

# Pharmacognostical, Phytochemical Screening and Evaluation of Anti-Ulcer Activity of Ethnomedicinal Plant: (Aerial parts of *Cynodon dactylon* (L) Pers.)

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## Abstract

*Cynodon dactylon* (L) Pers. (Family: Poaceae) is known to be a sacred tackler in Indian Hindu mythology is offered to Lord Ganesha and one of the 10 auspicious herbs that constitute the group Dasapushpam in Ayurveda. It is an creeping, graceful, enduring grass, abundantly founding in India. The intention of the present study was to investigate the pharmacognostical, preliminary phytochemical studies and anti ulcer activity of n-hexene, chloroform, methanol extracts of *cynodon dactylon* in wistar albino rats. Pharmacognostical studies including morphology, microscopy, total ash, acid insoluble ash, water insoluble ash, sulphated ash and loss on drying were determined. The preliminary phytochemical studies were performed to determine the various secondary metabolites. Carbohydrates, proteins, flavonoids, saponins, alkaloids were detected. The *cynodon dactylon* extracts in dose of 200,400 mg/kg body weight (given orally) were investigated for its potential to protect gastric mucosa against indomethacin induced ulcer model. The common parameter like ulcer index, % protection were used for evaluation of anti ulcer activity. The significant anti ulcer activity was found at the dose of 400 mg/kg of methanolic extract. But 200 mg/kg dose was found to be potent comparable with standard drug Pantoprazole. This study provides scientific evidence that aerial portion of *cynodon dactylon* extracts have potential anti-ulcer effect which could be either cytoprotective action of the drug.

**Keywords:** *Cynodon dactylon*, Pharmacognostical, Phytochemical screening, Ulcer.

## Introduction

According to the world Health Organization medicinal plants are the best source to obtain a variety of new herbal drugs than the modern synthetic drugs which show minimum or no side effects and are considered to be safe. Pharmacognosy is a simple a reliable tool, can be given the complete information of the crude drug [1-4]. Today several medicinal plants and their products have been used for treating various illness, being employed as home remedies, over the counter drugs as well as raw materials for the pharmaceutical Industry and they represent a substantial proportion of the global drug market [5].

*C. dactylon* most commonly known as Bermuda grass, is an hardy, variable, with long rapid growing, creeping runners or stolons forming a dense tuft on the surface of the soil, runners 20 m long, leaves 2.5-10 cm long with numerous spikelets. The genus *Cynodon* was derived from the Greek Kuan, dog and odous, a tooth. The epithet dactylon was derived from the Greek dactulos, a finger, and refers to the inflorescence which is digitate (arranged like fingers on the hand). In ethno medicinal practices, the fresh juice of the plant is demulcent, astringent, diuretic and is applied externally to fresh cuts & wound healing property [6-7], used as a

folk remedy for chronic diarrhoea and dysentery and also in catarrhal ophthalmia and irritation of Urinary organs [8]. The plant is a beneficial for the treatment of cancer, convulsion, cough, cramps, dropsy, epilepsy, haemorrhage, hypertension, laxative, snakebite, sores, warts, toothache.

Ulcers are open sore of the skin or mucus membrane characterized by sloughing of inflamed dead tissue [9]. The gastric ulcer is regarded due to imbalance in mucosal offensive and defensive factors [10]. Helicobacter pylori is one of the most common causes of peptic ulcer. The gastric mucosa is continuously exposed to potentially injurious agents, food ingredients, & NSAID drugs [11]. These agents have been implicated in the pathogenesis of gastric ulcer including enhanced gastric acid & pepsin secretion, inhibition of prostaglandin synthesis, diminished gastric blood flow and gastric motility, stress or due to Zollinger Ellison Syndrome [12].

The present objective is intended to evaluate pharmacognostical, preliminary phytochemical studies and anti ulcer activity of aerial portions of *cynodon dactylon* in indomethacin induced Wistar albino rats.



**Figure 1: Macroscopic characters of the *Cynodon dactylon***

## Materials and Methods

### Collection of the plant material

Aerial parts of the *C. dactylon* (L) pers. were collected from local area of Karimnagar, A.P, India. in the month of January 2012. The botanical identity was confirmed by the joint Director of Forest Range Officer, Sircilla, Karimnagar. A.P, (voucher specimen number is FRO/84/2012).

