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Pharmacognostic, physicochemical analysis and phytochemical screening of the leaves of W. trilobata L.

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Abstract : Objective: To evaluate the pharmacognostic properties, including the macroscopic, microscopic, physicochemical characteristics and phytochemical screening of the leaves of *Wedelia trilobata* (*W.trilobata*) **Methods:** Microscopic and macroscopic characteristics of fresh and dried leaf samples were analyzed. Organoleptic evaluations and physicochemical studies were performed using WHO-recommended parameters, and fluorescence behavior of the leaf sampleswas also analyzed. serial exhaustive extraction was done with various of solvents: Aqueous, Chloroforms, Ethanol, Methanol, Acetone, Benzene, Petroleum ether with increasing polarity using soxhlet apparatus. The phytochemical analysis was done by using the standard procedure. **Results:** Microscopic studies revealed the presence of three-lobate leaves, with the arrangements of spongy and palisade tissues. Physicochemical parameters such as foreign matter, moisture content, extractive values, ash content, pH, and fluorescence behavior of leaf powder were also determined. The results revealed that the leaves extracts contain Flavonoids, Terpenoids, Tannins, Phlobatannins, Saponins, Cardiac glycosides, Carbohydrate, Protein and Anthraquinones in major proportion. **Conclusions:** This is the first report on the pharmacognostic studies of *W.trilobata* and helpful in the characterization of the crude drug. Further phytochemical research is needed to identity the active product of *S. alata* may serve as leads in the development of new pharmaceuticals.
Keywords : *W.trilobata*, Fluorescence behavior, Pharmacognostic, Physicochemical, phytochemical screening.

Introduction

India has a rich heritage of traditional medicine constituting different components such as Ayurveda, Siddha, and Unani. The development of these traditional systems of medicines with the perspectives of safety, efficacy, and quality will help in the preservation oftradition in healthcare [1]. Pharmacognostic study is a preliminary step in the standardization of crude drugs. An in-depth pharmacognostic evaluation provides valuable information regarding the morphology and microscopic and physical characteristics of crude drugs.

Plants have formed one of the sophisticated traditional medicine systems and have been in existence for thousands of years [2-4],dating back to early humans [5]. They constitute an effective source of traditional and modern medicines and play an important role in health care programs.

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Most of the pharmaceutical industry is highly dependent on wild population for the supply of raw material for extraction of medicinally important compounds. The genetic diversity of medicinal plants in the world are getting endangered at an alarming rate because of ruinous harvesting practice and over-harvesting for production of medicines, with little or no regard to the future. Also, extensive destruction of the plant-rich habital as a result of forest degradation, agriculture encroachments, urbanization. In modern medicine, plants are used as sources of direct therapeutic agents, as model for new synthetic compounds and as a taxonomic marker for the elaboration of more complex semi synthetic chemical compounds [6].

Pharmacognosy is a simple and reliable tool by which complete information of the crude drug be obtained [7-10]. Today, with the current surge of interest in phytotherapeutics, the availability of genuine plant material is becoming scarce. Since crude plant drugs form the basis for the manufacture of numerous medicinal preparations, accurate determination of drug identity becomes an essential part of its study. It is extremely important to make an effort toward standardization of plant material as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies [11]. These studies help in identification and authentication of the plant material.

Wedelia trilobata (*Asteraceae*) is a creeping evergreen perennial with roots at the leaf nodes that spread widely. Itis a tropical perennial medicinal herb, with deeply lobed fleshy leaves, growing up to 10 inch height, spreading like a mat; it makes a dense cover, blossoms profusely, and the flowers are orange-yellow. It is a long lived (perennial) herb with a creeping or climbing habit [12].

Authentication and standardization are prerequisite steps, especially for herbal drugs and their formulations in traditional systems of medicine [13]. The present study is focused on the pharmacognostic standardization parametrics such as organoleptic, microscopic, and macroscopic analyses, along with the determination of ash and moisture content, extractive values, foreign matter, and fluorescence characteristics of the leaves of *W.trilobata* as described in the World Health Organization guidelines.

Material and Methods

Plant material

Leaves of *W. trilobata* were collected from Thanjavur, Tamil Nadu. The plant was identified, authenticated, and certified (CKOO1) by Dr. S. John Britto SJ, The Rapinat Herbarium and Centre of Molecular Systematics, St. Joseph College, Trichirappalli-620002.

Organoleptic evaluations

Organoleptic evaluations were performed according to the color, size, odor, and taste parameters.

