OPEN ACCESS Pharmacologia

ISSN 2044-4648
DOI: 10.5567/pharmacologia.2017.83.89

Research Article
Pharmacognostic Standardization of *Wedelia chinensis* Merrill Leaf

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Abstract

Background and Objectives: *Wedelia chinensis* (family: Asteraceae), commonly known as Pilabhangra has been traditionally used for the treatment of various ailments like jaundice, cholagogue, diarrhoea, cephalalgia and respiratory disorders to reduce the mental tension and anxiety. Despite a long history of uses, no scientific pharmacognostic evaluation has ever been carried out on this plant, hence the objective of study was to investigate pharmacognostic studies and physico-chemical analysis of *Wedelia chinensis* (*W. chinensis*) leaf. **Methodology:** Transverse sections of *W. chinensis* leaf under the microscope showed scattered vascular bundles, various types of multicellular covering trichome and occasional glandular trichome etc. The powdered drug under the microscope showed various types of multicellular covering and occasional glandular trichomes, uniseriate (collapsed) covering trichome, paracytic stomata, xylem vessels etc. **Results:** Phytochemical screening showed presence of mainly phenolic, flavonoids, saponins and tannin compounds in hydroalcoholic extract of *W. chinensis.* In physico-chemical analysis, ethanol and water soluble extractive value were estimated to be 2, 7.4, 2.75 and 4.25%, respectively. Moisture content of air dried leaves of *W. chinensis* was found to be 8.25%. The total ash, acid insoluble ash, water soluble ash and sulphated ash was estimated to be 14.66, 1.32, 9.79 and 7.18%, respectively. **Conclusion:** The standardization parameters evaluated in the present study would provide a way for the standardization of raw materials and formulation of herbal origin. Further, the findings of the present investigations could also serve in the correct identification and preparation of a monograph on this plant.

Key words: *Wedelia chinensis,* pharmacognostic evaluation, physico-chemical analysis, glandular trichomes, hydroalcoholic extracts

Citation: Kundan Singh Bora and Ankita Pant, 2017. Pharmacognostic standardization of *Wedelia chinensis* Merrill leaf. Pharmacologia, 8: 83-89.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant parts or extracts obtained there of. To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in standardization of selected medicinal plants of potential therapeutic significance. Despite the modern technique, identification of plant drugs by pharmacognostic studies was more reliable[1-2]. According to WHO, the first step towards establishing the identity and degree of purity of a medicinal plant was the macroscopic and microscopic description and should be carried out before undertaken any test[3].

The plant *Wedelia chinensis* Merrill (Family: Asteraceae) is an aromatic perennial, has been traditionally used as a cholagouge, in jaundice, diarrhoea, cephalahagia, mental tension, inducing sleep and in the treatment of anxiety throughout the world. The plant was reported to relieve tension and stress reactions and widely valued for its calming properties[4-7]. An exhausted literature survey on *W. chinensis* revealed that sporadic pharmacognostic, phytochemical and pharmacological reports are available on this plant. As *W. chinensis* has been used traditionally for the treatment of various ailments, this plant holds great potential for in depth pharmacognostic and phytochemical evaluation. The present study deals with the macroscopical, microscopical, physico-chemical and phytochemical evaluation of *W. chinensis*.

MATERIALS AND METHODS

Morphological/organoleptic evaluation: In this analysis, the material was evaluated by studying color, odour, taste, size, shape and special feature like bit, texture etc. It is a technique of qualitative evaluation based on the study of morphological and sensory profiles of leaves.

Microscopical evaluation: In microscopic studies, transverse sections (TS) and pulverized material were evaluated for internal structure, vascular bundles, epidermis, metallic element salt crystals, trichomes etc.[8].

Transverse section (TS) of leaves of *W. chinensis*: Healthy fresh leaves of *W. chinensis* were taken and soaked in water for 60 min. Blank test cross sections were cut with a razor blade. The clear sections were elite, decolorized and mounted on a clean glass slide and coated with plate glass victimization glycerol. Routine staining with safranin was done. Sections were cut by blank test sectioning and diverse sections were examined microscopically.

Powder microscopy: Powder microscopic studies were performed following the Dutch method. Foremost, 2 g of powdered drug was taken and 10% nitric acid solution (50 mL) was added and warmed for 2 min. Then, the solution was filtered and residue was obtained, it was washed with hot water and then filtered. Again residue was taken and 10% sodium hydroxide solution (50 mL) was added, warmed for 2 min. Again the solution was filtered, residue washed with hot water and again filtered. Finally, residue was taken for powder microscopic studies[8-9].

