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Preliminary pharmacognostic and phyto-chemical evaluation of *Darbha* (*Desmostachya bipinnata* Stapf.)

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ABSTRACT

Background: *Darbha* (*Desmostachya bipinnata* Stapf.) is found in the plains of India, throughout the Middle East to Indo-China, North and tropical Africa. It is a species of open habitat, arid regions with water table near surface. According to Ayurveda its roots are used in conditions such as *Mootrakruchchra¸ Ashmari, Raktapitta, Pitaprakopa,* etc. **Aim:** To investigate preliminary pharmacognostical and phytochemical parameters of plant to standardize the drug. **Materials and Method:** Identification of the plant was done as per the standard guidelines given in the floras. Preliminary physico-chemical and phyto-chemical screening was done and after achieving the idea of phytoconstituents group, quantitative test of sugar content and volatile oil content and thin layer chromatography studies were carried out for different organic solvent extracts. **Results:** Presence of air cavities in root but their absences in stolon suggest that *Darbha* is a halophytic plant. Aqueous extracts showed the presence of tannins, carbohydrates and sugars. **Conclusion:** The findings of the study will be helpful in the identification of *Darbha* plant.

Key words: *Darbha, Desmostachya Bipinnata Stapf., Diuretic.*

INTRODUCTION

Darbha (*Desmostachya bipinnata* Stapf.) is found in the plains of India, throughout the Middle East to Indo-China, North and tropical Africa. It is a species of open habitat, arid regions with water table near surface. This grass grows usually in moist sandy loams, sand dunes and is very common on Coromandel coast and in the Deccan districts.[1] The plant is known by the names of *Saved Gram, Darbh, Dabhdo, Dab, Darbaipul*,[2] etc. in different parts of India and is one of the constituents of *Trina Panchmoola*[3],[4] which is used for dysentery, menorrhagia, etc. in compound form with other drugs.[5] The culms of the plant are said to possess diuretic and stimulant properties.[6] The aerial parts contain flavonoid glycosides which are identified as kaempferol, quercetin, quercetin-3-glucoside, trycin and trycin-7-glucoside.[7]

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MATERIALS AND METHODS

Collection of Sample

Darbha (*Desmostachya bipinnata* Stapf.) (Family - Poaceae)[8] was collected from 'Sasoi' region of Jamnagar district and authenticated by Shri A.P.G. Pillai, (OSD), PGT-SFC Cell I.P.G.T. & R.A., Gujarat Ayurved University. The rhizomatous roots were made into small pieces, shade dried, pulverized to fine powder (mesh number 80) and stored in airtight glass container for experimental purposes.

Preparation of Herbarium

Plant sample of *Desmostachya bipinnata* Stapf. was identified and authenticated with the help of The Flora of Gujarat State[9] and The Flora of Maharashtra

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State.[10] Herbarium was prepared and deposited in the herbarium section of the Institute.

Preparation of wet sample

Freshly collected and thoroughly washed plant sample was kept in a glass bottle containing solution of formalin-aceto-alcohol (FAA).

Microscopy

A. **Root, Stolon and Leaf:** Transverse sections of root, stolon and leaf were taken and photomicrography was done after proper mounting and staining.

B. **Powder microscopy:** Powder of drug was studied microscopically and microscopic characters were photographed by using Canon digital camera attached to Zeiss microscope.

Phyto-chemical and physico-chemical analysis

Dried rhizomatous powder was used for analysis of physico-chemical parameters such as loss on drying, ash value, acid insoluble ash, water soluble extractive, alcohol soluble extractive, pH, particle consistency and phyto-chemical tests for alkaloids, tannins, triterpinoids, carbohydrate, flavanoids, saponin and glycosides as well as sugar content and volatile oil content.

Phyto-chemical screening

Preliminary phyto-chemical screening was done and after achieving the idea of phytoconstituents group, Alkaloids, Tannins, Triterpenoids, Carbohydrate, Flavonoids, Saponin, Glycoside; quantitative test of Sugar content and Volatile oil content.

TLC fingerprinting profiling

TLC glass plates were prepared using silica gel-G (E-Merck) and were activated at 110°C for 30 minutes. Petroleum ether, chloroform and ethanol, all of LR grade, distilled under normal atmospheric pressure were employed for extraction of the plant material. All the solvents employed as mobile phase for thin layer chromatography were of AR grade.

Preparation of sample

1gm of powder was extracted with 10ml methanol by warming, it was filtered and the solvent was concentrated to 5ml. This solution was used for spotting.

