Research Paper

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ISOLATION AND BIOACTIVITIES OF CYNODON DACTYLON : A CASE STUDY

Pharmacy

KEYWORDS : Cynodon dactylon, Charka Samhita, Sushrutha Samhita, Chromatography, Methanolic, Ayurveda medicine, Ergonosinine etc.

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ABSTRACT

Medicinal plants are a source of a very rich botanical wealth. A large number of diverse types of plants grow in different parts of India. Mainly in developing countries herbal medicine is still the mainstay of about 75-80% of the whole population, for primary health care, better cultural acceptability, better compatibility with the human body and fewer side effects.

Cynodon dactylon (Linn.) Pers. commonly known as "durva" of Poaceae family is a perennial creeping herb found almost all over India. It has many pharmacological activities such as diuretic, anti diabetic, anti microbial and wound heeling and many constituents like β-sitosteral, ergonosinine. A complete understanding of medicinal plants involves a number of disciplines including commerce, genetics, quality control and pharmacology. A large portion of the Indian population even today depends on the Indian system of medicine Ayurveda, 'an ancient science of life'. The well known treatises in Ayurveda are charka samhita and Sushrutha samhita. A pharmacist is able to do chromatography and other procedures necessary for the identification and determination of purity in addition to the study of macroscopically and histological characters of the dried plant. An anticancer drug paclitaxel from taxus bronifolia emerged up to the clinical trial stage with help of this method.

Cynodon dactylon is commonly available grass with more medicinal uses. Five different phyto constituents were isolated from crude methanolic extract by column chromatography using ethyl acetate (0-20): n-hexane (100-80) solvent system, the Rf values of the isolated components are 0.86, 0.58, 0.48, 0.34, 0.26 respectively. These are identified and confirmed with HPTLC and TLC analysis.

It can be noticed that the better anti ulcer activity in leaf extract of Cynodon dactylon than stolon extract. The methanolic extract of Bermuda grass does not show action for CNS stimulant. The remaining isolation of other constituents was going to be performed. The ulcer induced stomach tissues are allowed for histopathological studies and diuretic activity to be performed.

PRELUDE

Medicinal plants are a source of a very rich botanical wealth. A large number of diverse types of plants grow in different parts of India. Mainly in developing countries herbal medicine is still the mainstay of about 75-80% of the whole population, for primary health care, better cultural acceptability, better compatibility with the human body and fewer side effects. Pharmacognosy may be defined as a branch of bioscience which treats in detail medicinal and related products of crude or primary type obtained from plant, animal and mineral origins.

Cynodon dactylon (Linn.) Pers. commonly known as "durva" of Poaceae family is a perennial creeping herb found almost all over India. It has many pharmacological activities such as diuretic, anti diabetic, anti microbial and wound heeling and many constituents like β-sitosteral, ergonosinine. A complete understanding of medicinal plants involves a number of disciplines including commerce, genetics, quality control and pharmacology. A large portion of the Indian population even today depends on the Indian system of medicine Ayurveda, 'an ancient science of life'. The well known treatises in Ayurveda are charka samhita and Sushrutha samhita. A pharmacist is able to do chromatography and other procedures necessary for the identification and determination of purity in addition to the study of macroscopically and histological characters of the dried plant. An anticancer drug paclitaxel from taxus bronifolia emerged up to the clinical trial stage with help of this method.

AIM AND PLAN OF WORK

In continuous search of new bioactive molecules from the plant sources we selected ***CYNODON DACTYLON*** **(Bermuda grass, Garika)** for the bioactive moieties. Being it is widely distributed and its applications for the poor people from the various regions from the ancient time. The main aim is to identify possible chemical moieties through isolation with chromatographic techniques and pharmacological activities for its extract.

PLAN OF WORK

1. Collection, drying, size reduction of crude extract.
2. Extraction for possible isolates from crude through chromatography.
3. Identification of isolates through TLC and HPTLC.
4. Studies of pharmacological actions for the following activities
   - Anti-ulcer activity.
   - Action on central nervous system.

