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Pharmacognostic and phytochemical investigation of *Wedelia trilobata* leaves

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Abstract

Objective of present work is to study Pharmacognosy of *Wedelia trilobata* leaves (WTL). It was investigated for morphological and microscopic characters, determination of leaf constants, ash values, extractive values, total phenol, tannin, flavonoid and alkaloid content and preliminary phytochemical study. Microscopical studies indicated the presence of single layer epidermis with cuticle, unicellular covering trichomes and anisocytic stomata. Stomatal number, stomatal index and palisade ratio was 6, 19.3% and 1:5 respectively. Total ash value, acid insoluble ash, water soluble ash and sulphated ash was 6.79%w/w 1.23%w/w, 2.61%w/w and 1.09%w/w respectively. Extractive value in methanol was found to be 7.39%w/w. Total phenol, total tannin, total flavonoid and total alkaloid content was found to be 10.67 mg gallic acid equivalent/g, 7.8 mg gallic acid equivalent/g, 5.8 mg quercetin equivalent/g and 1.22 mg atropine equivalent/g respectively. Preliminary phytochemical investigation showed the presence of alkaloids, glycosides, tannins, triterpenoids, carbohydrates and flavonoids.

Keywords: Pharmacognostic study, phytochemical investigation, *Wedelia trilobata* roxb

1. Introduction

Wedelia trilobata (L.) Hitchc (Synonym: *Sphagneticola trilobata* (L.) Pruski), belonging to the Asteraceae family and native to South America, is a perennial creeping herb which is known as an invasive plant at many tropical and subtropical areas including southern China [1-3]. *W. trilobata* is an herbaceous creeping perennial shrub, up to 70cm height, forms dense mounded mats over the ground. Leaves are glossy green, paler green below, with simple coarse white hairs, serrated margins, sometimes with a pair of lateral lobes. The stem is rounded, rooting at nodes, stolon's up to 2m in length or more. Flowers are borne on solitary or branched inflorescences, ray florets yellow (8-13 per head), central disc florets yellow and tubular, flowers freely produced throughout the year in warm tropics. Fruits are 3-cornered nuts, very small (3 - 5mm), with corky covering and topped by short scales, mature from green to brown, dispersed by water. New plants arise from nodes that root at the soil surface. Seed production is low and generally does not reproduce prolifically via seed [4]. It really is utilized by Indians as traditionally in the treatment of backache, muscle cramp, rheumatism, stubborn wounds, sores, swelling and arthritic pain, fever and malaria [5-7]. Phytochemical studies have revealed some structurally diverse chemicals from this plant, including terpenoids (sesqui-, diter-, and triterpenoids), steroids, flavonoids and phenolics, some of which showed significant bioactivities [7-13]. Numerous pharmacological activities of *W. trilobata* offers been reported such as antimicrobial [14], antioxidant [15], anti-inflammatory [15], wound healing [16], anthelmintic [17] and anticancer [15]. For the standardization and quality assurance purpose authenticity, purity and assay must be verified. Hence, the objective of the present study is to evaluate various pharmacognostic parameters such as macroscopy, microscopy, physicochemical and phytochemical studies of WTL.

2. Material and methods

2.1 Plant material

Fresh leaves of *Wedelia trilobata* Roxb. were collected in the month of January from local region of shirdi, Maharashtra, India and authenticated from Botany department of Sanjivani College of Pharmaceutical Education and Research, Kopergaon and voucher specimen is maintained. The fresh leaves was removed and dried in shade. The fresh leaves were used for the study of macroscopic and microscopic characters, whereas the dried leaves powder was used for determination of physicochemical investigations.

2.2 Macroscopic and organoleptic studies

The macroscopic study of a medicinal plant was helpful in rapid identification of plant material and also plays an important role in standardization of drug.

The fresh leaves was subjected to macroscopic studies, which comprised of organoleptic characters *viz.*, color, odour, appearance, taste, texture etc.

