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Comparative account of Leaf studies between *E. alba* (L.) Hassk. & *W. trilobata* (L.) A.S. Hitchc

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Abstract

The diversity of medicinal plants has been observed and used for medicinal and therapeutic efficacy from time to time. These plants having potential to produce wide forms of chemical constituents and secondary phytochemicals which imparts medicinal values to the plants. The current paper deals with comparative studies of leaf of E. alba and W. trilobata because now a days W. trilobata is known and used as Bhringaraja instead of an original plant E. alba, therefore the main aim of comparative study of leaves of E. alba and W. trilobata is to avoid adulteration for this purpose the number of modern technique have been used for standardization of these two medicinal plants includes pharmacognostic study. In the present research work, important phytochemical constituents were extracted from the methanol, water and chloroform respectively by using standard procedures. Histological study dealing with transverse section of leaf, qualitative microscopy includes vein-islets, palisade ratio, no. of stomata, type of stomata, stomatal index study and physicochemical characters such as analysis of total ash values, acid insoluble and water soluble values were determined, while on the other hand various extractive values also determined. Histochemical tests for alkaloids, flavonoids, saponins, tannins, steroids, glycosides, terpenoids, starch etc. were conducted for both the plants leaf sample and results are summarized in current paper.

Keywords: *E. alba, W. trilobata, pharmacognostic, Histochemical, Ash values.*

Introduction

In herbal drugs and Indian traditional system of medicine, medicinal plants play an important role such as fight against free radicals to avoid the wide spread of infectious diseases, has led to quick and sever investigation of such medicinal plants with its healing and therapeutic potential (Husain and kumar 2015). The different extracts of medicinal plants are referring the appearance of significant compound used in treatment of anti-microbial, anti-inflammatory, analgesic, anti-cancerous and antioxidant properties (Uniyal *et al.* 2006). Asteraceae, the largest family of angiosperms includes & 23000 species of 1535 genera from 3 subfamilies & 17 tribes. The member of this family are mostly herbaceous, while sometimes shrubs as well as trees (Perveen *et.al.* 2016). The family Asteraceae found dominantly in wide range of tropical and subtropical regions. Including more than 1900 genus of flowering plant species of order Asterales (wath *et.al.* 2022). The most exceptional taxonomic character expresses by all members of Asteraceae is inflorescence in the form of small floral heads or blooms called Capitulum, having tiny bracts which are protective in function, the ray florets and disc florets are observed. The calyx in Pappus is characteristics feature of Asterales. Function like parachute for dispersing of fruits. In addition to the small hairy projections arises from outer epidermal layer called trichrome. (Syah *et. al.* 2014).

Material and Methods:

1. Pharmacognostic evaluation:

The comparative study of *E. alba* and *W. trilobata* is performed on the basis of observation of freshly collected plant species from different location of Parbhani District. The identification and authentication was confirmed through qualified taxonomist and by floras. The specimen plants were mounted in herbarium form and deposited in Department of Botany. The fresh plant material of leaf *E. alba* and *W. trilobata* was collected form their natural habitat (Photoplate A and B), prepared free from foreign impurities. Leaves are separates and shade dried, make fine powder and stored in air tight container which is used for pharmacognostic study. For transverse section, cut the mid rib of leaf with blade, take enough section and transferred to Petridish having water. Select section of leaf stained with double staining technique and mount it on slide. Observe slide under microscope and take photographs (Pawale 2019).

Powder analysis of leaves:

To study the powder analysis of leaves, the shade dried leaves were grind to form fine powder by using electric grinder, sieve it through sieve No. 60 and

used to observe under objective of microscope (Langhi *et.al.* 2020).

2. Macroscopic and Organoleptic evaluation:

The analysis on the basis of sensory organs includes teste, odour, colour, texture, size etc. of plant drug was done. The fresh leaf of *E. alba* and *W. trilobata* were collected and use to study the transverse section, number of stomata, stomatal index etc. while the dried leaf powder was used to study the starch granules (Langhi *et.al.* 2020).

3. Microscopic evaluation:

The study of leaf was done to investigate type of stomata, anatomical structure, No. of stomata and stomatal index. The index of stomata was calculating by peeling transparent epidermal layer on abaxial and adaxial surface. The layer was cut with blade put it on slide, remove chlorophyll debris and then slide was observe under high power objective [45X] of microscope. Structure of stomata was drawn by observing through camera lucida and then the stomatal index as well as no. of stomata calculate (Radhika 2017).

4. Physicochemical evaluation of leaves:

The physicochemical properties like ash values, extractive values, fluorescence analysis and moisture content in leaf were determined (Mandal *et.al.* 2018).