EXPERIMENTAL

1) PLANT PROFILE: Scientific classification

Kingdom: Plantae; Division: Magnoliophyta; Class: Liliopsida; Order: Poales;

Family: Poaceae; Genus: *Cynodon;* Species: *C. dactylon;* Common names: Bermuda grass, durva grass, Garika; Binomial name: *Cynodon dactylon* (L.) Pers.

Morphology description

A hardy perennial grass with creeping clums, rooting at nodes and forming spreading mats on the surface of the soil.

Chemistry: Bermuda grass is reported to contain cyanodon, hydrochloric acid and triticin.

COLOUR: The blades are grey-green colour, stem is in purple colour.

SHAPE: Blade like shape, stem are slightly flattened

SIZE: Leaves: 4-15cm long with rough edges.

Stem: 1-30cm (rarely 90cm) tall.

Roots: can grow 2m deep in to soil.

SOLUBILITY: Soluble in ethyl acetate, methanol, n-hexane and water partially soluble.

SEED : Heads are produced in cluster of 3-7 spikes together at top of stem.

Each spike 3-6cm long.

Cynodon dactylon

2) CRUDE PREPARATION

Plant collection

Fresh plants/plant parts were collected. The plant material was compared with authentic sample and confirmed. The fresh plant material washed under running tap water, air dried, and then homogenized to fine powder and stored in air tight bottle. The procedure is as follows

Drying:

Drying consists of removal of sufficient moisture content of drug, so as to improve its quality and make it resistant to the growth of micro-organisms. *Cynodon dactylon* dried by either direct sun-drying or in shed. If the natural colour and volatile principles are retained, shed is preferred. If the contents of drugs are quite stable to temperature and sunlight, sunlight is preferred.

Size reduction:

The present plant material was powdered by using Hammer mill. The size and shape of hammers may be square-faced, tapered to a cutting edge or have a stopped form are preferred.

3) EXTRACTION

The pharmaceutical preparations known as extracts are prepared by using alcoholic or hydro-alcoholic solutions and adjusting the product to a standard strength and followed methanol extraction process.

Methanol extraction

10 g of air dried powder in 100 ml of methanol is taken in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 200 rpm for 24 hrs. After 24 hrs the supernatant was collected and the solvent was evaporated to make the final volume (¼th of original volume) and stored at 4º c in air tight bottle.

HPTLC ANALYSIS FOR EXTRACT

Finger printing of extract of grass was carried out by HPTLC. The extract was spotted on a precoated HPTLC plate at different concentrations using CAMAG LinomatIV, an automatic sample spotter and the plate was developed in the solvent system ethyl acetate and n-hexane (20:80). The plate was dried at room temperature and scanned using CAMAG –TLC scanner 3 at U.V-254nm and RF values, spectra and peak area of the dissolved bands were recorded and reported.

4) ISOLATION OF PRINCIPLES

The isolation of various compounds from the crude was done with the column chromatography by using (0-20%) Ethyl acetate and n-hexane as solvents with various proportions which are recorded in the table.

The crude was admixture with silica gel (1:2) (mesh 60-120). Column was packed with silica gel 12gm (mesh 70-325) using n-hexane and the admixture was loaded over the column. The column was eluted with increasing order of polarity using ethyl acetate: n-hexane to afford the pure compounds.

The fractions were evaporated to get solid mass and it was washed repeatedly with n-hexane and allows crystallizing. The crystalline products were identified by TLC (20:80) ethyl acetate and n-hexane as solvent system and tabulated the isolated compounds based on their R_f values.

5) PHARMACOLOGICAL ACTIVITY STUDIES

A) Anti-ulcer activity

Materials and methods used

Drugs : Anaesthetic ether, Famotidine.

Chemicals : Sodium hydroxide (0.01N).

Topfer's reagent (Dimethyl-amino-azo-benzene with Phenolphthalein)

Dose : 10mg/kg; stock solution containing 2mg/ml of the drug and inject

0.5ml/100g body weight of the animal.

Equipments : Projecting microscope, 10X magnification, PH-meter, burette, surgical equipment.