2.3 Microscopic studies

2.3.1 Leaves microscopy

Fresh leaves of *Wedelia trilobata* was selected for the microscopical studies. Microscopic sections were cut by free hand sectioning. Numerous temporary and permanent mounts of the microscopical sections of the leaves specimen were made and examined microscopically. Histochemical reactions were performed with Hydrochloric acid - Phloroglucinol to reveal the lignified elements, weak iodine solution for starch. Photographs of the microscopical sections were taken with the help of MOTIC photomicroscope provide with MOTIC IMAGE PLUS 2.0 software [18].

2.3.2 Powder microscopy

To study the presence or absence of various types of tissues or structures, the dried leaves is powdered using electric grinder, passed through sieve No. 60 and then subjected for microscopic studies [19-20].

2.4 Physicochemical parameters

Physiochemical values such as the percentage of ash value and extractive values were determined according to the official methods and as per WHO guidelines on quality control methods for medicinal plant materials. Fluorescence analyses of the powdered leaves were carried out by standard methods [21-28].

2.5 Determination of ash values

For determining ash content of drug, about 5 gm of powder was spread in a pre-ignited and weighed silica crucible. Then the crucible was incinerated gradually to make the crucible free from carbon. After cooling, the crucible was weighed to get the total ash content and then the ash was subjected for determining the acid insoluble and water soluble ash. The percentage of total ash was calculated by taking the air dried sample as standard [21-28].

2.6 Determination of extractive values

Considering the diversity and chemical nature of the drug, different solvents *viz.* petroleum ether, chloroform, ethyl acetate and methanol were used for determination of extractive values. About 5 gm of powdered material was subjected continuous Soxhlet extraction with 100 ml of petroleum ether, chloroform, ethyl acetate and methanol as solvents. Determination of extractive values of a crude drug is beneficial in its evaluation process wherever evaluation of chemical components is applicable. After extraction, the extracts are concentrated in rotary evaporator and dried in vacuum desiccators. Then the extractive values are calculated as percentage w/w of solvent soluble extractive with reference to the air dried drug [21-28].

2.7 Determination of moisture content

The moisture content was determined by loss of weight on drying (LOD) method. For this 5 gm of powdered drug was taken and kept in an oven at 105 °C till a constant weight was obtained. Amount of moisture present in the sample was calculated as a reference to the air dried material [21-28].

2.8 Fluorescence analysis

Crude drugs show their own characteristic fluorescence when exposed to ultraviolet radiation and is dependent on its

chemical constituents. This analysis is useful to identify adulterants during crude drug evaluation. In the present study, 5 gm of crude drug was taken in a watch glass and subjected to fluorescent analysis as such and after treatment with different reagents [21-28].

2.9 Preliminary phytochemical analysis

A systematic preliminary phytochemical screening of plant material is essential for identifying plant constituents and to establish a chemical profile of a crude drug for its proper evaluation. Extracts obtained by continuous soxhlet by using different solvents *viz.* petroleum ether, chloroform, ethyl acetate and methanol were subjected to standard qualitative phytochemical tests to identify the presence of chemical constituents (*viz.*, alkaloids, glycosides, tannins, flavonoids, sterols, fats, oils, phenols and saponins) present in them [21-28].

2.10 Determination of total phenol content

The concentration of phenolics in methanolic extract of leaves was determined using spectrophotometric method. Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consists of 1 ml of extract and 9 ml of distilled water was taken in a volumetric flask (25 ml). One millilitre of Folin-Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 ml of 7 % Sodium carbonate (Na₂CO₃) solution was treated to the mixture. The volume was made up to 25 ml. A set of standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Incubated for 90 min at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an Ultraviolet (UV) /Visible spectrophotometer. Total phenol content was expressed as mg of GAE/gm of extract [29-31].

2.11 Determination of tannin content

The tannins were determined by Folin - Ciocalteu method. About 0.1 ml of the methanolic extract of leaves was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35 % Na₂CO₃ solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of gallic acid equivalent /g of extract [32].

2.12 Determination of alkaloid content

Methanolic extract of leaves (1mg) was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of atropine equivalent /g of extract [32].

2.13 Determination of total flavonoid content

Total flavonoid content of methanolic extract of leaves was measured by the aluminium chloride colorimetric assay. The reaction mixture consists of 1 ml of extract and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 minutes, 0.3 ml of 10 % aluminium chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. A set of reference standard solutions of quercetin (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of quercetin equivalent /g of extract ^[32].

