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PHARMACOGNOSTICAL AND PHYSICOCHEMICAL EVALUATION OF *CYNODON DACTYLON* LINN. PERS. LEAF EXTRACT

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ABSTRACT: From ancient time, plants serve as vast source for varied phyto-constituents exhibiting varied pharmacological property. Identifying such potential plants is of significance in medicine. So it becomes necessary to study the pharmacognostic characteristic and physicochemical parameters of the plant before they are use in the field of research and also in pharmaceutical formulations. Moreover it also helps in distinction from other allied species and adulterants, guarantee the safe use of quality products. In this connection, the pharmacognostical characteristics of the leaf of the *Cynodon dactylon* (L.) Pers. was examined. The macro and microscopical characteristics like, vein islet numbers, palisade ratio, stomatal index (upper and lower surfaces of the leaf) etc. were studied. Physicochemical parameters evaluated include ash values, extractive values and loss on drying. These findings will be helpful towards establishing pharmacognostic and physicochemical standards on identification, purity, quality and classification of the plant, which is gaining relevance in plant drug research.

INTRODUCTION: In the indigenous system of medicine, the plants in crude form, either fresh or dried are utilized for their curative effects against a variety of mankind's ailments. However, due to some morphological similarities and lack of correct identification, the crude drugs are often adulterated or substituted in commerce, which obviously results in the loss of drug efficacy. Correct identification of herbal drug is the foundation of the safe use of plant based natural health products. Without proper identification as a starting point, the safe use of quality products cannot be guaranteed¹. Hence, adulteration in market samples has become one of the greatest drawbacks in promotion of herbal products².

Dried products sold in the market are generally difficult to identify, as many useful diagnostic characters are lost during drying. At the same time other numerous problems are confronted to taxonomists in the identification of traded herbal drugs. The existence of several common names for the same plant species in different areas may confuse end users for selection and utilization of genuine drug. Another problem is superficial resemblance of plant species within the same tribe or family³.

Problem of adulteration in medicinal plants arose due to the potential use of different species for similar ailments⁴. The quality control of herbal drugs and their bio-constituents is of paramount importance in justifying their acceptability in modern system of medicine. One of the major problems faced by user industry is non-availability of rigid quality control profiles for herbal raw materials and their formulations. With the advent of new analytical tools and sophisticated instrumental

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technology, it is possible to suggest a practicable quality assurance profile for a crude drug or its bioactive constituent⁵.

Over the years, nature and degree of evaluation of crude drugs has undergone a systematic change. Initially, the crude drugs were identified by comparison only with standard description available. Due to advancement in the chemical knowledge of crude drugs, at present, evaluation also includes method of estimating active constituent present in the crude drug, in addition to its morphological and microscopic analysis. With the advent of separation techniques and instrumentation analysis, it is possible to perform physical evaluation of a crude drug, which could be both of qualitative and quantitative nature. The biological behaviour of crude drug extracts constitutes pharmacological evaluation. The crude drugs can be identified on the basis of their morphological, histological, chemical, physical and biological studies.

Each and every plant has its unique external and internal body structure (morphology) whether it is from the same or different genus. Various types of tissues / cells from the plants. These cell types can become markers for their identity. For confirmation of the same, the evaluation of the external and the internal morphology of the leaves of *Cynodon dactylon* (L.) Pers. in fresh and powdered form was done using standard procedures from Indian Herbal Pharmacopoeia⁶.

Physicochemical analysis includes the study of various parameters such as determination of foreign organic matter, ash content, water and ethanol extractives, loss on drying and percentage moisture content. The evaluation of these parameters gives a clear idea about the specific characteristic of the medicinal plant under examination, besides its macro - morphological or cyto - morphological characters. So also most of the crude drugs (plant material) are usually put in quarantine store and they remain there for long time. During storage proper ventilation, humidity controls, suitable temperature and light conditions should be ensured to maintain their original pharmacological action; however, it is observed that, crude plant materials, before being taken for processing, are not analyzed and can lead to changes in their original

characteristics. To avoid this, the crude drugs should be tested for all the physicochemical parameters as per the United State Pharmacopoeia (USP)⁷ and Indian Herbal Pharmacopoeia (IHP)⁶. This paper reports the pharmacognostical standardization and physicochemical parameters which includes macroscopical, microscopical and quantitative microscopy studies. The study also includes determination of physico-chemical constants of leaves of *Cynodon dactylon* (L.) Pers.

