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# PRELIMINARY PHYTOCHEMICAL STANDARDIZATION OF *CYNODON DACTYLON* LEAVES

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**ABESTRACT**

Plant *Cynodon dactylon* (L.) Pers. Family (Graminae/Poaceae) is extensively used in clinical symptomes but it has various pharmacological activities have been investigated. It is a very familiar plant almost available in the perfect world. In ethno medicinal practices, the plant *Cynodon dactylon* used in the treatment of various diseases and has pharmacological action. The present reviews represent the different pharmacological activities and medicinal properties of *Cynodon dactylon* (L.). The determination of chemical evaluation alkaloid, glycoside, saponin are present in *cynodon dactylon* powder of leaf and The result of fluorescence analysis was summarized. The powder from the leaf fluoresced green under daylight and short UVlight (254 nm), dark green under long UV-light (365 nm). The result of TLC ethyl acetate:Benzene:water (2:2:1) Rf value showed 0.98 and ethyl acetate:toluene:Benzene (2:2:1) Rf value showed 0.95. It is used for treatment of various diseases like diarrhea, gastroenteritis, dysentery, diabetes, hypertension, caries, wounds, pain and fever. It also possesses anti-microbial, anti-malarial, antitussive, hepatoprotective effects etc.

**KEYWORDS:** *Cynodon dactylon* (L.), Graminae/Poaceae, steroids, covering trichomes.

## 1. INTRODUCTION

Since the ancient age, different medicinal plants were extensively used for treatment of different diseases. Those were utilized in different systems of medicines all over the world which include Ayurveda, Chinese medicine, Homeopathy, Siddha, Unani and many others. Though amazing development could be possible through advanced research in Allopath system of medicine but even today nearly eighty percent of individuals from developing countries are using traditional medicine obtained from medicinal plants because of lack of affording capacity and others.[1]

*Cynodon dactylon* is hardy, perennial grass, very variable, with long rapid growing, creeping runner or stolons, rooting at nodes, forming a dense tuft on the surface of the soil, runners sometimes 20m long, 2-6mm broad, flat or sometimes folded or convolute; inflorescence on clums 15cm to 1m tall consisting of 2-12 spikes arranged star like at apex of stem; spikes 2.5-10cm long with numerous spikelets, arranged in 2 rows on one side of spike; spikelets flat, 2-2.5mm long, awnless, with 1 floret; glumes unequal, the upper longer and one third to three fourth length of floret. It is used for treatment of various diseases like diarrhea, gastroenteritis, dysentery, diabetes, hypertension, caries, wounds, pain and fever. It also possesses anti-microbial, anticonvulsant, anti-inflammatory, anti-malarial, antitussive, hepatoprotective effects etc.[2,3]

In traditional medicine it is used for indigestion and the treatment of wounds. According to an old Venda tradition, it is used in the fermentation process to make beer sour.[4] It is reported to be alterative, antiseptic, aperients, astringent, cyanogenetic, demulcent, depurative, diuretic, emollient, sudorific and vulnerary; it is reported to be photosensitizing in animals, to cause contact dermatitis and hay fever. It is folk remedy for anasarca, calculus, cancer, carbuncles, convulsions, cough, cramps, cystitis, diarrhea, dropsy, dysentery, epilepsy, headache, hemorrhage, hypertension, hysteria, insanity, laxative, measles, rubella, snakebite, sore stones, tumors, urogenital disorders, warts and wounds.[5,7]

## 2. MATERIAL AND METHODS

### Plant Material

Fresh leaves of *Cynodon dactylon* were collected from fields of Saranath, Varanasi, and medicinal garden in Prasad institute of technology, Jaunpur. The leaves parts were dried under shade and powdered (40 mesh size) and stored in airtight containers. The macroscopic characters were studies as per given procedure in WHO guidelines on quality control

methods for medicinal plants materials.[6] Fluorescence analysis of powdered leaves was carried out.[7,8]

**Macroscopical Studies**

In this method of evaluation crud drug evaluation by their color, order, shape, size, test, character and compare the standard one.

**Color:** grey-green.
**Order:** astringent,
**Test:** sweet
**Size:** 2-15cm
**Flower:** greenish
**Fruit:** tiny grains

The leaves of the plant were studied for their macroscopic characters such as size, shape, colour, odour, taste in nature.[9,10]

**Determination of Extractive Value/ Extractabli Method**

It is the amount of active constituents after extraction with suitable solvents. Solvent which is used for extraction is called as MENSTRUME and exhausted plant material is called as MARK.