3. Results and discussion

3.1 Macroscopic and organoleptic characters

The morphological studies revealed that WTL is glossy green in color paler green below, with simple coarse white hairs, serrated margins. The fresh leaves has an agreeable odor, the taste is slightly acid.

3.2 Microscopic studies

3.2.1 Transverse section of the leaves

The transverse section of the leaves shows the typical morphoanatomical characteristics as Upper epidermis: single layered compactly arranged parenchymatous cells covered with cuticle (Fig.1). It shows presence of multicellular uniseriate covering trichomes (Fig.2). It also shows presence of anomocytic stomata (Fig.3). Lower epidermis: single layered compactly arranged parenchymatous cells covered with cuticle. It shows presence of multicellular uniseriate covering trichomes. It also shows presence of anomocytic stomata. Spongy mesophyll: loosely arranged parenchymatous big size cells than epidermal cells. It shows presence of starch grains and volatile oil (Fig.4). Palisade cells: tubular compactly arranged palisade cells are present only below upper epidermis in lamina region; it is not continued in midrib region (Fig. 5).

Midrib: it shows presence of upper epidermis, collenchyma, palisade cells, lower epidermis. Vascular bundles are present in the central part of transverse section of leaf passing through midrib which is composed of xylem on ventral side and phloem on dorsal side. Both are lignified in nature (Fig.1).

3.2.2 Powder characteristics under microscope

Starch grains: prism and cuboidal starch grains are observed when power stain with iodine solution.

Trichomes: Multicellular uniseriate covering trichomes.

3.3 Physicochemical investigation

Determination of physicochemical parameters of a crude drug is essential as it helps in identification and estimation of mishandling, adulteration and also in setting of proper standards. Various physicochemical parameters like ash values, extractive values, moisture content and fluorescence analysis on reaction with various chemical reagents were investigated and the results are presented in Table 1 and 2. Ash values of the drug gives an idea about the earthy matter

or inorganic composition and other impurities present along with the drug. The extractive values are primarily useful for the determination of the exhausted or adulterated drug and results are given in Table 3.

3.4 Preliminary phytochemical screening

Preliminary phytochemical screening mainly revealed the presence of steroids and triterpenes in petroleum ether extract; alkaloids in chloroform extract; flavonoids in ethyl acetate extracts, carbohydrate, reducing sugars, tannin and glycoside in methanol extract (Table 4).

3.5 Estimation of total phenol, tannin, flavonoid and alkaloid content

The total phenolic contents in the methanolic extract of leaves using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: $y = 0.0003x + 0.0113$, $R_2 = 0.9911$). The values obtained for the concentration of total phenols are expressed as mg of GA/g of extract. The total phenolic contents in the examined extract was found to be 10.67 mg gallic acid equivalent/g. The tannins contents was examined in the methanolic extract of leaves using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: $y = 0.0005x + 0.0017$, $R_2 = 0.9947$). The values obtained for the concentration of tannin contents are expressed as mg of gallic acid equivalent/g of extract. The concentration of tannins was found to be 7.8 mg gallic acid equivalent /g. The concentration of flavonoids in the methanolic extract of leaves was determined using spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of quercetin equivalent (the standard curve equation: $y = 0.0004x + 0.0029$, $R_2 = 0.9901$), and was found to be 5.8 mg quercetin equivalent /g. The alkaloid contents was examined and expressed in terms of atropine equivalent /g of extract (the standard curve equation: $y = 0.0004x + 0.0079$, $R_2 = 0.9977$). The concentration of alkaloid was found to be 1.22 mg atropine equivalent/g. The quantitative determinations of some pharmacognostic parameters are useful for setting standards for crude drugs. The physical constant evaluation is an important parameter in detecting adulteration or improper handling of the drug. Various ash values are important to determine the purity of the drug i.e. the presence or absence of foreign inorganic matter. Since the plant *Alianthus excels* is useful in the traditional medicine for the treatment of some ailment, it is important to standardize it for use as a drug. The pharmacognostic constants, diagnostic microscopic features and the numerical standards reported in this work could be useful for the compilation of a suitable monograph for its proper identification.