Therefore the present work has been undertaken to establish the various pharmacognostical and physicochemical parameters, which could serve as a measure of authentication and quality control for commercial samples of the crude drug.

MATERIALS AND METHODS: Leaves of *Cynodon dactylon* (L.) Pers. were collected from Mumbai and Talegaon – Dabhade (district - Maval, Pune). The plant samples *Cynodon dactylon* (L.) Pers. (Acc. no. - 83143) was authenticated by the expert taxonomist of St. Xavier's College, Mumbai.

Pharmacognostical Study: The external and the internal morphological characteristics of the leaves of *Cynodon dactylon* (L.) Pers. were studied in fresh form. For the microscopic study of the plant powder, the plant material was dried and processed as explained under collection.

Macroscopic Studies of Fresh Plant Leaves: External morphology of the said plant leaves were studied by naked eye and with the help of dissecting microscope.

Microscopic Analysis of Fresh Plant Leaves: The fresh leaves of *Cynodon dactylon* (L.) Pers. were separated and washed thoroughly. Thin transverse sections of the leaves were taken and temporarily double stained with safranin and hematoxyline by the standard procedure. The slides were observed under microscope with different magnifications, anatomical characteristics of the four leaves were noted down and photo-micrographic records were made.

Quantitative Microscopy of Fresh Plant Leaves: Determination of Stomatal Index: Leaf fragments of the plants under study (about 5 x 5 mm in size) were placed in test tube containing about 5ml

chloral hydrate solution and heated in a boiling water bath for about 15 minutes or until the fragments became transparent. A fragment was transferred to a microscopic slide mounted, in chloral hydrate solution and a small drop of glycerol, ethanol solution on one side of the cover glass to prevent the preparation from drying. Examination was done at 240x. A microscopical drawing apparatus was attached to the eyepiece A cross (x) was marked on the drawing paper for each epidermal cell and a circle (o) for each stomata.

The Stomatal Index was calculated by using following formula -

$$\text{Stomatal Index (I)} = \frac{S}{S + E} \times 100$$

Where, S: Number of stomata per unit area; E: Number of ordinary epidermal cells in the same unit area.

For each sample of leaf, minimum of ten determinations were made and the average index was calculated.

Determination of Palisade Ratio: Leaf fragments of the plants under study (about 5 x 5 mm in size) were placed in a test tube containing 5 ml of chloral hydrate solution and heated in a boiling water bath for about 15 minutes or until the fragments became transparent. A fragment was transferred to a microscopic slide mounted; the upper epidermis in chloral hydrate solution and a small drop of glycerol solution was put on one side of the cover glass to prevent the preparation from drying. Examination was done at 240x. A microscopical drawing apparatus was attached to the eyepiece was marked on the drawing paper. Four adjacent epidermal cells on paper were traced; gently focused downward to bring the palisade into view and sufficient palisade cells were traced to cover the area of the outlines of the four epidermal cells. When the cell is intercepted, it was included in the count only when more than half of it was within the area of epidermal cells. The average number of palisade cells beneath one epidermal cell dividing the count by 4 was calculated.

Determination of Vein - Islet Number: Pieces of leaf lamina were taken with an area of not less than 4 square millimeters from the central portion of the

lamina and excluding the midrib and the margin of the leaf. The pieces of lamina were cleared by heating in a test-tube containing chloral hydrate solution on a boiling water bath for 30 to 60 minutes or until clear and a mount on glycerol solution was prepared or, if desired, stained with saffranin solution and the mount prepared in Canada Balsam. The stage micrometer on the microscope stage was placed and examination was done at 240x by drawing a line representing 2 mm on sheet of paper by means of a microscopical drawing apparatus and construct a square on the line representing an area of 4 square millimeters.

The paper was moved so that the square was seen in the center of the field of the eyepiece. The slide was placed with the cleared leaf piece on the microscope stage and drawn in the veins and veinlets included within the square, completing the outlines of those vein-islets, which overlap the two adjacent sides of the square. The number vein-islets within the square including those overlapping on two adjacent sides and excluding those intercepted by the other two sides were counted. The result obtained is the vein islets in 4 square millimeters. For each sample of leaf make no fewer than three determinations and the average number of vein islets per square millimeter was calculated.

Fluorescence Analysis and Powder Microscopy of the Dry Plant Powders: For fluorescence analysis, the plant leaf powders were examined directly under UV light and day light by adding different reagents (**Table 1**). The powder microscopy was done by staining the dry plant powders with dilute aqueous saffranin for two minutes. At the end of two minutes, the stained material was washed to remove the excess stain and mounted in Dextrin plasticizer xylol (DPX) to make a permanent mount. The slides were studied under light microscope at different magnifications and photomicrographs were taken.