Track - B: *Desmostachya bipinnata* Stapf.

Stationary phase - Silica gel G

Mobile phase

1. Chloroform : Toluene : Isopropyl alcohol : Acetic acid : Water (22:8:1:0.5:1)
2. Hexane : Diethyl ether : Acetic acid (7 : 1.5 : 0.1)

Detection

For mobile phase 1:

1. Long UV (366nm)
2. Short UV (254nm)
3. Iodine chamber
4. Spraying with 5% methanol-sulphuric acid and heating up to 110°C. for 10 minutes.

For mobile phase 2:

a. Long UV (366nm)

b. Short UV (254nm)

c. On spraying with LB (Liebermann – Burchard reagent) and heating for 10 minutes at 110°C.

The volume of both the Samples was made equal with methanol and then same quantity was spotted on the TLC plate. For development, the plate after spotting was kept in a chamber saturated with the solvent system. The plate after development was viewed under long and short UV, then placed in Iodine chamber for 10 minutes and finally sprayed with 5% methanol-sulphuric acid and observed for spots for the first phase. While for the second phase the plates were observed under long and short UV as well as after being sprayed with LB reagent for the spots.

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OBSERVATIONS AND RESULTS

Macroscopy

Root and stolon

Drug occurs in 6-20 cm long, 0.3-0.5 cm thick cut pieces, almost cylindrical, internodes smooth, stout, mostly covered with shining sheath, having distinct nodes, brownish yellow, a few thin, fibrous, yellowish brown rhizomesarise at nodes, fracture, short (Figure 1) and (Figure 2).

Leaf

The leaves are fascicled, very long, rigid along acuminate tips filiform, margins hispid, leaf blades narrow, flat with parallel venation and coarse (Figure 3) and (Figure 4).

Microscopic evaluation

T.S. of Root

Epiblema: Outermost region of epidermii made up of two layers, thin walled, pentagonal to hexagonal, lignified and covered with abundant root hairs. (Figure 5)

Cortex: Made up of several layers and can be distinguished into three regions: outermost region single layered thin-thick walled, lignified, circular or pentagonal to hexagonal pericyclic; central single layered aerenchymatous with large air cavities, at places mass of parenchyma is seen between the region; innermost region is 2-3 layered, oval to rectangular, thin walled, lignified, parenchymatous. (Figure 6)

Endodermis: Innermost layer of cortex single layered, oval to rectangular with marked presence of Casparian strips. (Figure 7)

Pericycle: 3-5 layered, stony, pentagonal to hexagonal, lignified. (Figure7)

Vascular Bundle: Vascular tissue is 6-9 in number, polyarch, mostly covered with xylem region, xylem exarch, surrounded by thick walled stony fibers, phloem negligible. (Figure 7)

Pith: Central most region, thin-thick walled, both lignified and non-lignified, parenchymatous. (Figure 7)

T.S. of Stolon

Epidermis: Outermost, single layered, almost rectangular, thin walled covered with thin cuticle. (Figure 8)

Hypodermis: 3-5 layered, thick walled, lignified, stony. (Figure 8)

Cortex: Many layered, oval to circular, thin walled, parenchymatous consisting of starch grains. Outer vascular bundles arranged in one series, variable in sizes, collateral, endarch and closed. (Figure 8)

Endodermis: Consisting of single layered, barrel shaped, oval to rectangular cells. (Figure 9)

Pericycle: Composed of 10 - 15 layers, compactly packed, thick walled, lignified, sclerenchymatous. (Figure 9)

Vascular Bundle: Typical monocotyledonous, polyarch, collateral, scattered through out the stele region, surrounded by ground tissue of parenchyma, each vascular bundle is covered with 2-3 layers of sclerenchymatous sheath. At places presence of single isolated or in groups of 2-3 pitted parenchymatous cellscattered through out the region. Presence of yellow coloured material in some parenchymatous cells. (Figure 10)

T.S. of Leaf

Transverse section of this typical iso-bilateral shows the following structures;

Epidermis: Both upper and lower epidermis is single layered, thin walled, oval to rectangular, covered with thin cuticle and simple trichomes. Presence of stomata (sunken) on both the layers without any bulliform cells. (Figure 11)

Mesophyll: Palisade cells are chlorenchymatous, mostly located on both the sides of vascular region. Parenchymatous cells are spongy, thin walled, oval to

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circular or rectangular with few intercellular spaces. A large number of cells occur between the upper and lower epidermis. The cells are round to iso-diametric with chloroplasts and compactly arranged.(Figure 12) (Figure 13)