1. Total Ash value:

Weight 5gm of powder drug and kept in a silica crucible over the burner for incineration. The charred material was heated in a muffle furnace for six hours at 600-650^{0}C for formation of white carbon free ash, kept it for cooling and weight it on ash-less filter paper. Determination of total ash value was evaluated with dried drug (Mandal *et.al.* 2018).

2. Acid-insoluble ash:

The obtained ash was boiled for 5 min. in 25 ml of dilute HCL. Filter the solution and residue was collected on ash-less filter paper and then washed with hot water. The residue remained on filter paper was insoluble ash. Weighed it to determine the percentage of acid insoluble ash with reference to air dried drug (Radhika 2017).

3. Water soluble ash:

The ash remained in total ash was boiled with 25 ml of distilled water for 5min., then filter the mixture and residue was collected in a crucible. Washed off the residue by using hot water, ignited and weighted. Weight of insoluble matter was subtracted from the weight of ash. It gives water soluble ash and percentage was evaluated with dried drug (Radhika 2017).

4. Moisture content analysis:

The freshly collected leaves of each samples were weighted (5gm) and placed in a china dish dried at 140^{0}C for 2-3 days at 80^{0}C. The dry matter weight was recorded and water content was calculated (Jin *et al.* 2017).

5. Determination of fluorescence behavior: -

The nature of *E. alba* and *W. trilobata* leaf powder was observed under ultraviolet and visible radiation spectra after treating with different chemical reagents (Pattar and Jayaraj 2012).

6. Histochemical tests:

The histochemical tests were carried out to check the presence of alkaloids, flavonoids, saponins, tannins, steroids, glycosides, terpenoids, starch etc. It is essential for identification and specific evaluation of plant phytochemical constituents to demonstrate chemical profile of crude drugs (Husain & Kumar 2015).

Results and Observations:

Anatomical characters of *Eclipta alba* (L.) Hassk and *Wedelia trilobata* (L.) Hitchc: -

Description of anatomical character is an effective character of plant evaluation and systematic explanation. In leaf, anomocytic stomata is observed at larger quantities in the abaxial surface (Photoplate-E, F) than the adaxial surface with trichomes in the cuticle layer.

In leaf transverse section of *E. alba* and *Wedelia trilobata* (L.) Hitchc. shows anatomical characters such as upper epidermis consisting of compact arrangement of parenchymatous cells and having outer covering of cuticle. Externally the dermal tissue system has an outer uniserriate trichomes in *E. alba* while multicellular stomata and secretory ducts are present.

In the T.S. *E. alba* (L.) Hassk leaf shows presence of 3-4 layers of compactly arranged tubular palisade tissue below the upper epidermal layer. 5-7 layers of loosely arranged spongy parenchymatous cells at lower as well as upper epidermal side and within this 3-separate vascular bundles are found, while in *Wedelia trilobata* anatomical structures are similar only the difference is presence of resin ducts near to the vascular bundle. (Photoplate showing T.S of Leaf- C, D)

Pharmacognostic analysis:

In study of organoleptic analysis of leaf includes taste, size, odour, color and dried leaf powder. The external characters of leaves of *E. alba* and *W. trilobata* are typical and completely different from each other (Table 1).

Table 1: Quantitative microscopy of leaves / leaf powder of *E. alba* and *W. trilobata*:

Sr. No.	Parameters	Value	
		E. alba	***W. trilobata***
1	Stomatal No. (Lower epidermis)	21.5-25	22-25
2	Stomatal No. (upper epidermis)	16-20	12-14
3	Stomatal index (Lower epidermis)	21-25	22-25
4	Stomatal index (upper epidermis)	16-20	12-14
5	Type of Stomata	Anomocytic	Anomocytic
6	Vein islet no.	3- 13.6	3.6
7	Palisade ratio	3.8-4.5	1-5

Physicochemical evaluation:

The physical analysis includes moisture content, fluorescence analysis, ash value, total ash, water soluble, acid insoluble and other extractive values of leaves as well as leaf powder of *E. alba* and *W. trilobata*. The results were shown as below, it gives correct identification of the plant and avoids adulteration (Table 2).

Table 2: Physicochemical evaluation of leaf powder of *E. alba* and *W. trilobata*

Sr. No.	properties	Value % (w/w)	
		E. alba	*W. trilobata*
1	Moisture content	2.4%	3.18%
2	Total ash	18%	17.4%
3	Acid insoluble ash	3.6%	6%
4	Water soluble ash	13.2%	15.2%
5	Alcohol soluble extractive	6%	8%
6	Water soluble extractive	5%	5%
7	Ether soluble extractive	10%	16%

Fluorescence characteristics:

The fastest method to analyze the study of crude drug of uncertain plant specimen is done for identification of original form their adulterants by its fluorescence characteristics (Table 3).