Physico-Chemical Parameters:

Foreign Organic Matter: Medicinal plant materials should be entirely free from visible signs of contamination, *i.e.* moulds, insects, and other animal contamination, including animal excreta. It is seldom possible to obtain marketed plant materials that are entirely free from some form of innocuous foreign matter. However, no poisonous,

dangerous or otherwise harmful foreign matter or residue should be allowed. Any soil, stones, sand, dust and other foreign organic matter must be removed before medicinal plant materials are cut or ground for testing. Macroscopic examination can conveniently be employed for the determination of foreign matter in whole or cut plant materials.

In the present study, foreign organic matter of the plant leaf powders was determined as per WHO guidelines (1998) ⁸.

Calculation:

$$\% \text{ Foreign organic matter} = \frac{(M_1 - M)}{M_2} \times 100$$

Where, M = Weight of empty dish in gm.

M₁ = Weight of dish with foreign matter in gm.

M₂ = Weight of sample (whole plant material) in gm.

Extractable Matter: This method determines the amount of phyto-constituents extracted with solvents from a given amount of medicinal plant material. Here, according to Indian Herbal Pharmacopoeia (1998) ⁶, British Pharmacopoeia (2009) ⁹, British Herbal Pharmacopoeia (1990) and United States Pharmacopoeia (1994) ⁷, ethanol and water were used as solvents to determine the extractable matter ¹⁰.

Ash Content: The ash remaining after the ignition of medicinal plant materials is determined by three different methods, which measure -

- Total ash
- Acid-insoluble ash and
- Water-soluble ash

The total ash method measures the total amount of material remaining after ignition including both 'physiological ash', which is derived from the plant tissue itself, and 'non-physiological ash', which is the residue of the extraneous matter (*e.g.* sand and soil) adhering to the plant surface.

Acid-Insoluble Ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. This measures the amount of silica present as sand and siliceous earth.

Water soluble ash is the difference in weight between the total ash and the residue after treatment of the total ash with water.

In the present study, the total ash value, acid insoluble ash value, water soluble ash values of the four plant leaf powders were determined as per WHO guidelines (1998) ⁸⁻¹¹ and Indian Pharmacopoeia, (1996) ¹¹.

Loss on Drying: Loss on drying and the moisture content of the plant samples were determined according to Indian Herbal Pharmacopoeia (1998) ⁶, British Herbal Pharmacopoeia (1990) and United States Pharmacopoeia (1994) ⁷ as mentioned in (Mukherjee, 2002) ¹⁰.

Moisture Content:

Karl-Fischer Titrmetric Method: Moisture content of the *Cynodon dactylon* (L.) Pers. was estimated using Digital Automatic Karl Fischer Titrator (microprocessor based). The powder of the leaves of *Cynodon dactylon* (L.) Pers. was weighed and added to the titration vessel and the titration was allowed to complete. The readings were noted and the percentage moisture was calculated using following formula.

$$\text{Percentage of moisture} = \frac{\text{Titre factor} \times \text{reading}}{\text{Weight of sample (in mg)}} \times 100$$

RESULTS AND DISCUSSION:

Pharmacognostical Study: The observations and the results of the pharmacognostic study of the leaves of *Cynodon dactylon* (L.) Pers. are discussed as follows -

Macroscopic Analysis of the Fresh Plant Leaves:

The leaves of *Cynodon dactylon* (L.) Pers. are simple, alternate and ribbon shaped, long 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, entire lower leaves usually flat, upper complicate, parallel veined, with sheathing leaf bases attached at the node and surrounding the internode like a tube to varying lengths; often splitted at base; ligule, a projecting portion of sheat, very short, fine ciliate rim; hairy and erect **Fig. 1.**



FIG. 1: SHOWING EXTERNAL MORPHOLOGY OF THE LEAVES OF *CYNODON DACTYLON* (L.) PERS.