Vascular Bundle: The median portion of the lamina is thickened or somewhat broader than the rest of the region and can be differentiated by the presence of collenchyma at its upper side. Vascular tissues of variable sizes are present on the lower region of the leaf arranged in a parallel series. Each bundle is collateral and closed. Distinct parenchymatous bundle sheath, presence of cells with plastids and starch grains, sclerenchymatous cells present above and below the vascular bundles and extend up to upper epidermis. In between the vascular bundles presence of wide parenchymatous cells along with vacant spaces. Larger bundles have distinct and more amount of xylem and phloem than the smaller ones. In vascular bundles, xylem is towards the upper epidermis and phloem is towards the lower epidermis. (Figure 14)

Stomata: Presence of lengthy, Graminaceous stomata, in large numbers, arranged parallel to each other, subsidiary beaded and wavy parenchymatous cells. (Figure 15)

Powder microscopy

The powder of root exhibits simple and compound starch grains, stone cells, lignified fibers, pitted parenchyma, pitted vessels, annular vessels, isolated sclereids, group of sclerenchymatous fibers and yellowish content.

Physico-chemical content

Powder shows loss of drying at 110^{o}C is 2.5 % w/w, ash value 12.85 % w/w, acid insoluble ash 3.6% w/w, water soluble extractive 9.6 % w/w, alcohol soluble extractive 9.8 % w/w and pH 5.87. The particle size consistency above 60 mesh is 20.21% w/w; between 60-85 mesh it is 36.72% w/w; between 85-120 mesh it is 35.71% w/w and below 120 mesh it is 5.65% w/w. (Table 1)

Table 1: Physico-chemical parameters of *Desmostachya bipinnata* Stapf. root powder samples

SN	Parameters	Results
1.	Loss on drying	2.5 % w/w
2.	Ash value	12.85 % w/w
3.	Acid insoluble ash	3.6 % w/w
4.	Water soluble extractive	9.8 % w/w
5.	Alcohol soluble extractive	7.6 % w/w
6.	pH value	5.87
7.	Particle consistency	
	a. above 60 mesh	20.21 % w/w
	b. between 60-85 mesh	36.72 % w/w
	c. between 85-120 mesh	35.71 % w/w
	d. below 120 mesh	05.65 % w/w

Preliminary phyto-chemical screening

Preliminary phytochemical screening of Root powder of *Desmostachya bipinnata* Stapf. was performed to have an idea of the phytoconstituents group present in the plant part as reported in Table 2. Aqueous extract of root showed the presence of tannins, carbohydrates and glycosides.

Table 2: Preliminary phyto-chemical screening of *Desmostachya bipinnata* Stapf. root powder

SN	Tests	Reagents	Results
1	Alkaloids	Wagner's reagent	-ve
		Dragendorff's reagent	-ve
2	Tannins	Lead acetate	+ve
		Gelatin test	+ve
		Dil. HNO_3	+ve
3	Triterpenoids	Leibermann-Burchard reagent	-ve

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4	Carbohydrate	Fehling's test	+ve
		Tollen's reagent	-ve
5	Flavonoids	Shinoda test	-ve
		Sodium hydroxide test	-ve
6	Saponins	Foam test	-ve
5	Glycoside	Legal test	-ve
		Keller Killiani	+ve
+ve – Positive		-ve – Negative	

Quantitative test

The samples were quantitatively tested for the estimation of Sugar content and Volatile oil content from the alcohol extract and dry powder of the sample. On applying the spectrophotometric method and observing the absorbance of the sample at 490 nm it was seen that the sugar content was 2.61 µg/mg. Volatile oil content was observed in trace amounts. (Table 3)

Table 3: Result of Sugar estimation of Alcohol Extract of *Desmostachya bipinnata* Stapf. root powder

SN	Parameter	Method used	Result
1.	Sugar	Spectrophotometric	2.61 µg/mg
2.	Volatile oil	Clevenger Apparatus	Trace

DISCUSSION

Macroscopic study

The root and stolon of *Desmostachya bipinnata* is 6-20cm long, 0.3-0.5 cm thick, almost cylindrical, internodes smooth, stout, mostly covered with shining sheath, having distinct nodes, brownish yellow, a few thin, fibrous, ash coloured roots arise at nodes, fracture.