**Principle of extraction:-** It is based on the principle of mass transfer; it is a unit operation method which involved transfer of active constituent from plant material to the solvent.

**Mechanism of extraction**

1. Contact of solvent to the plant material
2. Penetration of solvent into cell through the cell wall.
3. Dissolution of active constituents with in cell with solvent.
4. Removal of active constituent from the plant material.

**Extraction method**

- ❖ Micilation
- ❖ Parcolation
- ❖ Soxhlet extraction
- ❖ Infusion

- ❖ Decoction
- ❖ Acceleration

**Method**

1. Hot extraction
2. Cold masilation extraction

**1. Hot extraction**

Extraction Value with Water

**Procedure**

- Take 4gm of air dried plant material, transfer it into 100ml of specified solvent in 250ml conical flask & boiled.
- Shack vegrasely & allow to stand for 1hrs, Filter & filtrate as soon as transfer 50ml of previasly treat beaker (25 ml transfer in beaker)
- Evaporate up to dryness on water bath. Finally calculate the amount of extract and determine the extractive value.

**Calculation**

Weight of empty beaker = 113.27gm

Weight of beaker + extractive = 113.51gm

= Final – initial

= 113.51 – 113.27 = 0.24gm

25ml extract contains = 0.24

1ml extract contains = 0.24 ÷ 25

100ml extract contains = 0.24 ÷ 25×100 =0.96gm

5gm powder drug gives = 0.96gm extractive drug

1gm powder drug gives = 0.96÷5

100gm powder drug gives = 0.96÷5×100 = 19.2%

**2. Cold maceration extraction**

**Principle:** It is based on the principle of mass transfer.

Extraction Value with Diethyl ether

**Procedure**

- Take 4gm of air dried material transfer it into 250ml of treated it with 50ml of diethyl ether solvent into 250ml of conical flask.
- Shack it for 7hr. And allow standing for 18hr.Then filter it in 50ml of previously treated beaker (25 ml transfers in beaker).
- Evaporate up to dryness on water bath .Finally calculate the amount of extract and determine the extractive value.

**Calculation**

Weight of empty beaker = 82.04gm

Weight of beaker + extractive = 82.09gm

= Final – initial

= 82.09 – 82.04 = 0.05gm

25ml extract contains = 0.05gm

1ml extract contains =0.05 ÷ 25

50ml extract contains = 0.05 ÷ 25×100 =0.1gm

2.5gm powder drug gives = 0.1gm extractive drug

1gm powder drug gives = 0.1÷2.5

100gm powder drug gives = 0.1÷2.5×100 = 4%

**Extraction Value with Ethanol**

- Take 4gm of air dried material transfer it into 250ml of treated it with 50ml of diethyl ether solvent into 250ml of conical flask.
- Shack it for 7hr. And allow standing for 18hr.Then filter it in 50ml of previously treated beaker (25 ml transfers in beaker).
- Evaporate up to dryness on water bath .Finally calculate the amount of extract and determine the extractive value.

**Soxhlet/ continuous method** **Filtrate extractive**

**Calculation**

Weight of empty Petridis = 49.76 gm

Weight of Petridis + extractive = 50.49gm

=Final – initial

= 50.49- 49.76

=0.73gm

25ml extract contains = 0.73gm

1ml extract contains = 0.73 ÷ 25

100ml extract contains = 0.73 ÷ 25 ×100 = 2.92gm

20gm powder drug gives = 2.92gm

1gm powder drug gives = 2.92gm ÷ 20

100gm powder drug gives = 2.92gm ÷ 20 × 100

=14.6%

**Determination of Chemical Evaluation**

1. **Hager's test:-** Took 2-3 ml test solution with Hagers reagent observed for yellow precipitate.
2. **Legal's test (For cardenoloids):-** Took aqueous or alcoholic test solution, added 1 ml pyridine and 1 ml sodium nitroprusside observed for pink to red colour.

