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Research Article

PHARMACOGNOSTICAL STUDIES ON ROOT OF DIOSPYRUS FERREAE (WILLD.) BAKH AND AERVA LANATA LINN., A POTENT INDIAN MEDICINAL PLANTS.

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ABSTRACT

Diospyros ferrea (Willd.) Bakh., is small-sized tree belongs to the family Ebenaceae seldom reaching a diameter of 40 cm; stem is straight, fluted, short, attaining a height of 5 m; leaves are elliptic or obovate, often notched at the tip. Inflorescence is axillary, with 1-3 white or pale yellow flowers. Fruits turn from red orange to purple when mature. The tree is commonly known as the Batulinao, Ebony. Aerva lanata is a plant from the family Amaranthaceae , locally known as sirupoolai in tamil. Aerva lanata is endowed with chemical components such as flavonoids, alkaloids, Steroids, Polysaccharides, tannins and saponins etc. The plant has been documented earlier for its therapeutic effects in controlling kidney disorders, diuretic, anti-inflammatory, antidiabetic, antitumor and antimicrobial activity. Even though both the plant root has gained scientific importance recently, there is a need for the pharmacognostic standardization. Hence, in the present work the root were subjected to various microscopical and physical evaluations. In the microscopical studies, the different cell structures and arrangements were studied and in physical evaluation the ash values and extractive values were studied. The various pharmacognostical constants were obtained which could help in the development of a suitable monograph for the plant.

Keywords: *Diospyrus ferrea, Aerva lanata*, root constants, proximate analysis, powder analysis, fluorescence analysis.

INTRODUCTION

The tree is commonly known as the Batulinao, Ebony. It is naturally growing in the provinces of Eastern Samar particularly in Borongan, Guian and Catarman. The species was also sighted as ornamental and as bonsai. The wood is used in the manufacture of furniture, cabinet, inlaying eaves, tool handles, fingerboards and key of guitars and violins, drawing instrument, shuttles, bobbins, spindles and novelty products. The trees grow along rocky seashores and back of mangrove swamps and in some areas extending inland on dry slopes. In Guian, Eastern Samar, flowering starts in April, seed collection is from July to August. Propagation is through seed. Seeds are germinated in different media such as on sand, ordinary garden soil, and 1:1 mixture of sand and ordinary garden soil. The root is found to have promising anti inflammatory effect, immunomodulatoty effect, anti tumor activity, anti cariogenic, anti oxidant activity anti noniceptive, trypanosidal activity3-10. *Diospyrus ferrea* root mainly consists of steroids, tannins, phenolic compounds and flavonoids.

Aerva lanata (A. lanata) known as Polpala is a prostrate to decumbent, sometimes erect herb, found throughout tropical India as a common weed in fields. Traditionally, *A. lanata* leaves are used as sap for eye complaints, an infusion is given to cure diarrhea and kidney stone, and root is used in snake bite treatment. The root extract has been given orally for 11 days to degrade the toxic effect induced by the venom toxins. A root decoction is taken orally in empty stomach one day for one month to cure diabetes. The root is diuretic, demulcent, tonic and given to pregnant women. A leaf decoction preparation is used as gargle for treating sore throat and is also used in various complex treatments against guinea worm. A variety of pharmacological activities of this ethno medicinally important plant has been reported as follows: anthelmintic, demulcent, antiinflammatory, diuretic, expectorant, hepatoprotective and nephroprotective activities. Alcoholic extract of shoots of *A. lanata* has shown significant antidiabetic and antihyperglycaemic activities in rats. Antimicrobial cytotoxic, urolithiatic, hypoglycemic, antihyperlipidaemic, antiparasitic, antihelmentic activities have also been reported in *A. lanata* by various workers. Although the preliminary phytochemical studies revealed the presence of various bioactive compounds other than alkaloids, there is no detail study on phytoprofiling of *A. lanata* root in five different solvents.

Pharmacognostical parameters for easy identification like root constants; microscopy & physico chemical analyses are few of the basic protocol for standardization of herbals. Hence, in the present work the pharmacognostical standardization has been performed for the root of both the plant.