Physico-chemical analysis[9,3]

Determination of moisture (loss on drying): Firstly, 3 g of dried powder (*W. chinensis*) was taken in a tared porcelain dish. The crude drug was heated at 100-105°C in an oven till a constant weight. Cool in desiccators and calculate the loss in weight was typically recorded as moisture.

Calculated the percentage of moisture content of air dried material as:

$$\text{Moisture content}(\%) = \frac{\text{Loss in weight of the sample on heating}}{\text{Weight of total amount of drug taken}} \times 100$$

Ash value: The determination of ash was helpful for detection of low grade merchandise, exhausted drug and far more than sandy or earthy matter. The determination of ash value worth a lot in pulverized drug applicability. Following forms of ash value are given below.

Determination of total ash: Firstly weight and ignite flat, thin porcelain dish silica crucible and then weight about 2 g of pulverized drug into the dish/crucible and incinerated in a crucible at a temperature 500-600°C in a muffle furnace till carbon free ash will obtained then it's cool, weight and percentage of yield will calculated as per reference.

The percentage w/w of total ash was calculated as follows:

$$\text{Total ash (w/w\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of acid-insoluble value: As per the above step of ash, 25 mL of dilute-hydrochloric acid wash the ash from the dish using for total ash into a 100 mL beaker.

Then place wire gauze over a muffle furnace and boil for 5 min. Filter though an ash-less filter paper, wash residue twice with hot water. Ignite the crucible in the flame cool and weigh, at the moment place the filter paper and residue together into crucible, heat gently until vapors cease to be evolved and then more strongly until all carbon can take away. Cool in a desiccator weigh the residue and finally calculate acid insoluble ash of crude drug with reference to the air dried sample of the drug.

The percentage w/w of acid-insoluble ash was calculated as:

$$\text{Acid insoluble ash (w/w\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of water soluble ash: Total ash was boiled for 5 min with 25 mL water and insoluble matter which was collected on an ash-less filter paper, was washed with water and ignited for 15 min at a temperature not exceptional 450°C in a muffle furnace. Difference in weight of ash and weight of water insoluble matter gave the weight of water-soluble ash. The percentage of water-soluble ash can calculated with reference to the air- dried powder drug.

The percentage w/w of water soluble ash was calculated as:

$$\text{Water soluble ash (w/w\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of sulphated ash: A silica and platinum crucible was heated to redness for 10 min, allowed to cool down in desiccators and weigh. Unless otherwise, specific within the individual monograph, 1 g of substance was transferred to the crucible under examination and the crucible and the content was weighed accurately. Gently, ignite at 1st until the substance was totally burn. The residue was cooled and moistened with 1 mL of sulphuric acid, gently heated till the white fumes was no longer evolved and ignite at 800±25°C till all black particles can disappear. The ignition was conducted in a place protected type air currents. The crucible was allowed to cool down and few drops of sulphuric acid added and heated. Ignite again as before, allowed to cool down and weigh. The operation was repeated until 2 successive weighing do not differ by more than 0.5 mg[10].

The percentage w/w of sulphated ash was calculated as:

$$\text{Weight of sulphated ash (w/w\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Determination of extractive value

Cold maceration

Ethanol soluble extractive: Ethanol soluble extractive was useful for evaluation of crude drug and gives idea about the nature of the chemical constituents, soluble in the particular solvent. Macerate the 4 g of air dried leaves part of *W. chinensis* and 100 mL of ethanol of the specified strength was added in a closed flask for 12 h, then shaking frequently during the first 6 h and allowing stand for the 18 h. Thereafter, filter it rapidly take precautions against loss of ethanol. Take 25 mL of the filtrate and evaporate it to dryness in a tarred flat bottomed shallow dish and dry at 105°C and weigh. Then the percentage of ethanol soluble extractive was calculated the air dried drug.

Calculate the percentage of extractive value of air dried material as:

$$\text{Extractive value (\%)} = \frac{(\text{Final weight-Initial weight}) \times 4}{\text{Weight of drug}} \times 100$$

Water soluble extractive: Water soluble extractive was useful for evaluation of crude drug and given idea about the nature of the chemical constituents soluble in that particular solvent. Add 4 g leave part to 50 mL of water in a stoppered flask. Shake well and allow standing for 10 min, cool, add 2 g of kieselguhr in it and filter it, 5 mL of the filtrate was transfer to a tarred evaporating disk, diameter of disk is 7.5 cm, solvent was evaporated on a water bath, then drying was continued for 30 min, then dry in a steam oven for 2 h and the residue was weighed. The % of water soluble extractive was calculated with reference the air dried dry.