Microscopic Analysis of Fresh Plant Leaves:

Transverse section (T.S.) of the leaf is ribbon shaped with a centrally located conspicuous meristele each lying in the primary vein alternating with rows of 3 to 4 smaller sized meristele placed almost at equal distances in the secondary veins. (Fig. 2 and 3)

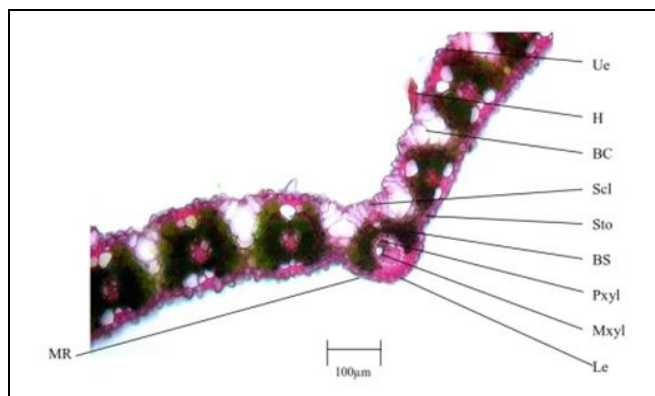


FIG. 2: TRANSVERSE SECTION OF THE MIDRIB PORTION OF THE LEAF OF *CYNODON DACTYLON* (L.) PERS. (H: HAIR; STO: STOMATA; UE: UPPER EPIDERMIS; BC: BULLIFORM CELLS; SCL: SCLERENCHYMA; PXYL: PROTOXYLEM; MXYL: METAXYLEM; BS: BUNDLE SHEATH; LE: LOWER EPIDERMIS; MR: MIDRIB REGION)

Detailed T.S. shows presence of Lamina which 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 parenchyma; row of vascular bundles nearly alike, except that the median bundle is larger; bundle sheath single, and consists of thin-walled more or less iso-diametric 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 stereome near upper and lower epidermis.

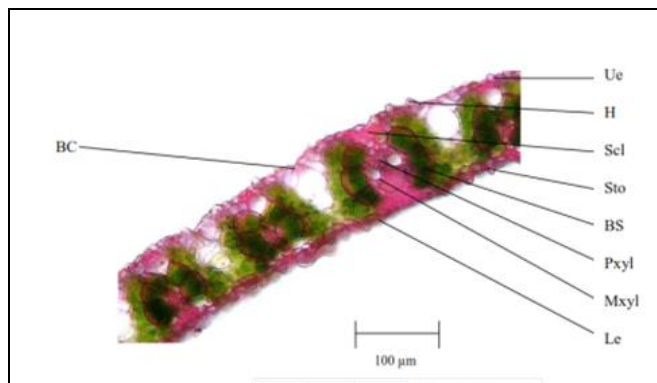


FIG. 3: TRANSVERSE SECTION OF THE LAMINA PORTION OF THE LEAF OF *CYNODON DACTYLON* (L.) PERS. (H: HAIR; STO: STOMATA; UE: UPPER EPIDERMIS; BC: BULLIFORM CELLS; SCL: SCLERENCHYMA; PXYL: PROTOXYLEM; MXYL: METAXYLEM; BS: BUNDLE SHEATH; LE: LOWER EPIDERMIS)

Quantitative Microscopy of the Plants under Study:

Monocot leaves have veins that are parallel to one another through the length of the leaf and do not branch. There may or may not be a mid vein present, but all other veins are of a single order. Stomata can be found on both leaf surfaces; however, the palisade layer is indistinct from the spongy mesophyll layer in monocots. Cells in both layers are roughly isodiametric. Hence, palisade ratio, is not applicable to monocot leaves due to a lack of consistent differentiation within the mesophyll; ¹⁰ the analysis of leaf constants (quantitative microscopy) was therefore not done for *Cynodon dactylon* (L.) Pers.

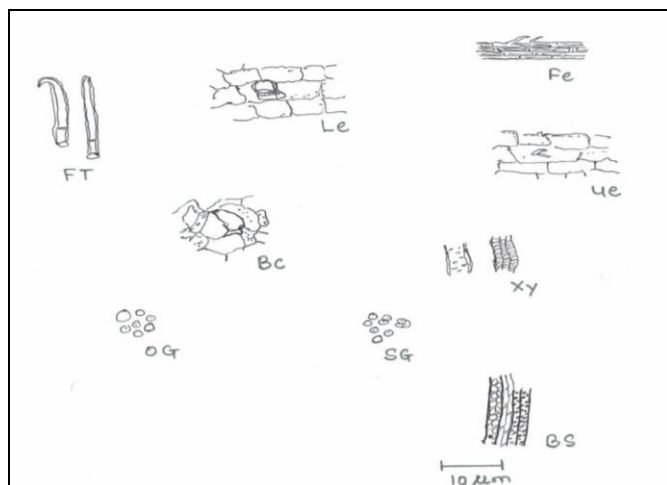
Fluorescence Analysis of the Plant Leaf Powders:

The fluorescence characters of the leaf powder of *Cynodon dactylon* (L.) Pers. is tabulated in **Table 1**.

