# Pharmacognostic Investigation of Cynodon dactylon Pers Roots

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## ABSTRACT

**Background:** *Cynodon dactylon* (L.) Pers. family (Graminae/poaceae) occupies its unique place and key position in ethnomedicinal practices and traditional medical (Ayurvedic, Unani, Nepalese, and Chinese) knowledge systems but according to best of our knowledge lack is done on its standardization of the herb for its quality control and authenticity. **Objective:** To evaluate the morphological and microscopical characters of *Cynodon dactylon* Pers roots collected from Maharashtra region and its phytochemical and physicochemical analysis. **Methods:** Microscopic, macroscopic characters and fluorescence analysis of roots samples were analyzed. The physicochemical properties such as loss on drying, total ash value, acid insoluble ash, water soluble ash value and extractive values of *Cynodon dactylon* were carried out. **Results:** The detailed microscopy revealed the presence wide cortex, intact epidermis, wide circular metaxylem and parenchymatous cells loaded with starch grain. Preliminary phytochemical investigation revealed the presence of carbohydrates, flavonoids, phenols and tannins. **Conclusions:** This is first report on the pharmacognostic studies of *Cynodon dactylon* and is helpful in laying down identification, standardization and pharmacopeial standards.

## INTRODUCTION

The proper identification of plants and drugs is the most important aspect of any crude drug research. The problem of identification is more pronounced in Ayurveda since much controversy exists due to one or more than several botanical species for the same drug.[1]Since many of the drugs in Ayurveda are sold as crude drugs, it is essential to study and understand the tissue systems which hold key in arriving at the correct identification. Thus, this original classical approach towards Pharmacognosy is essential for the proper characterization and identification of the drug and the plant.

Bermuda grass, Doob Ghas, or Durva or taxonomically the *Cynodon dactylon* (L.) Pers. family (Graminae/poaceae) occupies its unique place and key position in ethnomedicinal practices and traditional medical (Ayurvedic, Unani, Nepalese, and Chinese) knowledge systems. The herbal preparations of this grass are being based on folklore and traditional wisdom.[2]

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It is an inseparable part of religious rituals and is a valuable herbal medicine used as first aid in minor injuries.[3] In Indian households it is a common practice to place strands of this grass on eatables during eclipse. Folk wisdom claims it to be water purifier.[4] The juice of the plant is astringent and the fresh juice is used in the treatment of chronic diarrhoea and dysentery.[5] The plant occupies a renowned position in Ayurveda, Unani and Homoeopathic systems of medicine.[6] It possesses various medicinal properties such as antimicrobial, antiviral activity[7] and has significant application in treating dysentery, dropsy and secondary syphilis.[8]

## MATERIAL METHODS

### Plant collection and extraction

Fresh roots of *Cynodon dactylon* Pers were collected from the local area of Pune District of Maharashtra, India in month of June 2011. The plant specimen was identified and authenticated as *Cynodon dactylon* Pers by BSI, Pune, India. The voucher specimen (No. SRD-1) is preserved in the herbarium of Dept. of Pharmacognosy. The air-dried roots of C.dactylon were made into coarse powder. The powdered material was extracted with Soxhlet apparatus using different solvents like petroleum ether (60°–80°C), chloroform, methanol and water as per their polarity

successively. The extract was dried using rotary evaporator and was kept in a dessicator till experimentation.[9,10]

## PHARMACOGNOSTIC STUDIES

### Macroscopy evaluation

Different sensory parameters of the root material (like colour, odour, size, shape and taste) were studied.