Calculate the percentage of extractive value of air dried material as:

$$\text{Extractive value (\%)} = \frac{(\text{Final weight-Initial weight}) \times 4}{\text{Weight of drug}} \times 100$$

Hot extractive

Ethanol soluble: Four gram of coarsely powdered accurately weighed air- dried material was placed in a glass stoppered conical flask. 100 mL of ethanol was added and weighed, the total weight including the flask was obtained. Flask was shacked well and allowed to stand for 1 h. A reflux condenser was attached to the flask and gently boils for 1 h, cool and weighed. The original total weight with the solvent was readjusted to specify in the test procedure for the plant material was feared. Flask was shaken then filters rapidly through a dry filter 25 mL of the filtrate was transferred to a

tared flat-bottomed dish and evaporated to dryness on a water bath. Dried at 105°C for 6 h cooled in a desiccator for 30 min, then weigh without delay. The content of extractable matter was calculated in terms of mg g^{-1} of air dried material.

Calculate the percentage of extractive value of air dried material as:

$$\text{Extractive value (\%)} = \frac{(\text{Final weight-Initial weight}) \times 4}{\text{Weight of drug}} \times 100$$

Water soluble: Four gram of coarsely powdered accurately weighed air-dried material was placed in a glass stoppered conical flask. 100 mL of water was added and weighed, the total weight including the flask was obtained. Flask was shaken well and allowed to stand for 1 h. A reflux condenser was attached to the flask and gently boils for 1 h, cool and weighed. Shake well and filter rapidly through a dry filter 25 mL of the filtrate was transferred to a tared flat-bottomed dish and evaporated to dryness on a water-bath. Dried at 105°C for 6 h cooled in a desiccator for 30 min then weigh without delay. The content of extractable matter was calculated in mg per g of air dried material.

Calculate the percentage of extractive value of air dried material as:

$$\text{Extractive value (\%)} = \frac{(\text{Final weight-Initial weight}) \times 4}{\text{Weight of drug}} \times 100$$

RESULTS

Pharmacognostic evaluation of *W. chinensis*: The plant was evaluated macroscopically by studying color, odour, taste, size, shape, special feature like touch, texture etc. In microscopic study, the transverse sections (TS) and powdered plant material was evaluated for internal structure and cells of plant like type of vascular bundles, epidermis, stomata, trichomes etc. Macroscopic and microscopic characters of leaves of *W. chinensis* are described in Table 1 and Fig. 1-7, respectively.

Physico-chemical parameters of *W. chinensis*: Physico-chemical parameters help to authentic plant material and check adulteration. The extracts obtained by exhausting drugs are indicative of approximate measures of the chemical constituents. Presence of excess moisture in plant acts as an adulterant and may lead to deterioration of plant material and its phytoconstituents by promoting microbial growth. So, it was essential to study the moisture content and extractive value to maintain quality and check adulteration of plant material. Results of physico-chemical parameters (loss on drying, ash and extractive values) of *W. chinensis* are shown in Table 2-3. Every parameter was evaluated in triplicate.

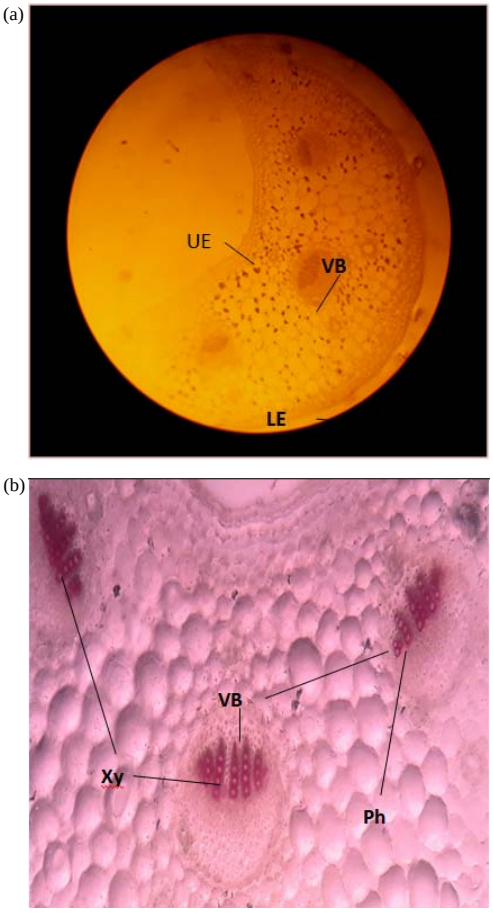

Fig. 1(a-b): TS of *W. chinensis* through midrib [10 and 45X], (a) UE: Upper epidermis, LE: Lower epidermis and (b) VB: Vascular bundle, Xy: Xylem, Ph: Phloem