Fig. 1: Whole plant *D. bipinnata*

Fig. 2: Dry yellowish root of *D. bipinnata*

Fig. 3: Lower surface of leaf, saw edged margins

Fig. 4: Upper surface of leaf, dark green colour and folded margins

Fig. 5: Transverse section of root, Tr-trichomes, Eb-epiblema, Ac-air cavities, Cor-ortex, Ed- endodermis, Xy-xylem, Vb-vascular bundles, P-pith

Fig. 6: Arrangement of Ed-endodermis, Tr-trichomes, Ac-air cavities, Cor-cortex, Cp-casparian strips, Pr-pericycle

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Fig. 7: Ed-endodermis, Cp-casparian strips, Pr-pericycle, Xy-xylem, P-pith

Fig. 8: Transverse section of Stolon, Ep-epiblema, Hd-hypodermis, Vb-vascular bundle, Cor-cortex, Ed-endodermis, Pr-pericycle, Xy-xylem

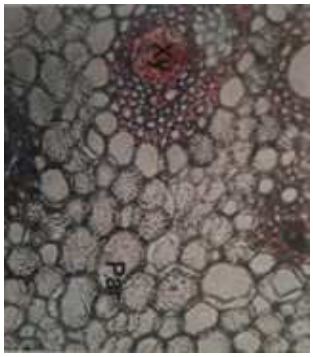

Fig. 9: Many layered Pr-pericycle, , Vb-vascular bundle, Ed-endodermis, Xy-xylem, Par-parenchyma

Fig. 10: Colouring material, pitted Par-parenchyma, Xy-xylem

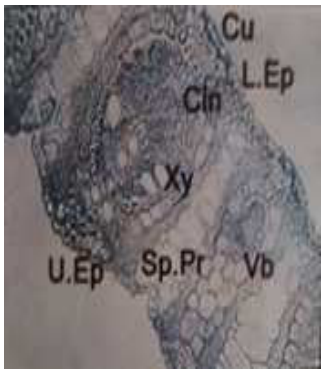

Fig. 11: Transverse section of leaf, Cu-cuticle, L.Ep-lower epidermis, Cln-collenchyma, Xy-xylem, Sp.Pr-Spongy parenchyma, U.Ep-upper epiblema

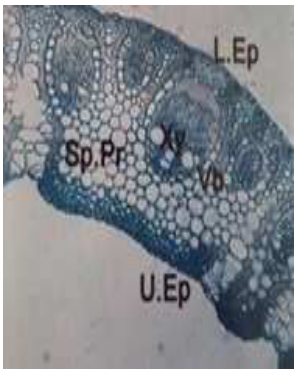

Fig. 12: Mid-rib region L.Ep-lower epidermis, , Xy-xylem, Sp.Pr-Spongy parenchyma, U.Ep-upper epiblema, Vb-vascular bundle

Fig. 13:Upper epidermis with stomata in between Cln-collenchyma , Xy-xylem, Vb-vascular bundle

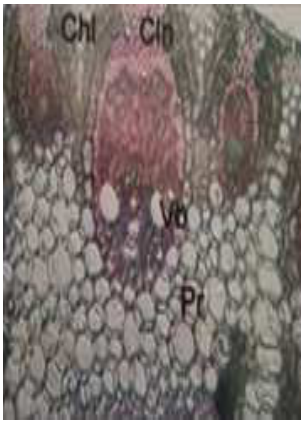

Fig. 14: Central Vb-vascular bundles with lateral bundles, Chl-collenchyma, Pr-parenchyma

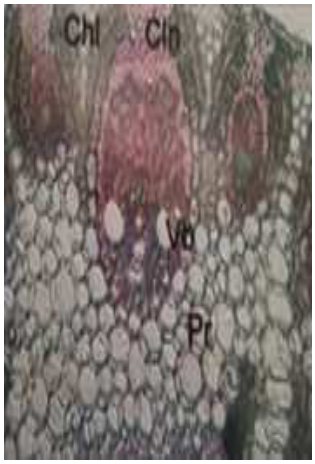

Fig. 15: B.Pr-beaded parenchyma and St-stomata

Fig. 16: Simple, compound, cup shaped starch grains in root powder

Fig. 17: Stone cells, yellow coloured material, sclerieds

Fig. 18: Annular and pitted vessels

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Fig. 19: Track B – Rf 0.44, 0.52, 0.79

Fig. 20: Track B – Rf 0.10, 0.52

The leaves of *Desmostachya bipinnata* are fascicled, very long, rigid along acuminate tips filiform, margins hispid, leaf blades narrow, flat with parallel venation and coarse.