### Preparation of the plant extracts

Aerial parts of *C. dactylon* were washed, shade dried, grounded to a fine powder in a mechanical blender passed through 80 mesh size and preserved in an air tight container at room temperature for further studies.

### Preparation of n-hexane extract

About 50 gm of aerial parts of *C. dactylon* powder (n-HECDL) and stem (n-HECDS) were extracted using n-hexane (63°C) in Soxhlet apparatus. Then the obtained extract was air drying.

### Preparation of Chloroform extract

The same amount of *C. dactylon* powder (CECDL) and stem (CECDS) were extracted by using chloroform (61.2°C) in Soxhlet apparatus and dried under reduced pressure to get mass product.

### Preparation of Methanolic extract

The specified amount of *C. dactylon* powder (MECDL) and stem (MECDS) were extracted by using methanol (64.7°C) in Soxhlet apparatus and kept for drying to get the solid mass.

### Phytochemical screening

Preliminary phytochemical exploration of the extracts for the presence of different phytoconstituents [13-15] like Glycosides, Alkaloids, Carbohydrates, Saponins, Flavanoids, were carried out.

### Determination of physico-chemical parameters

The leaves and stem of the plant were subjected for investigation of physico-chemical parameters like organoleptic evaluation, Microscopical studies, Determination of ash value, extractive values and Loss on drying [16- 17].

### Animals

Wister albino adult male rats, weighing 200±20 gm were selected and housed in polyacrylic cages under the standard experimental conditions (12 hrs photo period, 22±2°C, 45-60% RH). The animals were allowed to acclimatize to the environment for 1 week and fed with a standard pellet diet (Mahaveera enterprises, Hyderabad) and water *ad libitum*. The study was approved by Institutional Animal Ethical Committee (IAEC) .

### Acute toxicity study

Acute toxicity study was performed by mice by oral administration of various doses of n-hexane, chloroform and methanolic extracts of aerial portion up to 400 mg/kg. The mortality rate and behavioral changes were observed.

### Indomethacin induced ulcer model

This was produced by the method of Parmer and Desai<sup>[18]</sup>. Albino rats of weighing between 150-200 gm divided into 8 groups of 6 animals in a group.

Group 1: Disease control (DC) with 1% CMC (1ml / kg) + Indomethacin (IDM-200 mg / kg)

Group 2: Pantoprazole (4 mg/kg) with 1% CMC solution)+ Indomethacin (IDM-200 mg / kg)

Group 3: Indomethacin (IDM -200 mg / kg) + n-hexane extract (200 mg / kg)

Group 4: Indomethacin (IDM-200 mg / kg) + n-hexane extract (400 mg / kg)

Group 5: Indomethacin (IDM -200 mg / kg) + Chloroform extract (200 mg / kg)

Group 6: Indomethacin (IDM-200 mg / kg) + Chloroform extract (400 mg / kg)

Group 7: Indomethacin (IDM-200 mg / kg) + Methanolic extract (200 mg / kg)

Group 8: Indomethacin (IDM- 200 mg / kg) + Methanolic extract (400 mg / kg)

The animals were fasted for 24hrs. The test drugs were administered orally in 1% CMC solution 30 min prior to indomethacin at dose of 30mg/kg. Four hours later the rats were scarified by using anaesthetic ether and their stomachs were dissected, for the determination of gastric lesions.

## Parameters

Ulcer index [19]

$$\text{Ulcer Index} = \frac{\text{Number of ulcer} + \text{ulcer score} + \% \text{incidence}}{\text{Number of Animals}}$$

The number of ulcers / stomach were noted and severity of the ulcers were

Scored [20] microscopically with help of hands lens (10x) as follows-

- 0 = Normal Coloured Stomach
- 0.5 = Red Coloration
- 1 = Spot Ulcers
- 1.5 = Haemorrhagic Streath
- 2 = Ulcer > 3mm but < 5mm
- 3 = Ulcers > 5mm

$$\% \text{ protection} = \frac{(\text{ulcer index})_{\text{control}} - (\text{ulcer index})_{\text{test}}}{(\text{ulcer index})_{\text{control}}}$$

## Statistical analysis

The values were represented as mean  $\pm$  S.E.M, and statistical significance between treated and control groups were analyzed using of One way ANOVA, Analysis of Variance followed by Dunnett's test where  $P < 0.01$  was considered statistically significant.