### Microscopic analysis

Microscopic studies were done by simple microscope. Free hand section of root was taken and stained by safranine to confirm its lignifications. Powder microscopy was also carried out and the specific diagnostic characteristics were recorded.[11]

### Physicochemical parameters

Physicochemical parameters of powdered drug such as total ash, water soluble ash, and acid-insoluble ash were determined. Alcohol and water soluble extractives values were determined to find out amount of alcohol and water soluble constituents. Loss on drying method was employed to find moisture content.[12]

### Phytochemical analysis

Consistency, color, appearance of the extracts and their percentage yield were noted. The extracts obtained from successive solvent extraction were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, proteins and amino acids, saponins, and phytosterols using reported methods.[13,14]

### Fluorescence analysis

Powdered root material was analysed under visible light, short ultra-violet light, long short ultra-violet light after treatment with various organic/inorganic reagents like NaOH, HCl, $HNO_3$ and $H_2SO_4$. The colors observed by application of different reagents in different radiations were recorded.[15, 16]

## RESULTS

### Pharmacognostic studies

#### *Macroscopy evaluation*

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

#### *Microscopic analysis*

Transverse section of roots revealed the presence of smooth and even surface. It is nearly 4mm thick. It consists of continuous intact epidermis, fairly wide cortex and wide dictyostele (Fig 1.1). Epidermal layer includes small thick walled cells with heavy cuticle (Fig 1.2). Cortex has about 6 layers of circular or angular, compact parenchyma cells.

Inner to the cortex is a thick and continuous cylinder of fibres which posses thick lignified walls and narrow lumen (Fig 1.2, Fig 2.1, Fig 2.2). Some of the vascular strands are included within the sclerenchyma cylinder either in outer part or in the inner part. (Fig 1.2, Fig 2.1, Fig 2.2). The vascular strand located with outer part of the sclerenchyma cylinder are small and somewhat circular with tangential band of xylem elements and small cluster of phloem found on the outer part of the strand. (Fig 2.2).

The inner vascular strand and central strands are larger and more prominent (Fig 2.1, Fig 3.1). They are collateral

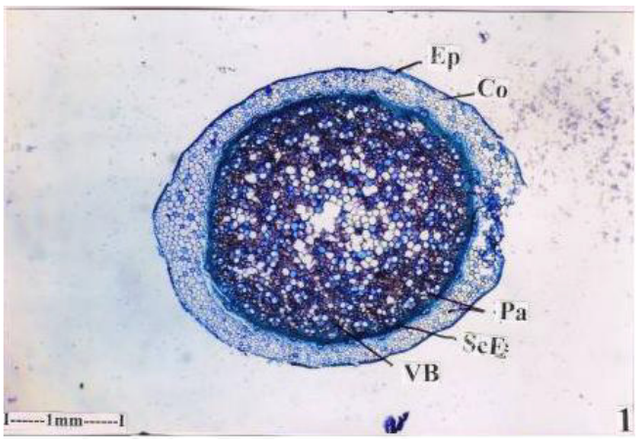


**Figure 1.1** TS of root-Entire view.

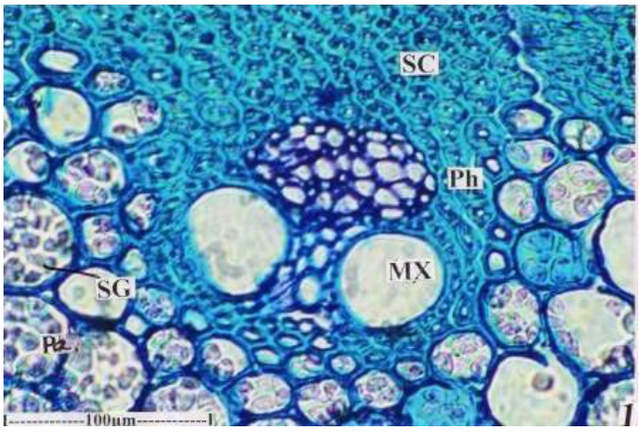


**Figure 2.1** Vascular bundle on the inner part of the sclerenchyma cylinder.

and closed. There are two wide circular metaxylem elements and narrow vertical row of protoxylem elements lying in between the metaxylem cells. Some of the protoxylem cells disintegrate form a small protoxylem lacuna (Fig 3.1). The medullary bundles which are free from the cortical sclerenchyma cylinder are surrounded by thin bundle sheet fibres.

The ground tissue is parenchymatous. The cells are large, circular and thin walled. The cells are heavily loaded with simple or compound starch grains (Fig 3.2).