Animals : Albino rats (n=27) weight 180± 10gm of either sex.

Procedure for anti-ulcer activity:

Ulcer induction

The albino rats were fasted for overnight and provided carbon tetrachloride (5% Ccl_4) water. Then all the animals were gavaged with aspirin suspended 0.2%. Agar (500mg/kg body weight). The animals were then left as such for 4 hrs.

Among 10 rats one is separated for gastric acidity estimation and remaining nine were grouped in to 3 batches. In each batch one rat is treated with Famotidine I.P., (10mg/kg), leaf extract of *Cynodon dactylon* (10mg/kg) and stolon extract of *Cynodon dactylon* (10mg/kg) and preceeded with following points.

- After four hours all the rats were anaesthetized with anaesthetic ether.
- Secure the rat on the operating table and cut incision of 1cm along in the abdomens just below the sternum and removed the entire stomach from the body of the animal and washed slowly under the running tap water.
- All the stomach were opened along the greater curative and washed with 10ml of water.
- Centrifuged the gastric content at 1,000rpm for 10minutes. Pipette out 1ml of supernated liquid. Note the PH of this solution with help of PH-meter. Titrate the solution against 0.01N sodium hydroxide using Topfer's reagent as indicator.
- Titrate to the end point when the solution turns to orange colour. Note the volume of sodium hydroxide which corresponds to the free acidity. Titrated further till the solution regains pink colour.
- The total volume of sodium hydroxide which corresponds to the total acidity was noted. Acidity (mEq/100g) can be expressed as:

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$$\text{Acidity} = \frac{\text{volume of NaOH} \times \text{Normality} \times 100}{0.1} \quad \text{mEq/1/100g}$$

Calculation of ulcer index

The mucosal layer of the stomach was observed under a magnifying lens and was checked for ulcers, hemorrhagic areas or perforations. The ulcer index was determined as described below.

Ulcer index == 10 / X

Where x = total area of stomach mucosa/ total ulcerated area. (OR)

Mean ulcer score of each animal is ulcer index.

Ulcer score mentioned as

0 = normal colored stomach, 0.5 = red coloration, 1 = spot ulcers, 1.5 = hemorrhagic streaks, 2 = ulcers ≥ 3 but ≤5, 3 = ulcers › 5. **The results were tabulated.**

B) ACTION ON CENTRAL NERVOUS SYSTEM

Materials and methods used

Drugs : caffeine, extraction of *Cynodon dactylon*
Equipments : Actophotometer, stop watch
Animals : Albino mice (50±5gms) of either sex.
Dose : 10mg/kg.

Method: Six mice's were taken and kept in Actophotometer for 5minutes for acclimatize that environment. The same mice are allowed to move in actophotometer and counted /observed the movements before and after power on for 10 min.

After 30 minutes the extract (10mg/kg) is given orally to the same mice. After 15 minutes again they were kept in Actophotometer and switched on and movement count was noted. After 30 minutes same repeated for caffeine drug (10mg/kg). Compare the movement count. **The results were tabulated.**

RESULTS AND DISCUSSIONS

1. ANALYSIS OF CRUDE EXTRACT

HPTLC Analysis conditions:

Sample : Bermuda grass

TLC Plate : HPTLC precoated plate, 10X15cm

Mobile phase: Ethyl acetate: n-hexane (20:80)

Detection : UV

Mode : absorbance or reflectants, wavelength254nm

TABLE -1: Phyto constituents present on *Cynodon dactylon* (Based on R_f values)

S.No	R_f VALUE	PERCENTAGE OF COMPOUND
1	0.09	26.63
2	0.10	26.81
3	0.18	8.81
4	0.26	11.07
5	0.34	9.74
6	0.48	7.66
7	0.58	6.99
8	0.86	2.31

From the above data the five types of compounds with R_f values of 0.26, 0.34, 0.48, 0.58, 0.86 and mixtures with 0.09, 0.10, 0.18 are found and the percentage of area of the each compound are calculated.