MATERIALS AND METHODS

Fresh roots of *Diospyrus ferrea and Aerva lanata* were collected from Southern Western Ghats, South India. A voucher specimen (FLOR 24. 144) was deposited in the Herbarium Botanical Survey of India Coimbatore for authentication of plant. After authentification roots were collected in bulk, washed, shade dried, macerated and extracted with hexane, chloroform, methanol, ethanol and aqueous for 48 hrs sequentially in a Soxhlet assembly. The extracts were concentrated, percentage yield calculated and subjected to preliminary phytochemical analysis.

Microscopical studies

Transverse section of root

Free hand sectioning was done for fresh root to obtain a thin section. Saffaranin was used as a stain and mounted on a glass slide and focused under a microscope [1].

Powder microscopy

Shade dried roots were powdered with the help of an electric grinder till a fine powder was obtained .This fine powder of the root was subjected to powder microscopy, as per standard procedures mentioned [2-4]. Small quantity of powdered drug sample was taken into the watch glass and mixed with different chemical reagents. The change in the color was observed under short UV, long UV and normal day light. Similarly extract were also subjected to UV chamber and fluorescence was observed and consistency was noted as an additional character for identification.

Determination of root constants

The different parameters like xylem tracheid, xylem parenchyma, vessel element, starch grain, cork cells, were determined as per standard procedure [5].

Fluorescence analysis

Powdered root parts were subjected to analysis under ultra violet light after treatment with various chemical and organic reagents like ethanol, 50% sulphuric acid, 10% sodium hydroxide and dilute hydrochloric acid [7]. Three parameters were taken into account

(1) Observation under long UV (365 nm),
(2) Short UV (256)
(3) Normal day light.

Similarly extract were also subjected to UV chamber and fluorescence was observed and consistency was noted as an additional character for identification.

Proximate analysis

The various physicochemical parameters like ash values, total ash, and acid insoluble, loss on drying, water soluble extract, alcohol soluble extract and extractive values were performed as per the standard procedures.

Qualitative determination of the chemical constituency of *Diospyrus ferrea* and *Aerva lanata* root

Determination of total phenol by spectrometric method

The fat free sample was boiled with 50ml of ether for the extraction of the phenolic component for 15 min. 5ml of the extract was pipetted into 50ml flask then 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol was also added. The samples were made up to the mark and left to react for 30 min for color development. This was measured at 505nm.

Alkaloid determination using Harborne (1973) method

5 g of the sample was weighed into a 250 ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4h. This was filtered and the extract was concentrated on a water bath to one quarter of the original value the concentrated ammonium hydroxide was added drop wise to the extract to the until the precipitation was complete. The whole solution was allowed to settle and the precipitation was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed [8].

Tannin determination by van-burden and Robinson (1981) method

500mg of the sample was weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken for 1h in a mechanical shaker. This was filtered into 50ml volumetric flask and made up to the mark. Then 5ml of the filter was pipette out into a test tube and mixed with 2ml of 0.1 m $Fecl_3$ in 0.1 N HCI and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min [9].

Flavonoid determination by the method of Boham and Kocipai Abyazan

(1974): 10 g of the plant sample was extracted repeatedly with 100ml of 80% aqueous menthol at room temperature the whole solution was filtered through what man filter paper No 42(125mn). The filtrate was later transferred into a crucible and evaporated into dryness over the bath and weighed to the constant weight [10].

Saponin determination: The method was that of Obadoni and Ochuko (2001). The samples were ground and 20g of each were put into a conical flask and 100 cm^3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55^0 C. The mixture was filtered and residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90^0C. The concentrate was transferred into 250 ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was reported while the ether layer was discarded. The purification process was repeated. 60ml of n- butanol was added. The combined n-butanol extract were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample were dried in the oven to a constant weight; the saponoin content was calculated as percentage [11].