3. **Molish's test (General test):** Took 2-3 ml aqueous extract, added few drops of naphthol solution in alcohol, shaken and added concentrated $H_2SO_4$ from sides of the test tube was observed for violet ring at the junction of two liquids.
4. **Baljet's test:-** A test solution observed for yellow to orange colour with sodium picrate.
5. **Test for deoxysugars (Kellar Killani test):-** Took 2 ml extract added glacial acetic acid, one drop of 5% $FeCl_3$ and concentrated $H_2SO_4$ observed for reddish brown colour at junction of the two liquid and upper layers bluish green.
6. **Saponin Glycosides (Foam test):** The drug extract or dry powder was shake vigorously with water. Persistent foam was observed.
7. **Tannin Test:** took Sample, add Pot. Ferric Cyanide solution and Ammonia observed deep brown color.

| S. No. | Test | Sample+ chemical | Color | Present or absent |
|---|---|---|---|---|
| **1.** | Hager's reagent | Sample+ Picric acid | Yellow ppt | Alkaloid present |
| **2.** | Molisch's reagent | Sample+ Molisch's reagent+ conc.$H_2SO_4$ | Violet color ppt. | Glycoside present |
| **3.** | Legal Test | Sample + Pyridine + Sod.nitropruside | Black color | Glycoside present |
| **4.** | Baljet Test | Sample + Picric acid | Yellow ppt | Glycoside present |
| **5.** | Killer-Killani Test | Sample + Fe. Chloride +Con.$H_2SO_4$ | Red color | Glycoside present |
| **6.** | Saponins Test | Sample + Dil water | Foam | Saponin present |
| **7.** | Tannin Test | Sample +Pot. Ferric Cyanide solution + Ammonia | Deep brown color | Tannin absent |

**Determination of Fluoresecene Analysis**

Fluorescence analysis The drug powder was treated with acids such as 1 N HCL and 50% H2 SO4 and alkaline solutions such as aqueous sodium hydroxide, alcoholic sodium hydroxide and other solvents such as nitric acid, picric acid, acetic acid, ferric chloride and nitric acid with ammonia. They were subjected to fluorescence analysis in daylight and in the ultraviolet (UV)-light (254 nm and 365 nm).[10]

| Powder +chemical | Long UV light(365) | Short UV Light(254) |
|---|---|---|
| Powder + dil $H_2SO_4$ | Brown | Green |
| Powder + Conc. $H_2SO_4$ | Light Brown | Light green |
| Iodine | Deep Brown | Deep green |
| 10% NaOH | Light Brown | Light green |
| 10% KOH | Light Brown | Light green |
| Toluene | Light Brown | Light Brown |
| Distilled water | Green | Brown |

| 5% Ferric chloride | Deep Brown | Deep green |
|---|---|---|
| 1N NaOH | Deep Brown | Deep green |
| Ruthenium red solution | Light Brown | Light green |
| Acetic acid | Light Brown | Light green |
| Pot. Ferric Cyanide solution | Deep Greenish Brown | Light green |
| Lead Acetate solution | Light Brown | Light green |

**Determination of TLC Profile**

Thin layer chromatograph (TLC) has become widely use method for the separation of substances in a great variety of inorganic and organic studies. The separation of solutes is carried out a layer of adsorbent i.e adhered to a flat surface of inert materials like a glass plate or a polyester film.

**Principle**

The Principle of separation is adsorbent. One or more compounds are spotted on a thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flow through because of capillary action. The compound with more affinity towards the stationary phase travels slower.

**Requirement of TLC**

**1. Stationary Phase**

A. Silica gel H

B. Silica gel G: Silica gel + $CaSO_4$

C. Cellulose with binder

D. $Al_2O_3$G: $Al_2O_3$ + binder

**2. Glass Plates**

Glass Plates which are specific dimension like 20×20cm (full plate), 20cm ×10cm (half plate), and 20cm×5cm (quarter plate) can be used.

**3. Preparation of TLC of Plates**

- Pouring technique: The slurry is prepared and poured on to a glass plate which is maintained on a leveled surface. The slurry is spread uniformly on the surface of the glass plate.
- Dipping technique: Two plates are dipped in to the slurry and are separated after removing from slurry and later dried.

**4. Activation of adsorbent**

After making thin layer on plates, this is done by drying the thin layer plate, for 30 min. in air and then in an over at 110˚C from another 30 min. This drying makes the adsorbent layers active. Silica gel & alumina plates can be heated to 150˚C for about & hrs.

**5. Development Tank**

In TLC, plate is placid in development chamber at an angle of 45˚C. The bottom of the chamber is covered upto nearly 1 mm by the solvent. Three sides of the tank are lined with solvent improved paper covered tightly with the lid. The development tank is perfectly saturated with solvent vapor.

**6. Mobile Phase**

The solvent or the mobile phase used depends upon various factors as mentioned in chromatography.

