ANATOMICAL AND PHYTOCHEMICAL STUDY ON DURVA (Cynodon dactylon Linn, Pers) – AN AYURVEDIC DRUG

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INTRODUCTION

Durva the whole herb is reputed as a remedy in epitaxis, haematuria scabies. It checks bleeding from cut and wounds and is useful in fever, burning sensation chronic diarrhea dysentery anasarca, dropsy catarrhal ophthalmia, dysuria, bleeding piles, eye infection, epilepsy, hysteria, insanity¹. It forms a chief ingredient of several important preparations like, Durvadi tailam², Durvadi ghrutam³, manaasmitravatakam⁴ etc.

Three varieties (Durva trayam) namely Nila Durva with bluish or greenish stems. Sweta Durva with whitish stem and branches and Ganda Durva with nodulose stems are mentioned. Bhavaprakash nighantu mentioned separate synonyms and different virtues to each but such a varietal distinction is not in vogue in practice and Cynodon dactylon is accepted source of the drug.⁵

As per WHO norms, botanical standards are the proposed as a protocol for the diagnosis of the herbal drug. The Phytochemical studies of drugs done by making use of various parameters help in standardizing the drug and authenticate it. It is expected an imminent need for a well coordinated research plan touching phytochemical study of drug. Like Physicochemical analysis, HPTLC etc. The present study puts forth a set of anatomical parameter of root, stem, leaf and which can be employed to distinguish the original drug as mention in the classical Ayurvedic drugs from the other adulterants. This study throws light on the need to properly identify the plant species to achieve standardization of drug and Ayurvedic preparation.

ABSTRACT

Durva is well familiar since vedic kala to the present era. It is used in various ailments as quoted by various lexicographic texts. In spite of having a confirmed botanical identity, this plants is trapped as a common weed throughout India A large proportion of medicinal knowledge is still unexplored. Moreover, it is easily available, less in price. Any plant which is used medicinally requires detail study prior to its use because the therapeutic efficacy is absolutely depends on the quality of the plant drug used. before using a drug it is very much essential to carry out its detailed pharmacognostic study as it is not only helpful for correct identification but also to get a clue for its phyto chemical, pharmacological and medicinal properties. here in this paper The anatomy of whole plant, (root, stem, leaf) and phytochemical analysis of Durva (Cynodon dactylon) has been studied in detail to identify the genuine drug. Keywords: Cynodon dactylon, root, stem, leaf microscopy, phytochemical screening
MATERIALS AND METHODS

Anatomical studies
Fresh green full-grown and healthy plant with root was collected from its natural habitat. The plant specimen was collected and authenticated in standard Herbarium specimen in the pharmacognosy section of Dept of Dravyaguna vijnanam in G.A.C. Tripunithura. The plant was washed in pure water to remove all the impurities and was photographed. The leaf, root and stem are separated by cutting with a sharp blade. For stem and root cylindrical portion of almost straight and of sufficient length to hold the sample was selected. For leaf lamina, using a sharp blade, part of the leaf passing through the midrib was cut. Enough number of sections was taken. The sections were carefully transferred to a Petridis containing water using a fine painting brush for selection of good sections. After Staining, and Mounting process the photographs of the sections were taken using digital camera.

Phytochemical analysis

A. Physio-chemical analysis; Total ash, Acid Insoluble Ash, Water Insoluble Ash, Moisture Content, Volatile oil, Fiber Content and Tannin Content were determined by using official methods. Results were mentioned in table 1.

B. HPTLC

Procedure: Accurately weighed 100 mg of Durva choorna(whole plant) were refluxed with 100 ml of methanol for 1hour separately and filtered using Whatmann filter paper and made up to 100 ml to get methanol extract at 1mg/ml. The stationary phase used was TLC Silica gel 60 F254. The mobile phase selected was Toluene: Ethylacetate: Diethylamine (7:2:1) for Durva powder extract. The samples were applied at 6 μl and 8 μl. Plate was developed and dried for five minutes. Plate was visualized under UV 366nm, 254nm. (Fig 1)

C. Qualitative Analysis of crude drug – Table 2

1) Alkaloids
Dragendroff’s test: To 0.5ml of alcoholic solution of extracts of Durva churna taken in separate test tubes, 2.0 ml of Hydrochloric acid solution was added. To this acidic medium, 1.0ml of Dragendroff’s reagent was added. An orange – red precipitate produced immediately indicates the presence of alkaloid.
Meyer’s test: To 10 ml of alcoholic extracts of Durva churna taken in separate test tubes, few drops of Meyer’s reagent was added. Formation of white or pale precipitate showed the presence of alkaloids.

2) Flavonoids: In test tubes containing 0.5ml of alcoholic extracts of churna each was taken, 5 – 10 drops of dilute Hydrochloric acid and small piece of magnesium was added and the solution was boiled for few minutes. Reddish pink color indicates positive test for flavonoids.

3) Saponins: In test tubes about 5ml of aqueous extracts of Durva churna was taken, a drop of sodium bicarbonate solution was added. The mixture was shaken vigorously and kept for 3min. A honey comb like froth formation in test tubes indicates the presence of saponins.