RESULTS

The standardization of the crude drug is an integral part of establishing its correct identity. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and in expensive means

Microscopic characters

Transverse section of *Diospyrus ferrea* root

Thick root with well developed secondary xylem was studied. The surface of the root irregular due to shallow dense fissures (Fig-4.1). The root is nearly 2mm thick. The periderm is six layered with narrow tubular cells of suberized walls (Fig-1) Inner to the periderm is a thick continuous cylinder of sclereids. The sclereid cylinder is followed internally by narrow zone of phloem with small groups of sieve elements. The secondary xylem cylinder is thick and dense. It has characteristic distribution of xylem parenchyma, sclerenchyma and vessels. The sclerenchyma (fibers) and wide rectangular Parenchyma cells are in several are in regular alternating co axial cylinders. Xylem rays are prominent, one cell thick straight and thick walled. Calcium oxalate crystals present in parenchymatous cells and scattered. Starch grains most are simple, oval or rounded, concentric with central helium. They are either simple type or compound comprising three or four grains combined into single unit. This anatomical study showed diagnostic features that revealed characteristic pattern of arrangement of the cellular components of root of *Diospyrus ferrea* Willd.

Transverse section of *Aerva lanata* root

Root measuring 850µm thick was studied. It consists of superficial, uniformly thicked 4 or 5 layers of periderm. The cortical zone is also narrow comprising 3 or 4 layers of compact parenchyma cells. The secondary phloem is fairly wide and continuous all around the xylem cylinder. The structure of the root is unusual (Anomalous) (Fig-2). There are two cylinders of xylem; one is circular, solid and central in position. This central cylinder is surrounded by a phloem cylinder. There is a second cylinder of xylem enclosing the central xylem and phloem. The number of xylem and phloem cylinders will increase when the root increases in thickness. The xylem cylinders consist of a few solitary vessels and highly thick walled and lignified xylem fibers.

Histochemical Screening

Histochemical screening showed the presence of starch, protein, fat, saponins, tannin, sugars, flavanoids and alkaloids (Table I).

Fluorescence analysis in *Diospyrus ferrea* root

The colored fluorescence obtained for the root powder was subjected to various analyses.

Fluorescence analysis was carried out to check the purity of the drug. The powder drug was observed in visible light as yellowish brown in color. The powder was then observed in ultraviolet light. It was treated with reagent like 1 N sodium hydroxide, 1 N sodium hydroxide and dry for 30 minutes and then it was observed under ultraviolet light and it emits the color as shown in (Table III).

Fluorescence analysis in *Aerva lanata* root

The colored florescence obtained for the root powders are tabulated in table no.III. Fluorescence analysis was carried out to check the purity of the drug. The powder drug was observed in visible light as yellowish brown in color. The powder was then observed in ultraviolet light. It was treated with reagent like 1 N sodium hydroxide, 1 N Hydrochloric acid and dries for 30 minutes and then it was observed under ultraviolet light and it emits the color as shown in (Table III).

Table. I: Histochemical study of D.ferrea and Aerva lanata.

Test	Reagent	**Color**	**Tissue**
Starch	I_2KI	Blue	Epidermis,Periderm,Xylem,Phloem
Protein	Potassium Ferrocynide+Water+acetic acie+60% alcohol+$Fecl_3$	Blue Light BrownYellow	Epidermis,Cortex,Xylem,Pith Xylem, Phloem Epidermis,
Tannin	Acidic $FeCl_3$	Pink	hairs,endodermis,pericycle,phloem,xylem
Saponin	Conc.H_2So_4	Yellow	Epidermis,
Fat	SudanIII	Brown	hairs,endodermis,pericycle,phloem,xylem
Sugar	20% NaOH	Colorless	Epidermis, phloem, xylem
Glycosides	Guignard,s Test	Orange	Epidermis., hairs, endodermis., Cortex
Alkaloid	Mayer,s Reagent	Dark Brown	Hair, Epi. Cort, xy. Phlo.
	Wagner's Reagent		Epi. Peri. Phlo. xy.
	Dragendorff's		Epi. Cort. Peri., Phlo., xy

Table II: Ash and Acid Insoluble Ash of D.ferrea and Aerva lanata.