Table 1: Macroscopic characters of *W. chinensis* leaf

Colour	Green
Odour	Characteristic
Taste	Bitter
Shape	Oblong to oblong-lanceolate
Size	2-5 cm in length
Apex	Acute
Margin	Entire or serrate
Petiole	Absent
Base	Wedge-shape

Table 2: Physico-chemical analysis of *W. chinensis*

Parameter	w/w (%)
Ash value	
Total ash	14.66
Acid insoluble ash	1.32
Water soluble ash	9.79
Sulphated ash	7.18
Loss on drying	8.25

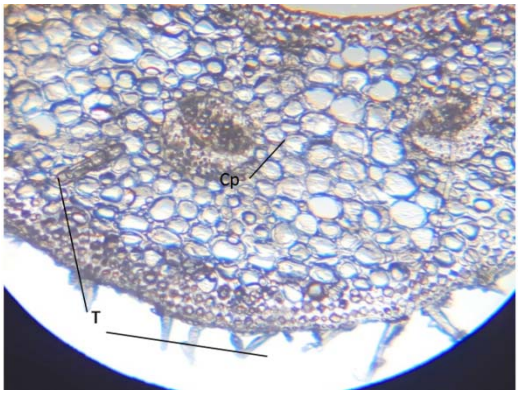

Fig. 2: TS of *W. chinensis* leaf through midrib [10X], T: Trichomes, covering, multicellular, Cp: Cortical parenchyma

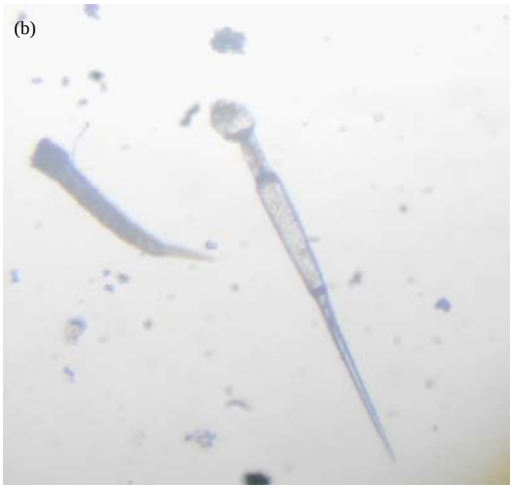

Fig. 3(a-b): Multicellular covering trichome [10X]

Fig. 4: Uniseriate [Collapsed] covering trichome [10X]

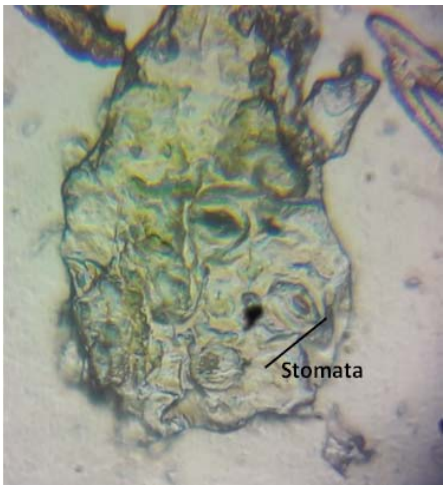

Fig. 5: Stomata [10X]

Fig. 6: Glandular trichome [10X]

Fig. 7: Xylem vessels [10X]

Table 3: Extractive values of *W. chinensis*

Extractive value	Method	w/w (%)
Ethanol soluble	Hot maceration	2
Water soluble	Hot maceration	7.4
Ethanol soluble	Cold maceration	2.75
Water soluble	Cold maceration	4.25

Table 4: Phytochemical screening of hydro-alcoholic extract of *W. chinensis*

Phytoconstituents-class	Hydro-alcoholic extract
Alkaloids	-
Flavonoids	+
Phenols	++
Steroids	-
Tannins	+
Glycosides	-
Saponins	-
Carbohydrate	+
Fixed oil/fat	-

+: Present, -: Absent

Phytochemical screening of *W. chinensis* extract: Phytochemical screening helps to investigate the phytoconstituents which are present in the plant. In the present study, qualitative chemical tests showed the presence of flavonoids, phenols, tannins, steroids in the hydroalcoholic extract of *W. chinensis*. Results are shown in Table 4.