## Results and Discussion

### Macroscopic characters

*C. dactylon* (Linn) Pers. is a perennial grass of poaceae family.

Leaf - 20 to 100 mm long and 1.25 to 3 mm wide, Leaves are short, subulate, glaucous narrowly linear or lanceolate, finely acute, soft, smooth, usually conspicuously distichous in the barren shoots. The nodes and leaf sheaths are glabrous, sheath light, bearded, ligules a very fine ciliate rim [21-22].

Stem -Slender, prostrate, up to 0.1 cm thick, leafy, very smooth, yellowish green in colour, Very ramified, spreading by long rhizomes and stolons[23]

Flower- The flowers are greenish or purple in colour.

Seed -The seed are spear shape, tiny, brown in colour and are produced in a cluster of 3-7 spikes at the top of the stem.

### Microscopic Characters

Stem- Epidermis is followed by endodermis. It shows conjoint, collateral fibro vascular bundle. The culm is erect or trailing on the ground, cylindrical, hollow, green to reddish and glarous, 1 to 3 mm large and 10 to 60 cm tall, rarely more than 1m numerous fertile culms; nodes dark and glabrous. Starch grains simple and

compounds having 2 to 4 components, present in cortex and ground tissue, simple grains measuring 4 to 16  $\mu$ .

Leaf - It is bilateral, mesomorphic, and stomatiferous. The lamina is smooth on the abaxil side and the region where a lateral vascular bundle is situated. The epidermal cells in between the vascular bundles are modified into bulliform cells. Leaf blades with a distichously disposition, linear, apex abruptly rounded ,margin scabrous to the apex; Leaf sheaths are smooth, carina rounded pubescent to glabrous. absence of hypodermal band of sclerenchyma [24-25].

Powder - Yellowish-green coloured showing the presence of simple pitted; short lignified, thick walled vessels, pointed fibres, paracytic stomata. Epidermis consists of elongated and rectangular cells. Both simple and compound starch grains measuring 4 to 16  $\mu$  in diameter are present.

The microscopic studies of the results are given in table no. 1



Figure 2: T.S of the stem showing Vascular bundle, fibro vascular bundle

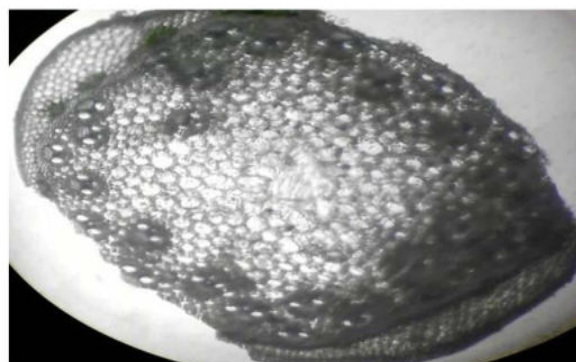


Figure 3: T.S of the stem showing epidermis, starch grain, cortex, endodermis



**Figure 4: T.S of the leaf showing Mesophyll, Vascular bundle with Y shaped xylem**

### Powder Analysis

### Organoleptic Characters

The Organoleptic characters of plant were mentioned in table no.2.

### Physico-chemical Parameters

Various physico-chemical tests were performed as per the standard procedures which were mentioned in ayurvedic pharmacopoeia and the results were shown in table no 3, 4 & 5.

### Preliminary Phytochemical Screening

Preliminary phytochemical screening was carried out on the n-hexane, chloroform and methanolic extracts of aerial portion of *Cynodon dactylon* for presence of various secondary metabolites are shown in table no.6.