Macroscopic and Microscopic analysis

Macroscopic analysis of the plant was carried out according to the method of Evans [14]. For microscopic studies, free-hand sections of the leaves were taken and stained with toluidine blue. Photomicrographs were taken using Image analyzer (OLYMPUS-BX51TF, Japan).

Physicochemical analysis

The leaves were shade dried and powdered using a mechanical grinder for powder analysis. The physicochemical characteristics of powdered leaves were determined as per the WHO guidelines [15]. The fluorescence characteristics of the plant material in different solvents were observed using visible, short UV, and long UV light [16]. Fluorescence behavior of leave powder and different extract with different chemical reagents such as sodium hydroxide, hydrochloric acid, nitric acid, and sulphuric acid was analyzed to detect the occurrence of phytoconstituents along with colour changes. The behavior of leaves power with different reagent and tested the staining of leaves power.

Preparation of Leaves Extract

The fresh, undamaged and disease-free leaves were selected and washed thoroughly with sterile double distilled water (DDW), shade dried and then coarsely powdered in a blender. The coarse powder was successively solvent extracted in a soxhlet extractor using different solvent such as ethanol, methanol, chloroform, acetone, benzene, petroleum ether, hexane, aqueous (Distilled water). The extracts so obtained were further dried in vacuum desiccators. The residue obtained from the extract was used for further studies by preserving it in refrigerator.

Phytochemical Screening

The freshly prepared different leaves extract were qualitatively tested for the presence of chemical constituents. They identified by characteristic colour changes and precipitation reactions using standard procedures [17, 18].

Results

Organoleptic characteristics

The powder of the dried herb of *W. trilobata* is dark green with a characteristic bitter odor and taste.The organoleptic characteristics of the plant are summarized in Table 1.

Table 1. Organoleptic characteristics of the plant

S.No	Organaleptic characteristics	Nature
1.	Color	Pale green
2	Sand & Silica	Absent
3	Odor	Pungent smell
4	Taste	Bitter
5	Insect infestation	Absent
6	Rodent contamination	Absent

Macroscopic and Microscopic analyses

The oppositely arranged leaves are stalkless or shortly petiolate, opposite-decussate, ovate dentate or three-lobed, irregularly toothed or serrate, usually with a pair of lateral lobes, fleshy, strigose on both surfaces, 4-7 cm long and 1.5-2.5 cm wide (Figure 1). The capitula are heterogamous, rayed, solitary on 3-10 cm long peduncles. Involucres are campanulate, hemispherical; bracts are 2 seriate, outer 1.0-1.2 cm long and 0.4-0.5 cm broad, ovate-lanceolate, chuffy, rigid, often recurved and exceeding the disk; inner shorter, lanceolate; receptacle convex, paleaceous. Paleae embrace the cypselas, concave. Ray florets 1-seriate, female,ligulate, 5-12 mm long; disk-florets many-seriate, tubular, bisexual. Corolla of the ray-florets are golden yellow with 2-3-fidlimb; that of disk-florets with 5-fid limb. Anthers are appendaged, and bases are sagittate with auricles. Stylar arms of outer florets are elongated, tip acute, hairy; florets flattened, with acute appendages, hairy. Cypselas of outer florets 3-angled, those of disk-florets sub-terete or sub-truncate, tuberculate. Pappus is a crown of shortfimbriate scales.

Figure 1. Morphological structure of *W.trilobata*

The transverse sections of the leaves of *W.trilobata* are generally thinner.*W.trilobata* usually has multiple vascular bundles in the midrib. It has three-lobate leaves, with arrangements of spongy and palisade tissues. The plant has trichomes on both the upper and lower epidermis (Figure 2). It also has resin ducts usually located near the vascular bundles in the midrib and veins (Figure 3).

Ads – Adaxial side
Ep – Epidermis
L La – Lower lamina
Mr – Midrib
Pa – Parenchymatous
Ph – Phloem
T – Trichomes
U La – Upper lamina
Vb – Vascular bundle
X – Xylem