4) Carbohydrates
Fehling’s test: To 2ml of aqueous extracts of churnas of Durva taken in separate test tubes, a mixture of equal parts of Fehling’s solution A and B were added. The test tubes were boiled for few minutes. Formation of red or brick precipitate indicates the presence of carbohydrates.
Benedict’s test: To 0.5ml of aqueous extracts of Durva churna taken in separate
test tubes, 5ml of Benedict’s reagent was added and boiled for 5 minutes. Formation of bluish green color in test tubes showed the presence of carbohydrates.

5) Proteins

Biuret's test: To 1ml of hot aqueous extracts of Durva churna taken in separate test tubes, 5-8 drops of 10% NaOH solution, followed by 1-2 drops of 3% CuSO4 was added. Formation of violet colored solution indicates the presence of proteins.

6) Phenols:

Ferric Chloride test: To 1ml of alcoholic extracts of Durva churna 2.0ml of distilled water followed by a few drops of 10% aqueous Ferric Chloride solution was added. Formation of blue or green color indicated the presence of phenols.

Lead acetate test: 1ml of alcoholic extracts of Durva churna was taken in separate test tubes and was diluted to 5ml distilled water. To this few drops of 1% aqueous solution of lead acetate was added. The formation of yellow precipitate in test tubes indicates the presence of phenols.

7) Steroids

Ferric chloride test: 1-2ml aqueous extracts of Durva churna was taken in test tube. Then, few drops of 5% Ferric chloride solution were added. A bluish black color formed which disappeared on addition of diluted Sulphuric acid, forming a yellow brown precipitate indicates the presence of steroids.

8) Tannins

Ferric chloride test: 1-2ml aqueous extracts of Durva churna was taken in test tube. Then, few drops of 1% solution of lead acetate was added. Formation of yellow or red precipitate indicates the presence of tannins.

OBSERVATIONS AND RESULTS

Anatomical Description

Macroscopic features:

i) Root - Fibrous, cylindrical, up to 4 mm thick, minute hair-like roots arise from the main roots; cream coloured.

ii) Stem - Slender, prostrate, up to 1.0 mm thick, jointed leafy, very smooth, yellowish green in colour.

iii) Leaf - 2 to 10 cm long and 1.25 to 3 mm wide, narrowly linear or lanceolate, finely acute more or less glaucous, soft, smooth, usually conspicuously distichous in the barren shoots and at the base of the stems; sheath light, glabrous or sometimes bearded, ligule a very fine ciliate rim.

Microscopic features

Histological characters:

i) T.S. of Root: Mature root shows epiblema or piliferous layer composed of a single layer of thin-walled, radially elongated to cubical cells; hypodermis composed of 1 or 2 layered, thin-walled, tangentially elongated to irregular shaped cells; cortex differentiated into two zones, 1 or 2 layers of smaller, thin-walled, polygonal, lignified sclerenchymatous and 4 to 6 layers of larger thin-walled, elongated parenchymatous cells; endodermis quite distinct, single layered, thick-walled, tangentially elongated cells; pericycle 1 or 2 layers composed of thin-walled sclerenchymatous cells; vascular bundles consisting of xylem and phloem, arranged in a ring on different radials; xylem exarch, having usual elements; centre occupied by wide pith, composed of oval to rounded thick-walled parenchymatous cells containing numerous simple, round to oval or angular starch grains measuring 4 to 16 μ
in dia., and compound starch grains having 2 to 4 components. (Fig.2)

ii) Stem: Oval in outline with a little depression on one side, shows a cuticularised epidermis single layered, having lignified walls; hypodermis 1 or 2 layers, sclerenchymatous; cortex composed of 3 to 5 layers of round to oval thin walled parenchymatous cells; endodermis not distinct; pericycle present in the form of Continuous ring of 2 to 5 layers of sclerenchymatous fibres; vascular bundle collateral, closed and scattered throughout the ground mass of parenchyma, each surrounded by sclerenchymatous sheath; vessels simple, spiral, scalariform, and annular; medullary rays not distinct; fibres short, thick walled, having narrow lumen and pointed tips; starch grains simple and compound having 2 to 4 components, present in cortex and ground tissue, simple grains measuring 4 to 16 μ in dia. (Fig.3)

iii) Leaf: Lamina shows nearly square to oval epidermis having irregularly cutinised outer wall, bulliform cells present on the dorsal side which are grouped together and lie at the bottom of a well defined groove in between the veins; these are thin walled and lack chlorophyll, extend deep into the mesophyll; mesophyll not differentiated into palisade and spongy aparenchyma; row of vascular bundles nearly alike, except that the median bundle is larger; bundle sheath single, and consists of thin-walled more or less isodiametric parenchyma cells containing chloroplast; mesophyll tissue broken by 1 or 2 thin-walled colourless cells which extend from bundle sheath to the thin walled parenchymatous band of stercome near upper and lower epidermis. (Fig. 4)

