysis was performed to achieve a comprehensive phytochemical characterization of *Wedelia chinensis*. Overall, the data generated from this study can be

effectively utilized for standardization, quality assurance, and further pharmacological exploration of *Wedelia chinensis* in herbal research and pharmaceutical development.

The manuscript is original, has not been published previously, and is not under consideration for publication elsewhere.

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ABBREVIATIONS

TPC: Total Phenolic Content; **TFC:** Total Flavonoid Content; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **H_2O_2:** Hydrogen Peroxide; **TLC:** Thin Layer Chromatography; **HPTLC:** High Performance Thin Layer Chromatography; **GC-MS:** Gas Chromatography-Mass Spectrometry; **UV:** Ultraviolet; **R_f:** Retention Factor; **LOD:** Loss on Drying; **TS:** Transverse Section; **API:** Ayurvedic Pharmacopoeia of India; **NIST:** National Institute of Standards and Technology.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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