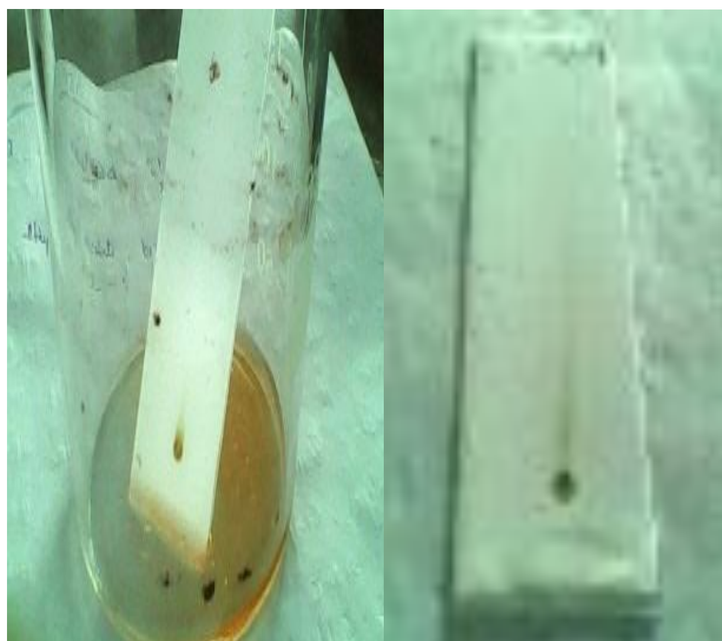
Petroleum ether, carbon tetrachloride, ether, Acetone, Benzene, Toluene, Ethyl acetate, Chloroform, Alcohols Water, organic acids, mixture ,etc. The solvent compositions are given in the application of TLC.

**Development Technique**

- One dimensional development
- Two dimensional Development
- Horizontal Development
- Multiple Development

**Procedure**

1. Preparation of stationary phase
2. Preparation of TLC of Plates
3. Activation of adsorbent
4. Preparation of Development Tank
5. Preparation of Mobile Phase
6. Travel by solvent
7. Measured the Rf value



**Development Tank** **Travel by solvent**

**Calculation**

| S. No. | Solvent system | Rf value | No. of spot | | |
|---|---|---|---|---|---|
| | | | UV light(254) | UV light(365) | Necked eye |
| 1. | Ethyl acetate : Benzene: Water (2:2:1) | 0.980 | 2 | - | 1 |
| 2 | Ethyl acetate :Toluene: Benzene (2:2:1) | 0.9508 | 2 | - | 1 |

Rf value = Distance travel by solute/ Distance travel by solvent

- Ethyl acetate : Benzene : Water(2:2:1)= gives one spot

Distance travel by Solvent = 5.2

Distance travel by solute = 5.1

Rf value = 5.1/5.2 = 0.980

- Ethyl acetate: Toluene : Benzene (2:2:1) = gives one spot

Distance travel by Solvent = 5.8

Distance travel by solute = 6.1

Rf value = 5.8/6.1 = 0.9508

## RESULT AND DISCUSSION

Preliminary pharmacognostical standardization study of the leaves of *Cynodon dactylon* including other physical values and parameters will help to identify the species of plant. The determination of chemical evaluation alkaloid, glycoside, saponin are present in *cynodon*

*dactylon* powder of leaf. The result of fluorescence analysis was summarized. The powder from the leaf fluoresced green under daylight and short UVlight (254 nm), dark green under long UV-light (365 nm). The leaf of *cynodon dactylon* showed the characteristic fluorescent green treated with dil. and conc. H2SO4, Iodine, 10% NaOH, 10% KOH, Toluene, 5% Ferric chloride, 1N NaOH, Distilled water, Ruthenium red solution, Acetic acid, Pot. Ferric cyanide, Lead acetate solution, under short UV-light (254 nm). The result of TLC ethyl acetate:Benzene:water (2:2:1) Rf value showed 0.98 and ethyl acetate:toluene:Benzene (2:2:1) Rf value showed 0.95.

## CONCLUSION

Preliminary pharmacognostical standardization study of the leaves of *Cynodon dactylon* including other physical values and parameters will help to identify the species of plant. *Cynodon dactylon,* the versatile traditional medicinal plant of India, is the rich source of bioactive compounds with diverse chemical structure. As of now, little work has been done on the biological activity and plausible medicinal applications of the photochemical compounds and hence extensive investigation is needed to exploit the bioactive principles of *Cynodon dactylon* for therapeutic utility. In the present of study Anti-inflammatory, Antiviral, Antidirrhea, CNS depressant activity of *Cynodon dactylon* extracts towards drug resistant/ clinically significant microbes of antibiotic of high potency.

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