Powder Microscopy of the Plant Leaf Powders: The leaf powder is yellowish-green in colour and shows simple pitted, scalariform, annular and spiral, vessels; short lignified, thick walled, pointed fibres, paracytic stomata; epidermis in surface view, of elongated, rectangular long cells and nearly square small cells having sinuous walls; simple and compound starch grains, measuring 1-3 μm in diameter. Oil globules and fragments of upper and lower epidermis (**Fig. 4**).

TABLE 1: SHOWING THE EFFECT OF DIFFERENT CHEMICAL REAGENTS ON THE FLUORESCENCE BEHAVIOUR OF CRUDE DRUG POWDER OF *CYNODON DACTYLON* (L.) PERS.

Sr. No.	Treatment	Day light	UV light (254 nm)	UV light (365 nm)
1	Distilled water	Transparent	Transparent	Fluorescent White
2	Alcohol	Yellowish Green	Green	Fluorescent light Orange
3	Acetic acid	Light Green	Light Green	Fluorescent light Orange
4	1N Hydrochloric acid	Transparent	Transparent	Fluorescent Green
5	2N Sulphuric acid	Light Green	Fluorescent dark Green	Fluorescent light Green
6	50% Nitric acid	Off White	Pale Green	Fluorescent light Green
7	10% Sodium hydroxide	Greenish Yellow	Light Green	Fluorescent Green

**FIG. 4: POWDER MICROSCOPY OF THE LEAF POWDER OF *CYNODON DACTYLON* (L.) PERS. (FT: FRAGMENTS OF LONG TRICHOMES; LE: LOWER EPIDERMIS; OG: OIL GLOBULES; BC: BULLIFORM CELLS; FE: FRAGMENTS OF EPIDERMIS WITH PRICKLE HAIR; SG: SINGLE AND COMPOUND STARCH GRAINS; XY: XYLEM VESSELS; BS: BUNDLE SHEATH CELLS ALONG WITH VASCULAR STRANDS; UE: UPPER EPIDERMIS)**

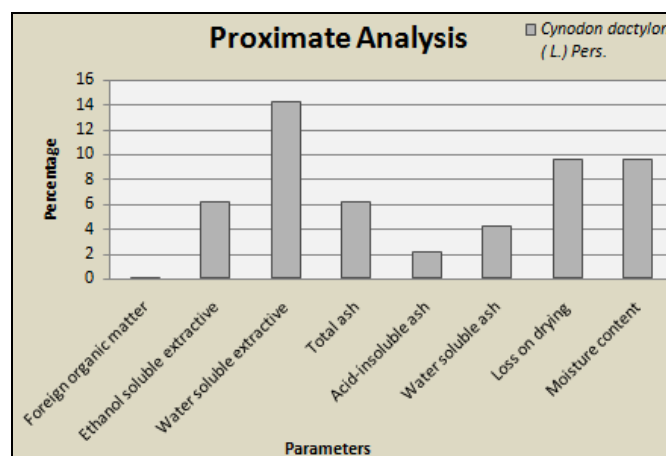
Physicochemical Parameters: The results of all the proximate parameters studied are presented in **Table 2** and **Fig. 5**.

TABLE 2: RESULTS OF THE PROXIMATE ANALYSIS OF THE LEAVES / LEAF POWDERS OF *CYNODON DACTYLON* (L.) PERS.

S. No.	Parameter	% Content in <i>Cynodon dactylon</i> (L.) Pers
1	Foreign organic matter	0.0366 ± 0.0087
2	Ethanol soluble extractive	6.14 ± 0.72
3	Water soluble extractive	14.33 ± 0.53
4	Total ash	6.15 ± 0.80
5	Acid-insoluble ash	2.08 ± 0.35
6	Water soluble ash	4.19 ± 0.33
7	Loss on drying	9.67 ± 1.26
8	Moisture content	9.626 ± 0.95

(*All values are expressed as mean ± SD for three determinations)

Study of these parameters is required for checking the quality of raw material of plant samples *i.e.* leaves of *Cynodon dactylon* (L.) Pers. before going for processing. However, these quality control parameters also depend on cultivation, handling of the raw material and storage condition.