Table 1: Physicochemical parameters of WTL

S. No.	Physicochemical Parameters	% w/w (Mean ± SD)
1.	Total ash	6.32±0.52
2.	Acid insoluble ash	2.19± 0.75
3.	Water soluble ash	2.83± 0.47
4.	Sulphated ash	0.95 ± 0.53
5.	Foreign organic matter	1.96±0.29
6.	Moisture content	3.38±0.49

Table 2: Fluorescence analysis of different extracts and powder of WTL with various reagents

Extract /powder	Color in		
	Daylight	Short UV (254nm)	Long UV (365 nm)
Petroleum ether extract	Light green	Green	Dark green
Chloroform extract	Pale brown	Dark brown	Greenish brown
Ethyl acetate extract	Brown	Yellowish brown	Greenish brown
Methanol Extract	Greenish black	Black	Black
Powder	Brown	Brown	Brown
Powder + sodium hydroxide in methanol	Greenish black	Greenish black	Greenish black
Powder + sodium hydroxide in water	Dark brown	Brown	Dark brown
Powder + 1 N hydrochloric acid	Dark brown	Fluorescent green	Dark Brown

Table 3: Extractive values of WTL

S. No.	Type of solvent	% Extractive value Mean± SD
1.	Petroleum ether (60-40)	2.632± 0.72
2.	Chloroform	1.523 ± 0.92
3.	Ethyl acetate	3.328± 0.96
4.	Methanol	5.923±0.95

Table 4: Preliminary phytochemical analysis of various extracts of leaves

Type of constituent	Petroleum Ether	Chloroform	Ethyl acetate	Methanol
Alkaloids	-	+	-	-
Glycosides	-	-	-	+
Carbohydrate	-	-	-	+
Reducing sugars	-	-	-	+
Flavanoids	-	-	+	-
Tannins and phenolic	-	-	-	+
Protein and amino acids	-	-	-	-
Gum and resin	-	-	-	-
Triterpenoids	+	-	-	-
Saponins	-	-	-	+
Steroids and sterols	+	-	-	+

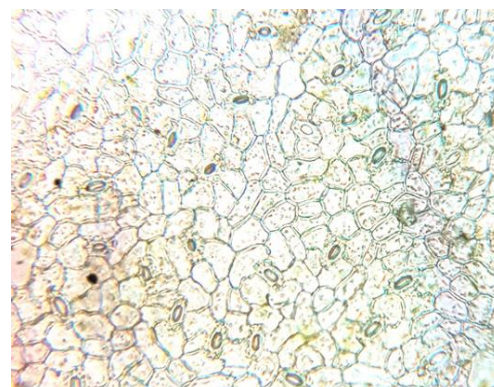
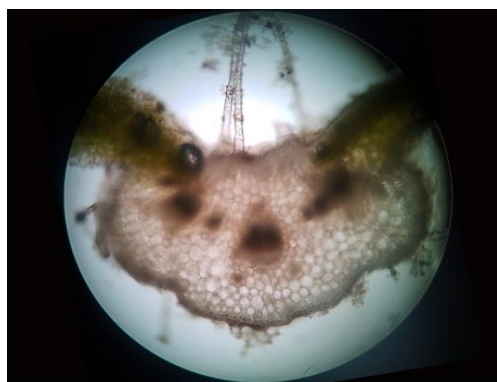
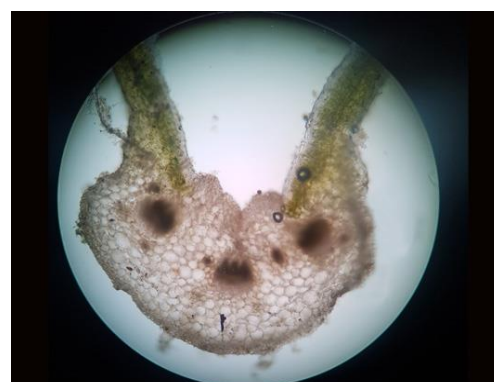
**Fig 1:** Transverse section of leaf**Fig 3:** Anomocytic type of stomata**Fig 2:** Transverse section of leaf showing multicellular uniseriate covering trichomes**Fig 4:** Transverse section of leaf showing palisade cells



Fig 5: Transverse section of leaf showing starch grains

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