Parameters-I	Results
Total ash	3.7%
Acid insoluble ash	0.8%
Loss on drying	8.29%
Water soluble extract	14.93%
Alcohol soluble extract	5.94%
Parameters-II	
Total ash	10.74%
Acid insoluble ash	1.95%
Loss on drying	9.11%
Water soluble extract	14.19%
Alcohol soluble extract	5.04%

Table III: Fluorescence analysis of D.ferrea and Aerva lanata

Root	**Diospyrus ferreae**			**Aerva lanata**		
Treatment	Coloremitsin ordinary light	Coloremitsin 254nm	Coloremitsin 365nm	Coloremitsin 254nm	Coloremits in365nm	Coloremitsin ordinary light
Powder as such	Dark brown	Olive green	Dark brown	Green	Dark brown	Pale brown
Powder as such in UV light	Dark brown	Olivebrown	Dark brown	Green	Dark brown	Pale brown
Powder + 1N Hcl	Pale brown	Darkgreen	GreenishBrown	Fluorescent gren	Grenish yellow	Light yellow
Powder+1NNaOHin Methanol	Dark brown	Dark Green	Greenish brown	Fluorescent green	Grenish yellow	Yellow
Powder+1NNaOH inWater	Dark brown	Dark brown	Dark brown	Dark green	Greenish yellow	Yellowish brown
Powder + HNO_3 (1:1)	Brownish yellow	Dark brown	Brownish yellow	Fluorescent ellow	Fluorescent green	Orangish yellow
Powder + H_2SO_4(1:1)	Dark brown	Deep blue	Dark brown	Deep blue	Dark brown	Dark brown
Powder + Acetic acid	Dark brown	Bluish green	brown	Orange	Yellow	Pale yellow
Powder + Picric acid	Brown	Dark brown	Brown	Yellow	Fluorescent green	Yellow
Powder + Water	Light brown	Green	Dark green	Light yellow	Fluorescent green	Yellow
Powder+Ferric chloride	Dark green	Dark green	Greenish rown	Dark brown	Dark brown	Dark brown
Powder + Iodine solution	Pale brown	Fluorescent yellow	Light brown	Light yellow	Pale green	Fluorescent green
Powder + alcoholic KoH	Dark brown	Dark blue	Dark brown	Dark green	Flourescencegreen	Yellow
Powder +alcoholic NaoH	Dark brown	Pale brown	Dark brown	Dark brown	Flouresecncegreen	Yellow
Powder + Methanol	Dark brown	Dark brown	Dark green	Orange	Fluorescenceyellow	Yellow
Powder + Ethanol	Pale brown	Pale blue	Fluorescence yellow	Yellow	Fluorescence yellow	Yellow
Powder + Ammonia	Dark brown	Dark brown	Light green	Fluorescence green	Fluorescence yellow	Yellow

The HPTLC analysis showed that, the Gallic acid from the *Diospyrus ferreae and Aerva lanata* root samples gave light brown bands in visible light and blue bands after derivatization in fluorescence light. The plates were scanned at 254 and 366 nm.

Powder microscopy in *Diospyrus ferrea*

Powder microscopy was done according to the standard procedure mentioned. The powder microscopy revealed the following

a) Fibers: lignified and non-lignified, long, slender and cylindrical shaped(Fig-1.1)..

b) Xylem vessels: it is lignified with simple pits(Fig-1.2)..

c) Calcium oxalate crystals are prismatic type are fairly abundant in the cortical parenchyma cells (Fig-1.3). The crystals scattered in distribution; they are located in ordinary parenchyma cells.

d) Starch grains: are abundant in xylem fissures (Fig-1.4). The starch grains are circular, concentric with central helium. They are either simple type or compound comprising three or four grains combined into single unit.

e) Xylem parenchyma was abundant in the tissues (Fig-1.5)

f) Xylem ray cells are scattered in between the parenchyma cells (Fig-1.6).

g)Cork cells were thick brown in color, distinctive fissure and cracks were observed (Fig-1.7)

h) Trichoscereid are present (Fig-1.8).

Powder microscopy in *Aerva lanata*.