DISCUSSION

The standardization of crude drugs has become very important for identification and authentication of a drug. But due to certain problems the importance was not up to the mark. Thus, the lack of standardization techniques fails to identify the drug from its originality which thereby exploits the usage of drug from its traditional system of medicine[11]. The medicinal plants which are abundantly found and their authentication and identification could not be a part of standardization but it is thoroughly accepted as per traditional methods.

The plant *Wedelia chinensis* Merrill is an aromatic perennial, has been traditionally used as a cholagouge, in jaundice, diarrhoea, cephalahagia, mental tension, inducing sleep and in the treatment of anxiety throughout the world[4-7]. An exhausted literature survey on *W. chinensis* revealed that sporadic phytochemical and pharmacological reports are available on this plant. As *W. chinensis* has been used traditionally for the treatment of various ailments, this plant holds great potential for in depth phytochemical and pharmacognostic evaluation. The present study deals with the macroscopical, microscopical, physico-chemical and phytochemical evaluation of *W. chinensis.*

The plant material was evaluated morphologically/organoleptically by studying color, odour, taste, size, shape, special feature like touch, texture etc. In this study, transverse sections (TS) and powdered plant material was evaluated for internal structure and cells of plant like type of vascular bundles, epidermis, calcium oxalate crystals, stomata, trichomes etc. Such descriptions form the basis for the identification of drugs. Transverse sections of *W. chinensis* leaf under the microscope showed scattered vascular bundles, various types of multicellular covering trichomes, occasional glandular trichome etc. The powdered drug under the microscope showed various types of multicellular covering and occasional glandular trichomes, uniseriate (collapsed) covering trichome, paracytic stomata, xylem vessels etc. (Fig. 1-7).

Physico-chemical parameters help to authentic plant material and check adulteration. The extracts obtained by exhausting drugs are indicative of approximate measures of the chemical constituents. Taking into consideration the diversity in chemical nature and properties content of drugs, various solvent are used for determination of extractives. In the present study, ethanol and water were used to evaluate the extractable constituents in the leaves of *W. chinensis.* Ethanol and water soluble extractive value were estimated to be 2, 7.4, 2.75 and 4.25%, respectively (Table 3). The high water extractive values probably revealed that water extract have the ability to extract more phytoconstituents than alcohol extract based on their polarity scale. This is in agreement with the study of Ajazuddin and Saraf[12]. Presence of excess moisture in plant acts as an adulterant and may lead to deterioration of plant material and its phytoconstituents by promoting microbial growth. So it was essential to study the

moisture content, helpful for determination of moisture present in plant and also controlled to maintain quality. Moisture content of air dried leaves of *W. chinensis* was found to be 8.25%. However, the average percentage of moisture content in crude drug should be within 12-14%[13,14] and the value obtained was within the permissible limits. The determination of ash is useful for detecting low-grade products, exhausted drugs and excess of sandy or earthy matter. The high value of total ash percentage could be used as criteria to judge the purity of drug[15]. The total ash was estimated 14.66%. The addition of dilute hydrochloric acid in total ash can removes all the variable constituents of the ash. The acid insoluble ash estimated 1.32%. The water soluble ash is used to detect the presence of material exhausted by water. The water soluble ash was found 9.79%. This finding was in agreement with Anonymous[14], WHO[13] and Chanda[16] that reported the permissible limits and the necessities for physicochemical evaluation of crude drugs. The determination of Sulphated ash is widely used to control the extent of contamination by non volatile inorganic impurities in organic substances. The sulphated ash was found to be 7.18% (Table 2). Phytochemical screening helps to investigate the phytoconstituents which are present in the plant. It showed presence of mainly phenols, flavonoids, tannins, steroids compounds etc. in hydroalcoholic extract of *W. chinensis* (Table 4). The percentage yield of hydro-alcoholic extract of *W. chinensis* was found to be 17.25 (w/w).

CONCLUSION

The standardization parameters evaluated in the present study would provide a way for the standardization of raw materials and formulation of herbal origin. Further, the findings generated from the current study could also serve in the correct identification and preparation of a monograph on this plant.

SIGNIFICANCE STATEMENTS

This study shows the pharmacognostic characteristics of *W. chinensis.* This study will help the researchers to comply the latest GMP (Good manufacturing practices) and FDA (Food and drug administration) guidelines on standardization of herbal drugs.

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