Table 3: Behavior analysis of drug with different chemicals under UV

Sr. No.	properties	*E. alba*		*W. trilobata*	
		Day light	**UV Light**	**Day light**	**UV Light**
1	Distilled water	light green	Faint green	light green	Dark green
2	**Powder + picric acid**	Dark yellow green	yellowish brown	Yellowish green	Light green
3	**Powder +$Fecl_3$**	Light green	yellow brown	Dark green	Dark green
4	**Powder + HCL**	Dark green	reddish brown	Dark green	Dark green
5	**Powder + NaOH**	Light green	Light green	light green	Green
6	**Powder + HNO_3**	Brown	Dark brown	Light brown	Yellowish green
7	**Powder +Iodine solution**	Dark green	Green	Blackish green	Dark green

Histochemical tests:

The histochemical tests mainly performed to revealed the occurrence of alkaloids, flavonoids, saponins, tannins, steroids, glycosides, terpenoids, starch etc in methanolic, aqueous and chloroform extract (Table 4).

Table 4: Comparative results of histochemical tests of leaf extract of *E. alba* and *W. trilobata*

Extract of plant	Leaf extract of *E. alba*			Leaf extract of *W. trilobata*		
Solvents / Phytochemical constituents	Methanol Extract	Aqueous Extract	Chloroform Extract	Methanol Extract	Aqueous Extract	Chloroform Extract
Steroids	+	-	-	+	+	+
Flavonoids	-	-	-	+	+	-
Saponins	+	+	-	+	+	+
Tannins	+	-	+	+	+	+
Terpenoids	+	-	+	+	+	-
Alkaloids	+	+	+	+	+	-
Starch	+	+	+	-	+	+
Glycosides	-	-	+	+	+	-

Discussion:

The plant *Eclipta alba* and *Wedelia trilobata* is used in treatment of various disease aliments. The standardization and analysis of specific plant is important before it added in pharmacognocy and herbal pharmacopoeia. The present study and its results are giving basic information for authentication, identification and collection of plants. The determined pharmacognostical evaluation and numerical values of leaves and dried powder of leaf is varies from other members of Asteraceae family.

The leaf transverse section of *E.alba* is dorsoventral. The unicerrate trichome in *E.alba* is different from multicellular unicerrate trichome in *W. trilobata* is arises from upper epidermal layer having outer cuticle covering in both plants. In plant *E. alba* 3 separate vascular bundles are present, while in *W. trilobata* resin ducts near to vascular bundle is observed. Anomocytic stomata is common in both the plants.

The pharmacognostic investigation shows both plants revealed values of stomatal no. on upper and lower epidermis, ranging from 21.5-25 in *E. alba* and 22-25 in *W. trilobata* which is slightly similar with each other. Vein islets no. of *E. alba* is 3- 13.6 greater than the *W. trilobata*.