### Pharmacological Screening

Preliminary phytochemical screening of the methanolic extract *C. dactylon* revealed the presence of alkaloids, carbohydrates, proteins, flavonoids, and glycosides. Different doses of n-hexane, chloroform, and methanolic extracts of aerial portion *C. dactylon* were screened for their oral toxicity study. No mortality was recorded up to 400 mg/kg with n-hexane, chloroform and methanolic extracts. Hence, the extracts were found to be safe up to the dose levels of 400mg/kg. The ulcer studies of the results are given in table no. 7

**Table 1: Microscopical studies of *Cynodon dactylon***

Test	Observation	Inference	
		Leaf	Stem
Phloroglucinol + conc. Hcl	Pink colour observed	Lignified cells, covering trichomes	Lignified cells of endodermis xylem and pith
Dil. Iodine	Blue colour observed	Starch grains in endodermis	Starch grains in endodermis
Alc. Picric acid	Yellow colour observed	Aleurone grains present in the cells	Aleurone grains present in the cells

**Table2: Organoleptic properties**

Parameters	Interference	
Appearance	Powder	Powder
Colour	Yellowish green	Green
Taste	Bitter	Bitter
Odour	Characteristic	Characteristic

**Table 3: Extractive values**

Solvent	In %w/w	
	Leaf	Stem
n-hexane	12	10
Chloroform	22	19
Methanol	15	11

**Table 4: Ash values**

In %w/w	
Leaf	Stem
5.75	4.01
3.7	3.1
9.7	6.9
3	2.12

**Table 5: Loss on drying**

Plant parts	Loss on drying (%w/w)
Leaf	12
Stem	9.85

**Table 6: Preliminary phytochemical screening of different extracts**

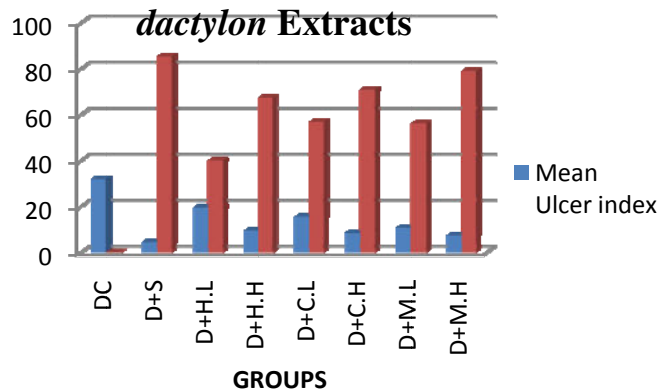
S.No.	PhytoConstituents	n-hexane		Chloroform		Methanol	
		Leaf	Stem	Leaf	Stem	Leaf	Stem
1.	Carbohydrates	+	+	+	+	+	+
2.	Proteins	-	+	-	+	-	+
3.	Amino acids	-	-	-	-	-	-
3.	Glycosides	+	+	+	-	+	+
4.	Alkaloids	+	+	+	+	+	+
5.	Tannins	-	-	-	-	-	-
6.	Volatile oils	-	-	-	-	-	-
7.	Flavonoids	+	+	+	+	+	+
8.	Steroids	+	-	+	-	-	-
9.	Saponins	-	-	-	-	-	-

**Table 7: Effect of *Cynodon dactylon* extracts on Indomethacin induced Ulcer rats**

Group No	Dosage of Drugs (mg/kg)	Mean Ulcer index $\pm$ SD	Percentage of Protection
1.	DC+IDM (200mg/kg)	32 $\pm$ 2.250926**	--
2.	IDM (200mg/kg)+PP (4mg/kg) With 1% CMC	4.7 $\pm$ 0.440076**	85.36
3.	IDM (200mg/kg) +n-HECD (200mg/kg)	19.5 $\pm$ 3.205464**	39.96
4.	IDM (200mg/kg) +n-HECD(400mg/kg)	9.5 $\pm$ 1.744037**	67.35
5.	IDM (200mg/kg) +CECD) (200mg/kg)	15.5 $\pm$ 2.300797**	56.84
6.	IDM (200mg/kg) +CECD) (400mg/kg)	8.6 $\pm$ 1.552632**	70.91
7.	IDM (200mg/kg) +MECD) (200mg/kg)	10.7 $\pm$ 2.34812**	56.09
8.	IDM (200mg/kg) +MECD) (400mg/kg)	7.5 $\pm$ 1.125611**	78.98

Values are expressed mean $\pm$ S.D of six rats. Significance value\*\* P<0.01 Vs Control.