Powder of the root consists of following elements.

Parenchyma cells: (Fig 4.1) wide and parenchyma cells are frequently seen in the powder. The cells are rectangular or squarish in shape. Most of the cells are seen in strands attached end to end. The cell walls are thin. The cells posses abundance of starch grains. The cells 40 × 50 μm in size. Fibres: Fibres are predominant elements in the powder. The fibres are of two types. Some are narrow, long and pointed at the ends. (Fig 4.1, 5.1) They have thick wall and narrow lumen. They are 650μm and 12μm

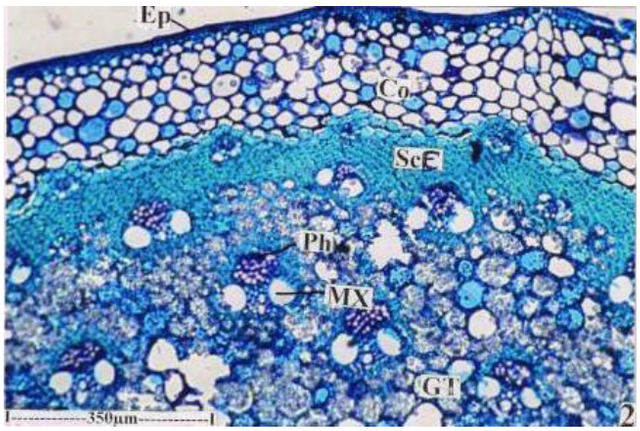


**Figure 1.2** TS of root-A sector (co–cortex, Ep-epidermis, GT- ground tissue, MX-metaxylem, Pa-parenchyma, Ph-Phloem, ScE-sclerotic endodermis, VB-vascular bundle).

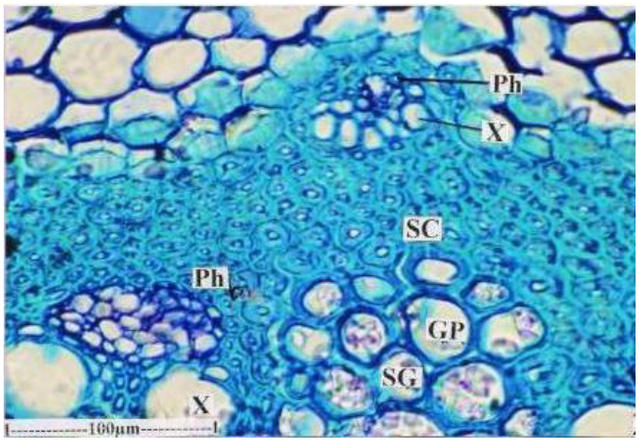


**Figure 2.2** Vascular strand situated in the outer part of the sclerenchyma cylinder. (Gp-ground plan, Mx-meta xylem, Ph-Pholem, Pa-Parenchyma, Sc-sclerenchyma, SG-starch grain, X-xylem).