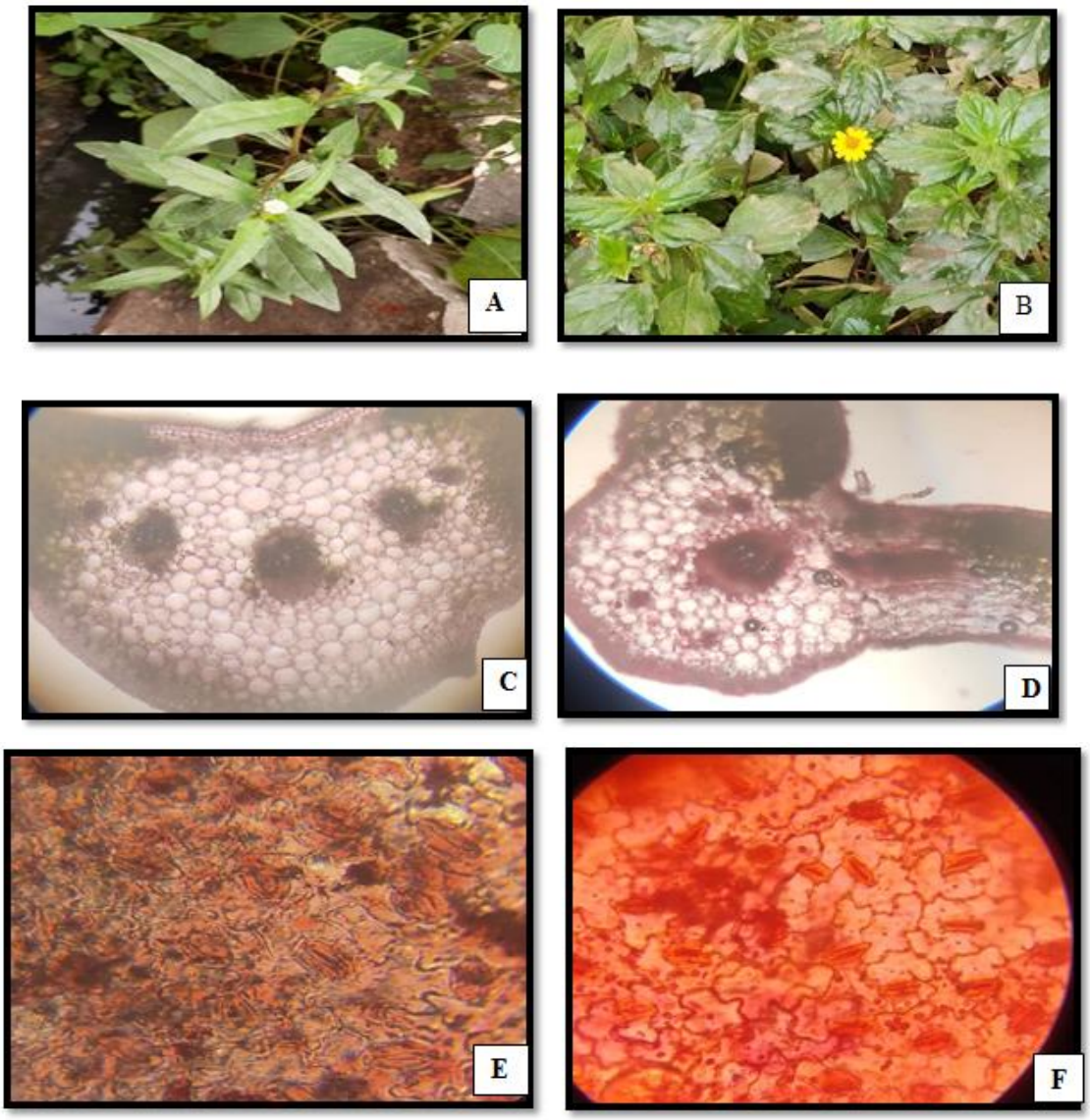
In the physicochemical evaluation, moisture content value of *W. trilobata* 3.18% is higher than the *E. alba*. Total ash in *E.alba* is 18% which is greater than *W.trilobata* 17.4%. The acid insoluble 3.6% ash found in *E. alba*, it is less than *W. trilobata* 6%. Water soluble value show the high percentage 15.2% in *W. trilobata* as compare to *E.alba* 13.2%. The water soluble extractive value of both plants have same and equal percentage 5%, while alcohol and ether soluble extractive have 6% and 10% in *E.alba*, on the other side *W. trilobata*. Have 8% and 16% which is also shows greater percentage.

The behavior analysis of *E.alba* and *W.trolobata* is observe in day light and under UV, It helps to identify the substitute during evaluation of original drug. The crude drug powdered is treated with different chemicals such as picric acid show change in colour dark yellow green in day light to yellowish brown in UV light. The leaf powder treating with HCL turns dark green to reddish brown in *E.alba* and in *W.trolobata* it remains dark green.

The methanol, aqueous and chloroform extract of leaf of both plants showed the appearance of variety of phytochemical compounds and some of them are absent. The methanolic extract of *E. alba* shows presence of steroids, saponins, tannins, teropenoids, alkaloids and starch while in *W. trilobata* except starch. The aqueous extract of leaf of *E. alba* shows presence of saponins, alkaloids and starch etc. while all phytochemical constituents were found to be present in *W. trolobata*. The chloroform extract of leaf of *E. alba* have tannin, terpenoids, alkaloids, starch and glyceriodes. While tannins, saponin, steroids and starch present.

Conclusion:

The Pharmacognostic, macroscopic and microscopic study of *E. alba* and *W. trilobata* are reveled which helps in identification of original drug plant. Histochemical tests study is also carried. Its results as well as observation obtained by following the experiments which used in authentication of genuine plant. The study of comparative account of plant is necessary to avoid the adulteration of original plant from the other substitute.



Photoplate-A: Plant of *E. alba* (L.) Hassak., **Photoplate-B**: Plant of *W. trilobata* (L.) A.S. Hitchc., **Photoplate-C**: T.S. of *E.alba* Leaf, **Photoplate-D**: T.S. of *W. trilobata* Leaf, **Photoplate-E**: Leaf Stomata of *E.alba*,**Photoplate-F**: Leaf Stomata of *W. trilobata*.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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