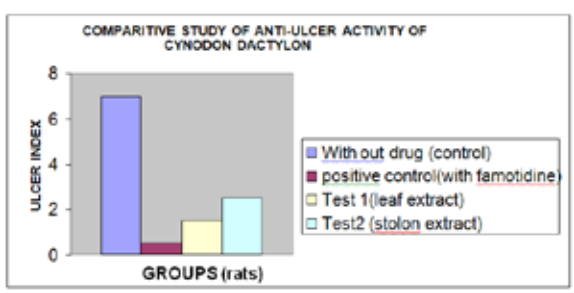
TABLE-2: Isolated fractions of phyto constituents by column chromatography.

S.NO	FRACTION	SOLVENT SYSTEM (Ethyl acetate : n-hexane)	IDENTIFIED ISOLATE BASED ON Rf-VALUE
1.	1 – 7	0 : 100	-----
2.	8 -14	1 : 99	-----
3.	15 -19	2 : 98	-----
4.	20 – 22	3 : 97	-----
5.	23 – 25	4 : 96	-----
6.	26- 28	5 : 95	-----
7.	29 – 33	8 : 92	0.86
8.	34 – 35	10 : 90	-----
9.	36 – 45	15 : 85	0.58
10.	46 – 51	17 : 83	-----
11.	52 – 60	20 : 80	0.48
12.	61 – 66	20 : 80	0.34
13.	67 – 72	20 : 80	0.26
14.	73 – 76	20 : 80	0.18 (mixture)
15.	77 – 85	20 : 80	0.10 (mixture)

From the above data five different compounds were noticed in isolation and separated at different fractions of solvent systems of ethyl acetate-hexane (8:92,15:85,20:80) by column chromatography and identified with R_f values of 0.86,0.58,0.48,0.34,0.26 by performing TLC. These R_f values are very nearer to R_f values of compound obtained in HPTLC.

3) ANTIULCER ACTIVITY

Figure-1 : Anti-ulcer activity performance of *Cynodon dactylon*



Graphical representation of ulcer index of *Cynodon dactylon* extract

It can be noticed from above tabular data that the leaf extract has better anti ulcer activity than the stolon extract of *Cynodon dactylon,* the acidities are 6.10 and 5.20 (mEq/1/100g) respectively. The ulcer index of leaf and stolon are noticed that 1.5 and 2.5 in albino rats. So the less ulcer index of leaf extract and reduced acidity of leaf extract shows more antiulcer activity and anti secretary activity than stolon extract.

S. NO	TREATMENT	DOSE (mg / kg, IP)	VOLUME (ml)	PH	ACIDITY (mEq/1/100g)	ULCER INDEX
1.	Control	—	1.1	2.52	3.00	7
2.	Famotidine	10	0.9	6.73	6.80	0.5
3.	Leaf extract	10	0.9	6.20	6.10	1.5
4.	Stolen extract	10	0.8	5.70	5.20	2.5

4) ACTION ON CENTRAL NERVOUS SYSTEM

TABLE-3: CNS stimulant action of mice with the *Cynodon dactylon* extract

S. NO	TREATMENT	TIME (MIN)	ACTOPHOTOMETER COUNT
1.	Control(without rug)	10 min	1462
2.	Extraction(10mg/kg)	10 min	1074
3.	Caffeine(10mg/kg)	10 min	1687

From the above data the extract of *Cynodon dactylon* does not show CNS stimulant being the movement count of mice in actophotometer is less than control and drug.

CONCLUSION

Cynodon dactylon is commonly available grass with more medicinal uses. Five different phyto constituents were isolated from crude methanolic extract by column chromatography using ethyl acetate (0-20): n-hexane (100-80) solvent system, the R_f values of the isolated components are 0.86, 0.58, 0.48, 0.34, 0.26 respectively. These are identified and confirmed with HPTLC and TLC analysis.

It can be noticed that the better anti ulcer activity in leaf extract of *Cynodon dactylon* than stolon extract. The methanolic extract of Bermuda grass does not show action for CNS stimulant. The remaining isolation of other constituents was going to be performed. The ulcer induced stomach tissues are allowed for histopathological studies and diuretic activity to be performed.

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