Powder microscopy was done according to the standard procedure mentioned. The powder microscopy revealed the following

a) Fibers: lignified and non-lignified, long, slender and cylindrical shaped (Fig-2.1).

b) Xylem vessels: it is lignified with bordered pits (Fig-2.2).

c) Calcium oxalate crystals: present in parenchymatous cells and scattered. The druces are 40µm in diameters (Fig-2.3).

d) Starch grains: most are simple, oval or rounded without any striation (Fig-2.4).

e) Parenchyma cells are intermingled between the xylem elements (Fig-2.5)

f) Secondary phloem was dominant with phloem parenchyma with embedded fibers (Fig-2.6)

g) Cork cells were commonly reported (Fig-2.7).

h) Tricho scelreid are present (Fig-2.8)

Proximate analysis of *Diospyrus ferrea* and *Aerva lanata*.

Ash values of the drug given an idea of the earth matter or the inorganic composition and other impurities present along with the drug [12]. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The extractive value of the crude drug determines the quality as well as the purity of the drug [13]. Thus alcohol and water soluble extractive values were determined. Loss on drying, percentage of total ash, acid insoluble ash, water soluble ash and different extractive values are tabulated in Table 4. It contains the total ash 3.7% and acid insoluble ash is 0.8% (Table II).

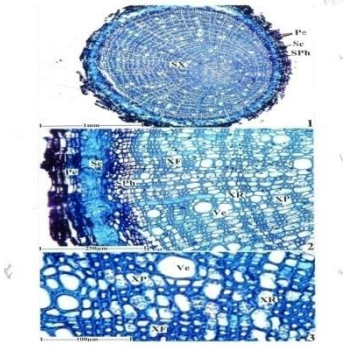

Figure 1:Transverse section of *Diospyrus ferrea root*

Figure 1.1: Fibers

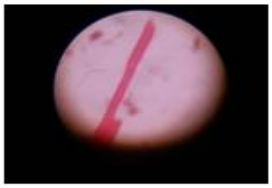

Figure1.2: Xyl

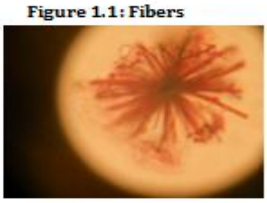

Figure1.3: Calcium oxalate crysatals

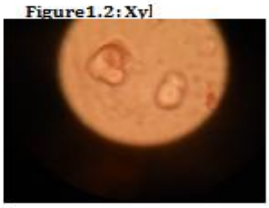

Figure1.4: Starch grains

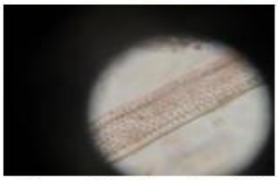

Figure 1.5 xylem with pitted vessels

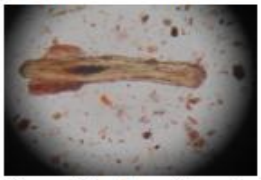

Figure 1.6 xylem with ray initials

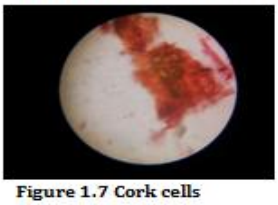

Figure 1.7 Cork cells

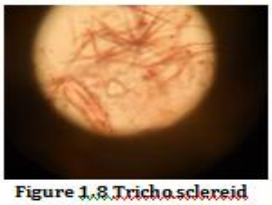

Figure 1.8 Tricho sclereid

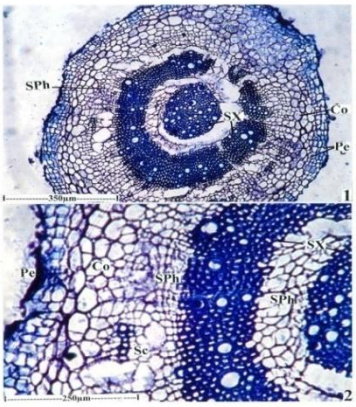

Figure 2:Transverse section of *Aerva lanata* root

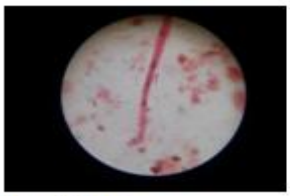

Figure 2.1: Fibers

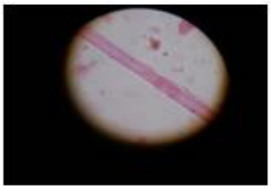

Figure 2.2: Fibers

Figure 2.3: Calcium oxalate crysatals

Figure 2.4: Starch grains

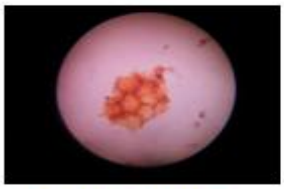

Figure 2.5 paren chyma

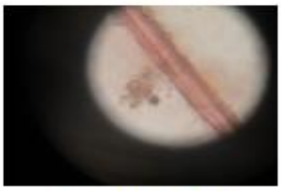

Figure 2.6 phloem fibre

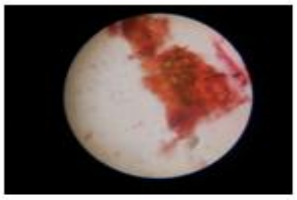

Figure 2.7 Cork cells

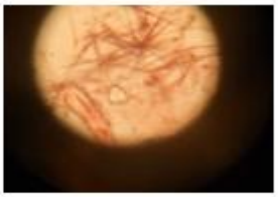

Figure 2.8 Tricho scelreid

DISCUSSION

The standardization of the crude drug is an integral part of establishing its correct identity. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and in expensive means. Transverse section of *Diospyrus* root shows the presence of thick periderm with well developed secondary xylem. Inner to the periderm thick continuous layer of sclereids. Pitted thickening of the vessels are prominent. Fibers are abundant with narrow lumen. Pith parenchymatous and prominently reduced. This anatomical study showed diagnostic features that revealed characteristic pattern of arrangement of the cellular components of root of *Diospyrus ferrea willd*. Histochemical tests revealed the presence of starch grains, tannin, lignin, oil globules and calcium oxalate crystals. The fluorescence characters of powdered of powder drug play a vital role in determination of quality and purity of the drug material. In the present study, powder treated with various reagents shows characteristic fluorescence at 254nm and 366nm wavelength.

To determine extent of adulteration as well as to establish the quality and purity of drug, ash and extractive values were calculated. Total ash was found to 3.77%of which 0.81% was acid in soluble

ash, and 14.93% was water soluble ash. The extractive values were found to be 5.94% and 5.94% for water and alcohol. The moisture content was found to be 8.29% in *Diospyrus ferrea* root.

In case of *Aerva* root the total ash content was determined as 10.74% and acid insoluble ash, water soluble ash were found to be 1.95 %,14.19% .The extractive values were found to be 14.19% and 5.04% for water and alcohol. Studies on physic-chemical constant can serve as a value source of information and provide suitable standard to determine the quality of the root. The crude fiber content so obtained can be implemented to determine nutritive values. Fluoresescence analysis is an important qualitative diagnostic tool to detect the presence of chromophore in crude powder drug under UV light. The fluorescence analysis of powder and extract were given in (Table-3)

Quantitative estimation of the percentage of crude chemical constituents in these medicinal plants studied is summarized in table-3. *Diospyrus ferrea* contained the highest percentage of phenol (0.91%), alkaloids (0.38%), flavonoid (0.54%) and tannin (0.78%). Aerva lanata root contained the highest yield of alkaloid (0. 94%), flavonoid (0.65%), tannin (0.79%) and phenol (0.49%). The phyochemical screening and quanititative estimation of the percentage crude yields of chemical constituents of the *Diospyrus ferrea* root studied showed the highest percentage of phenols and tannins. They were known to show the medicinal activity as well as the exhibiting physiological activity [14-15].

CONCLUSION

In last three decades, the scientist is keen in and sincere to evaluate many plant drugs used in medicinal purpose. I t is due to the specific healing properties, healthy action and non toxic effect. *Diospyrus ferrea* and *Aerva lanata* root is currently being used in the treatment of various disease without standardization. In this aspect, identification, collection and investigation of the plant pharmacognostical studies and phyto chemical screening can serve as a basis for proper standardization of the plant. These parameters which are being reported could be useful in the preparation of the herbal monograph for its proper identification.

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