**Anti-Ulcer Activity of *cynodon dactylon* Extracts**



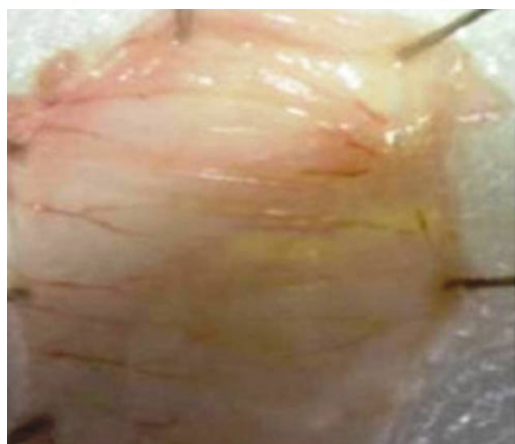
L- Low dose (200 mg / kg), H- High dose (400 mg / kg)



**Figure 5: Control**



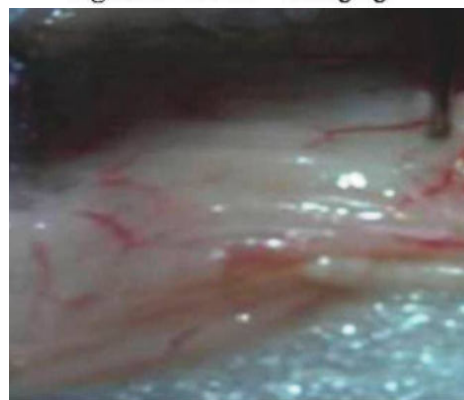
**Figure 6: Standard 4mg/kg**



**Figure 7: MECD 400mg/kg**



**Figure 8: CECD 400mg/kg**



**Figure 9: n-HECD 400mg/kg**

In fact plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants source of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times [26]. Over 50% of all modern clinical drugs are of natural product origin [27]. The preliminary phytochemical screening of n-hexene, chloroform, and methanolic extracts of aerial parts of *C. dactylon* revealed the presence of alkaloids, Flavonoids, Glycosides, Carbohydrates. Anti ulcer activity of extracts may be due to its high content of flavonoids and alkaloids. Organoleptic evaluation of crude drugs based on the study of morphological and sensory profile of whole drug. The various morphological, microscopical, physicochemical standards developed in this study will help for botanical identification and standardization of the drug in crude form.

Pharmacognostical evaluation of leaves and stem of *C. dactylon* were performed. Covering trichomes, Lignified cells of endodermis xylem, pith, starch grains, vascular bundles present in cells as important microscopic diagnostic characters. The determination of ash value gives an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Loss on drying measures the volatile substances and it is the index for volatile oils and moisture present in the drug.

The acid insoluble ash, water soluble ash, sulphated ash and loss on drying were found to be greater in leaves than stem.

In Antiulcer activity, the percentage of protection was calculated and analyzed for the significant reduction towards Ulcer control Group. There was significant reduction in the ulcer index on methanolic extracts (400mg/kg body weight) of *C. dactylon* ( $P < 0.05$ ) when compared with Ulcer control group. From obtained results, it was observed that, there was increase in percent of protection from ulcer and decrease in ulcer index in a dose dependent manner when compared with Ulcer control group.

*C. dactylon* shows defensive action on peptic ulcers by inhibiting cyclo oxygenase (Cox) inhibitors from arresting the mucous lining gastrointestinal tract.

## Conclusion

The present study indicated significantly decreased anti ulcer effect of methanolic, chloroform, and n-hexene extracts of different doses of leaves and stem of *C. dactylon* in indomethacin induced ulcer rats. From the preliminary investigation it has been concluded that the leaves and stem of *C. dactylon* have significant anti ulcer activity, due to high rich of flavonoids and alkaloids. Thus, the

result from our study suggested that the plant *C. dactylon* has the potent anti ulcer property. Further, extensive research work needed to isolate the active biocomponent responsible for the activity.

## Authors' Contributions

JS participated in the design of the study and preliminary phytochemical screening. VS carried out the pharmacognostical studies. VR worked on pharmacological studies. PM performed the study of statistical analysis.

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