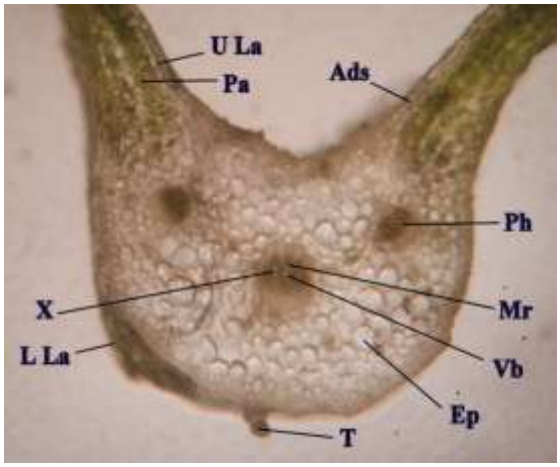
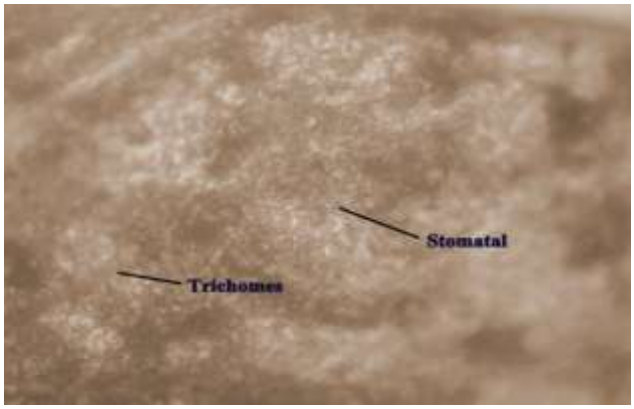


Figure 2.Transverse section of *W. trilobata*leaf



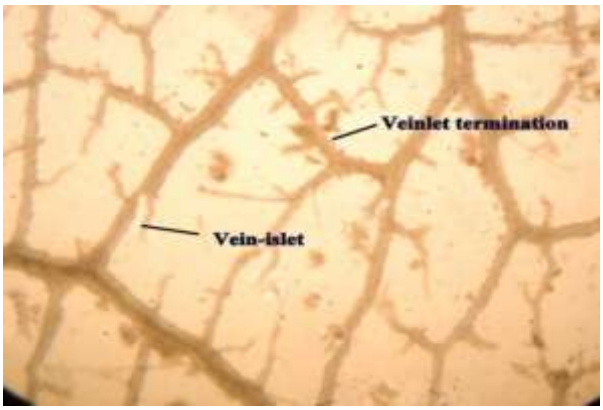


Figure 3.Vein terminal and islet of *W. trilobata* leaves

Physicochemical analysis

Powder analysis showed the presence of fibers, stomata, stone cells,and cork cells (Figure 4). The physicochemical characteristics, including foreign matter, moisture content, extractive values, and ash contents, were measured and are shown in Table 1. The pH of the sample was noted to be 7.5. Fluorescence characteristics of different solvent extracts under visible, short, and long light were determined and are shown in Table 2. Fluorescence behavior of the leaf powder with different chemical reagents was analyzed to detect the occurrence of phytoconstituents along with color changes as shown in Table 3.

Cork cells **Fiber** **Stone cells**

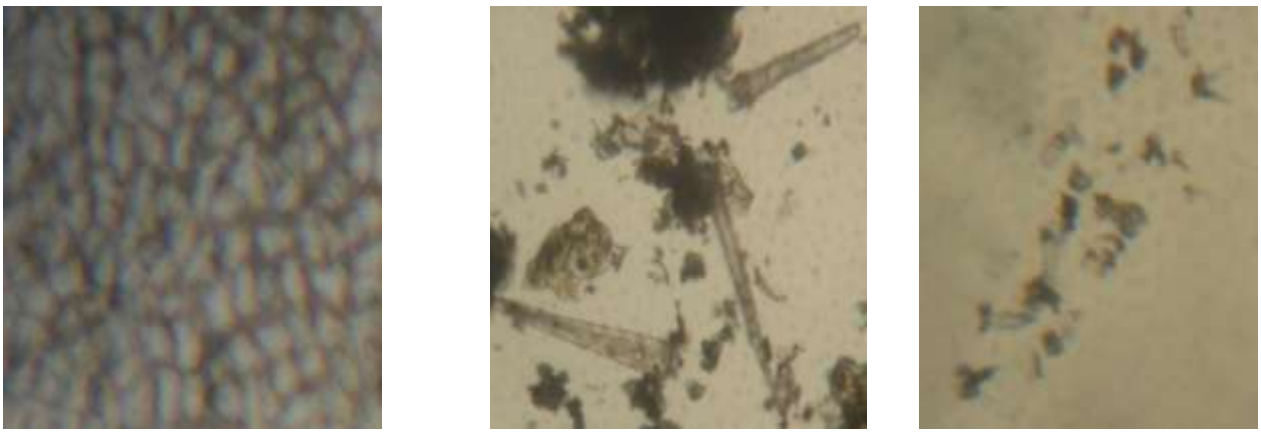


Figure 3. Powder analysis of *W.trilobata* leaf

Table 2.Determination of physic-chemical parameters of*W. trilobata* leaves

S.No.	TEST	RESULT (%)
1.	Total Ash	7.85
2.	Acid Insoluble Ash	1.30
3.	Water soluble Ash	0.85
4.	Alcohol extract value	9.35
5.	Water extract value	13.50
6.	Loss on dry	4.85
7.	Foreign matter	0.000