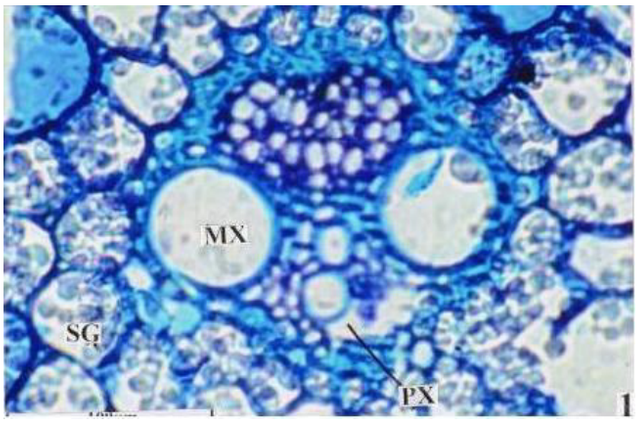


**Figure 3.1** Medullary vascular bundle-enlarged.

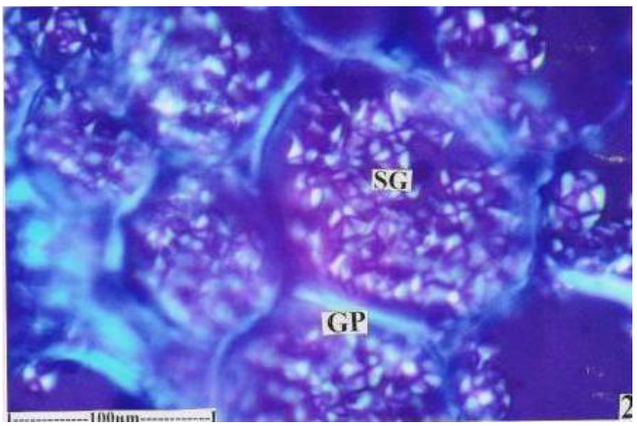


**Figure 3.2** Ground parenchyma cells with starch grains (Gp-ground plan, Mx-meta xylem, SG-starch grain, Px-proto xylem) Powder microscopy.

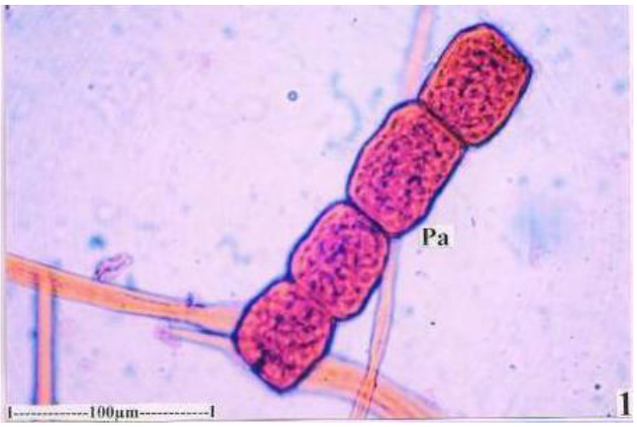


**Figure 4.1** Parenchyma strand.

thick. The second types of fibres are wide fibres. They are thin walled with wide lumen. The largest of the wide fibres ranges from 500–700µm, the thickness of the cells is 15–22µm (Fig 5.2) Vessel elements are long narrow and cylindrical (Fig 6.1, 2). The end wall perforation is simple, circular and horizontal in orientation. The lateral wall pits are horizontal elliptic, multiseriate and dense. The vessel elements are 430µm long and 30µm wide.