Microscopic study

Table 4: Showing the Characteristics of *Desmostachya bipinnata* Stapf.

Part	Component	*D. bipinnata*
Root	Epiblema	Pentagonal to hexagonal cells
	Root hairs	Abundant in number
	Exodermis	Absent.
	Cortex	Large air cavities.
	Passage cells	Absent
	Casparian thickenings	Less in number.
	Pericycle	3-5 layered, stony, pentagonal- hexagonal cells.
	Vascular Bundles	6-9 vascular bundles.
	Conjunctive tissue	More in number. Occupies large area towards the pericycle.
Stolon	Epidermis	Almost rectangular cells. Hairs – absent.
	Hypodermis	3-5 layered and stony.
	Cortex	Outer vascular bundles of variable sizes arranged in one series. No air cavities present.
	Pericycle	10-15 layered, compactly packed.
	Vascular Bundles	Presence of single, isolated or groups of 2-3 pitted parenchyma cells or stone cells.
Leaf	Epidermis	Comparatively less number of sunken stomata and absence of bulliform cells.
	Mesophyll	Large number of cells between upper and lower epidermis. Cells are round to iso-diametric.
	Vascular Bundle	1. Vascular bundles of variable sizes in all the regions. 2. Median portion of lamina is broader than the rest, differentiated by the presence of collenchyma on its upper side.
	Stomata	Parallel arrangement of stomata.

Table 4 gives the characteristics of the plant. The presence of air cavities in the root but its absence in the stolon of *Desmostachya bipinnata* indicates that the plant grows near water table but is not necessarily mesophytic as it is also found in arid regions. Also, the absence of bulliform cells in the leaf of *Desmostachya*

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bipinnata suggest that the plant grows more in the xerophytic conditions as bulliform cells are found mostly in mesophytic plants.

On comparing these observations with the classical referred plants *Darbha*, it has been quoted to grow on land and near sea in Atharvaveda (A.V. 6/43/2), which can be co-related with *Desmostachya bipinnata* which is a halophytic plant, found near water sources and also in desert regions.

Powder microscopy

Powder microscopy of the plant reveals that simple and compound starch grains, stone cells, lignified sclerenchymatous fibres, pitted parenchyma, pitted vessels, and yellow coloured material was found. *Desmostachya bipinnata* had starch grains with hilum and some were cup shaped.(Figure 16,17,18,19)

Physico-chemical parameters

The ash value indicates the inorganic load of a drug. The water soluble extractive value of Sample (9.8% w/w) indicates that the load of polar components. The alcohol soluble extractive value which is indicative of the load of non-polar components Sample (7.6% w/w). The pH is 5.87 which shows that the drug is slightly acidic. The overall particle size consistency of the sample suggests that the uniformity of therapeutic dose is medium. The acid insoluble ash suggest the amount of silica in the drug which can be present due to a number of reasons such as improper washing being one of them.

Phyto-chemical Analysis

The qualitative tests conducted by using different reagents for Alkaloids, Triterpenoids, Flavonoids was negative while tests for Tannins, Carbohydrates especially Sugars and Sugar part of Glycosides gave positive results and were considered to be present in. Qualitative test for Saponins was negative for the sample.

Quantitative Analysis

Volatile oil was found to be in trace amounts in the sample. Qualitatively and quantitatively sugar was found to be present in the sample. As sugar is considered to be diuretic, the plant with higher sugar content can be supposed to cause more diuresis. *Desmostachya bipinnata* has marginally higher sugar which suggests that therapeutically it can prove to be an effective diuretic.

TLC study

The TLC pattern of the sample showed that in mobile phase 1 under long UV 3 three spots at R_f value 0.44, 0.52 and 0.79 were found. (Fig.20). While on exposure to iodine vapours, 2 spots of R_f value 0.10 and 0.51 were found (Fig.21). On spraying with 5% methanol-sulphuric acid, 5 spots of R_f value of 0.24, 0.32, 0.41, 0.43 and 0.46were found (Fig.22).

Under long UV showed 3 spots with R_f values of 0.04 0.10 and 0.16 (Fig.23), while on spraying with LB reagent and subjecting to heat one spot was observed with R_f value of 0.12 (Fig.24).

CONCLUSION

Pharmacognostic and preliminary phyto-chemical investigation included with TLC finger print profile of the plant showed some unique diagnostic characters, which could be helpful to identify the plant of *Darbha* which is gaining relevance in herbal drug research for the identification and preparation of monograph. So the drug development from this plant through rational approach has wide scope in future.

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