Table 3. Determination of pH

S.No.	TEST	RESULT (%)
1.	pH 1%	6.57
	pH 10%	6.06

Table 4. Fluorescence behavior of powdered leaftreated with different reagents

S.NO.	Testing	Visible Light	Short –UV (254nm)	Long – UV (365 nm)
1.	Powder(P)	Green	Green	Blue
2.	P + 1N NaOH in methanol	Dark green	Light green	Dark blue
3.	P + 1N HCL	Maroon	Dark green	Violet
4.	P + HNO_3(1:1)	Dark maroon	Light green	Dark blue
5.	P + H_2SO_4 (1:1)	Green	Dark green	Black
6.	P + 50% H_2SO_4	Light green	Light green	Blue
7.	P + 50% HNO_3	Light sandal	Light green	Blue

Table 5.Fluorescence behavior of different extracts treated with different reagents

Extract of plant	Visible	Short –UV (254nm)	Long – UV (365 nm)
Ethanol	Black	Black	Black
Methanol	Brown	Dark blue	Black
Chloroform	Green	Dark green	Dark green
Acetone	Black	Black	Black
Hexane	Maroon	Dark maroon	Blue
Petroleum extract	Dark green	Dark maroon	Blue
Aqueous	Light yellow	Light green	Light green

Table 6. Behavior of *W. trilobata* (Drug) with different reagent

S.No	Tested	Result
1	Drug green powder treated with 5% aqueous KOH	Powder settles down slowly Colour – Dark brown
2	Drug green powder treated with 5% aqueous $Fecl_3$	Powder float on the surface Colour – Yellowish brown
3	Drug green powder treated with iodine solution	Powder settles down immediately Colour – Light green
4	Drug green powder treated with 5% NaoH	Powder settles slowly Colour – Yellowish brown
5	Drug green powder treated with Hcl	Powder settle down slowly Colour – Light green
6	Drug powder treated with Glacial acetic acid	Powder settle down immediately Colour- Greenish
7	Drug powder treated with H_2SO_4	Powder settle immediately Colour – Greenish black
8	Drug powder treated with HNo_3	Powder settle down slowly Colour – Reddish brown

Phytochemical screening

Phytochemical evaluation of various leaves extracts of *S. alata* were done for the presence of Alkaloids, Flavonoids, carbohydrate, protein, Saponins, Terpenoids, Tannins, Anthraquinones, Phlobatannins, Cardiac glycosides, and the result are presented in Table 7.

Table 7.phytochemical screening of different solvent extract

Phytochemical constituents	Ethanol extract	Methanol extract	Hexane extract	Acetone extract	Benzene extact	Petroleumether extract	Aqueous extract
Alkaloids	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+
Protein	+	+	-	-	+	+	+
Terpenoids	-	-	-	-	+	-	+
Tannins	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-	-
Phlobatannins	-	-	-	-	+	-	+
Cardiac glycosides	+	+	-	-	+	-	+
Oxalate	+	+	_	+	+	-	+
Quinones	-	+	+	+	-	+	+
Triterpenes	+	+	+	+	+	+	+
Phytosterols	+	+	+	+	-	-	+
Phenol	+	+	+	+	+	+	+
Steroids	+	+	+	_	-	+	+
Fixed oil and fats	+	+	+	+	+	+	+
Gums and Mucilages	+	+	+	+	+	+	+
Sterol	+	+	-	-	+	-	+

Discussion

Studies of physicochemical characterization can serve as a valuable source of information and are usually applied in judging the purity and quality of the drug. The extractive values give an idea about the chemical constitution of the drug.In the present study, the extractive value of alcohol was the highest, followed by water. The ash value determines the earthy matter or inorganic composition and other impurities present along with the drug. The pharmacognostic standard for the leaves of *W. trilobata* is laid down for the first time in this study. To conclude, this study could be used as a diagnostic tool for the standardization of this medicinal plant and will be helpful in the characterization of the crude drug.This study also concludes that leaves contain number of pharmaceutically important phytochemicals like Alkaloids, Saponins, Flavonoids, Terpenoids, Tannins, Anthraquinones, Carbohydrates, Protein. A further study of the extracts is in progress to isolate, characterize and elucidate the structure of the bioactive compounds present which were responsible for potent pharmacological activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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