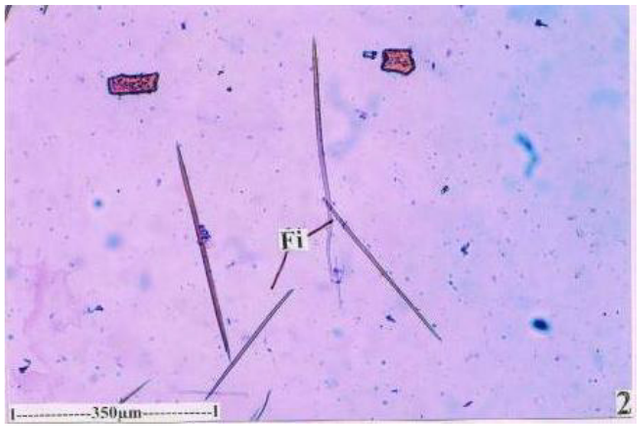


**Figure 4.2** Narrow fibers (F-fibers, Pa-parenchyma).

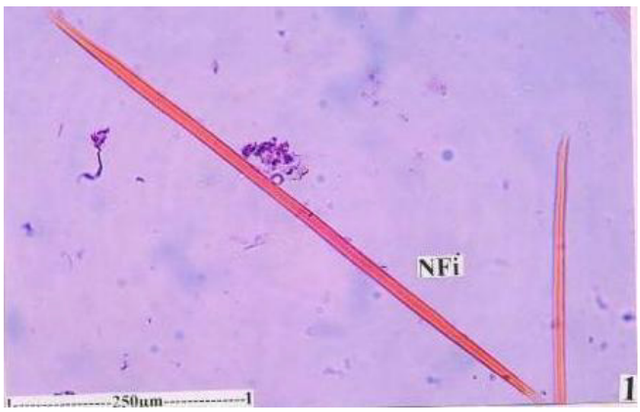


**Figure 5.1** Narrow fiber-enlarged.

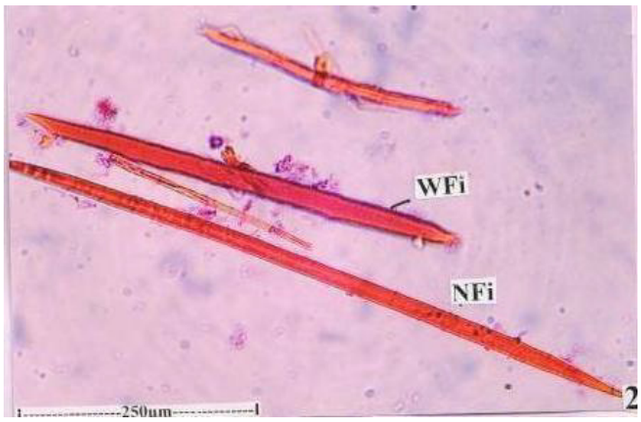


**Figure 5.2** One narrow fiber and one wide fiber (NFi-narrow wide fiber, WFi-wide fiber).

## *Physicochemical parameters*

Ash values of the drug give idea about earthy matter or inorganic composition and other impurities present along with the drug. Various physicochemical parameters such as total ash, water soluble ash and acid insoluble ash of *C.dactylon* root was found to be 7.15, 5.35 and 3.18% w/w, respectively.

Moisture content in the root was found to be 8.11% w/w. The extractive values are primarily useful for the determination of the exhausted or adulterated drug. Various extractive values such as petroleum ether soluble extract, chloroform soluble extract, methanol soluble extract and water soluble extract of *C.dactylon* root was found to be 3.61, 1.83, 8.11 and 7.85% w/w respectively given in Table 1.

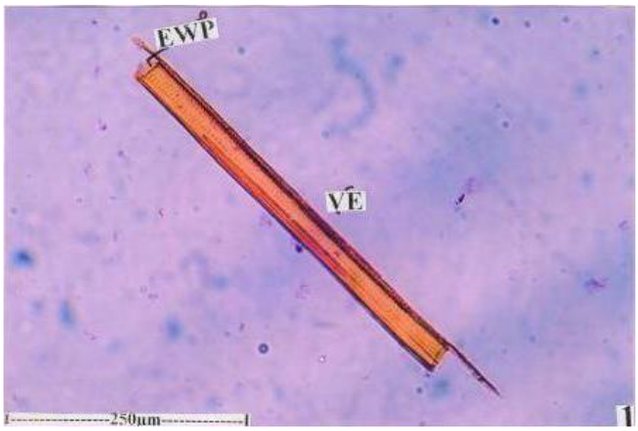


**Figure 6.1** Two vessel elements with simple end wall perforation (EWP-end wall perforation, Pi-pits, Ve-vessel elements).


**Figure 6.2** Two vessel elements with simple end wall perforation (EWP-end wall perforation, Pi-pits, Ve-vessel elements).

### *Phytochemical analysis*

Successive solvent extracts of root was studied for their phytochemical profile. Their % yield, color and consistency are recorded in Table 2. Preliminary phytochemical screening mainly revealed the presence of phenol and tannins in petroleum ether extract; carbohydrates, phenols and saponins in chloroform extract; carbohydrates, tannins and flavonoids in methanol extract and carbohydrates, tannins, flavonoids and carbohydrates in aqueous extract are mentioned in Table 3.

### *Fluorescence analysis*

The fluorescence analysis of the root powder with different chemical reagents is summarized in Table 4.