**FIG. 5: BAR DIAGRAM SHOWING THE RESULTS OF THE PROXIMATE ANALYSIS OF THE LEAVE/LEAF POWDER OF *CYNODON DACTYLON* (L.) PERS.**

Physico-chemical parameters *i.e.* ash value of a drug gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Since the ash value is constant for a given drug, this value is also one of the diagnostic parameters of the drug. In certain drug, the percentage variation of ash from sample to sample is very small and any marked difference indicates the change in quality. Unwanted parts of drug, some time possess a character that will raise the ash value. Ashing involves oxidation of the components of the product. A high value is indicative of contamination, substitution, adulterations or carelessness in preparing the crude drug for marketing. In the present study, the total ash value, acid insoluble ash value, water-soluble ash values of the four plant leaf powders were determined as per WHO guide lines.

The ash value of the leaf powders of *Cynodon dactylon* (L.) Pers. shown in **Table 2** revealed a high concentration of total ash. The total ash, water soluble ash and acid insoluble ash which are important parameters for detecting the presence of inorganic substances were found to be 6.15 ± 0.62 % w/w, 4.19 ± 0.33 % w/w and 2.08 ± 0.35 % w/w respectively in the leaf powder of *Cynodon dactylon* (L.) Pers. The acid insoluble ash value was found to be very low indicating that the plant drugs were collected afresh¹².

Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The water and ethanol soluble extractive values which are indicator of total solvent soluble component are - 14.33 ± 0.53 %) and (6.14 ± 0.72 %) for the leaf powder of *Cynodon dactylon* (L.) Pers. respectively. The extractive values of water were found to be more than the ethanol extractive values for the plant leaf powders.

The results of various types of ash and extractive values may provide a basis to identify the quality and purity of the drug¹³. Loss on drying of the leaf powders of *Cynodon dactylon* (L.) Pers. revealed an average of around 9.6% of moisture in the drugs. The results obtained in the present investigation are encouraging and can be used as reference data for the standardization of leaves of *Cynodon dactylon* (L.) Pers. and the formulations containing the plant leaves as a main ingredient. No previous study reports were found on the proximate analysis of the leaves of *Cynodon dactylon* (L.) Pers. and hence our present study can be considered as the first to report the same.

CONCLUSION: The microscopic evaluation is an indispensable tool for identification of medicinal herbs and is one of the essential parameters in modern monograph. The plants under study viz *Cynodon dactylon* (L.) Pers. is used extensively in the traditional system of medicine for the treatment of number of ailments. Although it is a very common plant having less possibilities of adulteration but to get highest efficacy of an herbal drug or its finished product cent per cent genuine plant material should be the source material.

In this regard the important microscopic features of the leaves of *Cynodon dactylon* (L.) Pers. has been

documented in this study; such as, T.S. of the fresh leaves, the study of the powder characters of the leaf powders and the quantitative microscopic studies.

Many phyto-compounds fluoresce when suitably illuminated. The fluorescence colour is specific for each compound. A non fluorescent compound may fluoresce if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analyze over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples¹⁴. Fluorescence analysis of the leaf powder of *Cynodon dactylon* (L.) Pers. showed the presence of fluorescence compounds which would serve as valuable information, for the scientist engaged in research, on the medicinal properties of these plants. All the above said characters of the leaf powders reflect the diagnostic features of the *Cynodon dactylon* (L.) Pers. plant parts in fresh and/or powdered form and can be used to check adulteration. No previous study reports were found on the proximate analysis of the leaves of *Cynodon dactylon* (L.) Pers. and hence our present study can be considered as the first to report the same.

Also the evaluation of the various proximate parameters for the leaves of *Cynodon dactylon* (L.) Pers. has given a clear idea about the specific characteristics of these crude drugs under examination, in their powder form. While these diagnostic features would enable the analyst to know the nature and characteristics of these plant drugs, further evaluation of different parameters is necessary to indicate their acceptability by criteria other than the proximate analysis. The data obtained in the present study is expected to serve as valuable tool for identification, authentication and detection of adulterants, standardization and quality control of the drugs. The developed technique was also thought to be useful for the standardization of formulations containing leaf powder of *Cynodon dactylon* (L.) Pers.

Hence, it can be concluded that the present study on the pharmacognostic and physicochemical characters can serve as a vital source of information and provide suitable standards to determine the

quality of leaf powder of *Cynodon dactylon* (L.) Pers. for future investigations.

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