Phytochemical Results

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Experiments</th>
<th>Result</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Total ash</td>
<td>9.1 %</td>
</tr>
<tr>
<td>2</td>
<td>Acid Insoluble Ash</td>
<td>3.7 %</td>
</tr>
<tr>
<td>3</td>
<td>Water Insoluble Ash</td>
<td>7.9 %</td>
</tr>
<tr>
<td>4</td>
<td>Moisture Content</td>
<td>15 %</td>
</tr>
<tr>
<td>5</td>
<td>Volatile oil</td>
<td>1 %</td>
</tr>
<tr>
<td>6</td>
<td>Fiber Content</td>
<td>30.46 %</td>
</tr>
<tr>
<td>7</td>
<td>Tannin Content</td>
<td>.80 %</td>
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</tbody>
</table>

Table 2: Qualitative analysis of crude drug powdered of Durva

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Result</th>
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<tbody>
<tr>
<td>1) Alkaloids:</td>
<td></td>
</tr>
<tr>
<td>a) Dragendorff’s test</td>
<td>Absent</td>
</tr>
<tr>
<td>b) Meyer’s test</td>
<td>Present</td>
</tr>
<tr>
<td>2) Flavonoids</td>
<td>Absent</td>
</tr>
<tr>
<td>3) Saponins</td>
<td>Present</td>
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<tr>
<td>4) Carbohydrates</td>
<td></td>
</tr>
<tr>
<td>a) Fehling’s test</td>
<td>Present</td>
</tr>
<tr>
<td>b) Benedict’s test</td>
<td>Present</td>
</tr>
<tr>
<td>5) Proteins</td>
<td>Absent</td>
</tr>
<tr>
<td>6) Phenols</td>
<td></td>
</tr>
<tr>
<td>a) Ferric chloride test</td>
<td>Present</td>
</tr>
<tr>
<td>b) Lead acetate test</td>
<td>Absent</td>
</tr>
<tr>
<td>7) Steroids</td>
<td>Present</td>
</tr>
<tr>
<td>8) Tannins</td>
<td></td>
</tr>
<tr>
<td>a) Ferric chloride test</td>
<td>Present</td>
</tr>
<tr>
<td>b) Lead acetate test</td>
<td>Present</td>
</tr>
</tbody>
</table>

DISCUSSION

_Durva_ one of the important drugs used in the various indigenous medicines and formulations of Ayurveda. Detailed pharmacognostical study of _Cynodon dactylon_ was decided to undertake to bring out the salient diagnostic features which would help in crude drug identification as well as standardization of the quality and purity of the drug in crude form. The generated data can also be used for the development of monograph on this plant. The following anatomical features are suggested to diagnose root stem and leaf.
**Root** - endodermis quite distinct, single layered, thick-walled, tangentially elongated cells; vascular bundles consisting of xylem and phloem, arranged in a ring on different radials; centre occupied by wide pith, composed of numerous simple, round to oval or angular starch grains.

**Stem** - Pericycle present in the form of continuous ring of 2 to 5 layers of sclerenchymatous fibres; vascular bundle collateral, closed and scattered throughout the ground mass of parenchyma.

**Leaf** - Irregularly cutinised outer wall, bulliform cells present on the dorsal side. mesophyll not differentiated.

While observing the physiochemical analysis, total ash represents the inorganic salts in the drug. Thus ash value is a general criterion to ascertain the purity of any drug. Here a total ash value was found as 9.1%. Acid insoluble ash gives the percentage of sand and impurities that remains insoluble in dil.HCl and it was found to be 3.7%. Lower the value higher the purity of the drug. Water insoluble ash mainly gives the percentage of organic matter present in the ash of the drug. It was found to be 7.9%. Moisture content of the shade dried plant was found to be 15% and the less amount of moisture indicates the proper drying of the materials. Volatile oil content was observed 1%. Fiber content was found to be 30.46%. Tannin content was found as .80%. The following HPTLC fingerprint profiles are suggested to diagnose the *Cynodon dactylon*; whole plant methonolic extract under UV366nm, it can be identify by the spot at Rf .18, Rf.24, Rf .76, Rf.86. The different extractive solution of crude drug powder of Durva showed the presence of alkaloids, saponin, carbohydrate, phenol, steroid, tannin.

**CONCLUSION**

This study on macro and microscopic features of *Cynodon dactylon*, revealed a set of anatomical parameters which may facilitate identification of genuine drug. Preliminary phytochemical study was also carried out and their details are mentioned along with the results, observation obtained in the experiments. These parameters help in standardizing the drug and give us an idea of phytochemistry of plant.

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Fig 1: HPTLC picture of the extract of powdered Durva

Fig 2: Cynodon dactylon(Linn)Pers. – T.S. of Root

Epiblema or piliferous layer
Hypodermis
Cortex
Endodermis
Pericycle
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Fig 3: *Cynodon dactylon* (Linn) Pers. – T.S. of stem

- Epidermis
- Cortex
- Vascular bundle
- Pericycle
- Starch grains

Fig 4: *Cynodon dactylon* (Linn) Pers. – T.S of the Leaf, entire view

- Cutinized outer wall
- Vascular Bundles
- Midrib region
- Bulliform cells