## DISCUSSION

Pharmacognostic study is the initial step to confirm the identity and to assess the quality and purity of the crude drug. It is necessary that standards have to be laid down to control and check the identity of the plant and ascertain its quality before use. According to World Health Organization (WHO) the macroscopic and microscopic description of medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.[17]

Microscopical evaluation is simplest and reliable tool for correct identification of herbs as well as small fragment of crude drugs or powdered drugs and detection of adulterants and substituents.[18–20]

Total ash values and extractive values are useful in identification and authentication of the plant material.[21,22] Extractive values are useful to evaluate the chemical constituents of crude drug.[23]

**Table 1. Physicochemical constant of root of Cynodon dactylon.**

| Sr. No | Parameters | Mean % w/w |
|---|---|---|
| 1. | Loss on drying | 8.11 |
| 2. | Total ash value | 7.15 |
| 3. | Acid–insoluble ash value | 3.18 |
| 4. | Water soluble ash value | 5.35 |
| 5. | Alcohol soluble extractive value | 2.48 |
| 6. | Water soluble extractive value | 8.21 |

**Table 2. Preliminary phytoprofile of root of Cynodon dactylon.**

| Sr. No | Type of extract | Day light | UV short light | UV long light |
|---|---|---|---|---|
| 1. | Pet ether (60°–80°C) | Yellow | Brown | Greenish |
| 2. | Chloroform | Green | Dark green | Black |
| 3. | Methanol | Brown | Deep brown | Black |
| 4. | Aqueous | Brown | Deep brown | Black |

**Table 3. Qualitative chemical test on extracts of roots of Cynodon dactylon.**

| Sr. No | Phytoconstituents | Pet ether (60o–80o C) | Chloroform | Methanol | Aqueous |
|---|---|---|---|---|---|
| 1. | Alkaloids | - | - | - | - |
| 2. | Carbohydrates | - | + | + | + |
| 3. | Glycosides | - | - | - | - |
| 4. | Flavonoids | - | - | + | + |
| 5. | Phenol & tannins | + | + | + | + |
| 6. | Steriods | - | - | - | - |
| 7. | Triterpenoids | - | - | - | - |
| 8. | Saponins | - | + | - | - |
| 9. | Proteins | - | - | - | + |
| 10. | Amino acids | - | - | - | - |

– Negative; + Positivew

**Table 4. Fluorescence analysis of root powder of Cynodon dactylon with various reagents.**

| Sr. No | Reagent + Drug | Colour of powder at Day light | UV Light Short | UV Light Long |
|---|---|---|---|---|
| 1. | Untreated powder | Yellowish brown | Brown | Black |
| 2. | Powder + saturated Picric Acid | Pale green | Green | Dark brown |
| 3. | Powder + Nitric acid | Brown | Brown | Blackish |
| 4. | Powder + 1 N HCl | Brownish | Greenish brown | Dark green |
| 5. | Powder + conc. H2SO4 | Light brown | Blackish brown | Black |
| 6. | Powder + Glacial Acetic Acid | Brownish | Brownish | Blackish |
| 7. | Powder + 1N NaOH | Brownish green | Light green | Blackish green |
| 8. | Powder + Iodine | Brownish | Blackish brown | Blackish |
| 9. | Powder + Ferric chloride | Yellowish brown | Dark green | Black |

Fluorescence is an important phenomenon exhibited by various chemical constituents present in plant material. Some constituents show fluorescence in the visible range in day light. The ultra violet light produces fluorescence in many natural products, which do not fluoresce in daylight. If the substance themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is important parameter of pharmacognostic evaluation.[21,22]

Preliminary phytochemical screening mainly revealed the presence of phenol and tannins in petroleum ether extract; carbohydrates, phenols and saponins in chloroform extract; carbohydrates, tannins and flavonoids in methanol extract and carbohydrates, tannins, flavonoids and carbohydrates in aqueous extract. T.S of the root confirmed the presence of wide cortex, intact epidermis, wide circular metaxylem and parenchymatous cells loaded with starch grain.

In conclusion the detail study was undertaken with an aim of pharmacognostic standardization and preliminary phytochemical analysis of *C.dactylon* roots established in the present study will useful in identifying the genuine drug and will also be useful in development of pharmacopeial standards for further studies.

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