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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND IN VITRO ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS EXTRACT OF *CYNODON DUCTOLON*

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ABSTRACT

The aim of present work to carried out extraction, phytochemical analysis and In –Vitro anti-inflammatory activity of aqueous extract of Cynodon dactylon using UV Vis. Spectroscopy. Extraction of the selected plant namely *cynodon ductolon* (Arial part) was carried out by maceration method using water as a solvent for about 24 h at room temperacture. The % yield was found to be 11.12% w/w of crude drug. In the phytochemical test flavonoids and Diterpenes were present. The in vitro anti inflammatory activity was performed by using protein denaturation method in which Diclofenac sodium was used as a reference drug. The results obtains from this study indicates that the aqueous extract of *cynodon ductolon* (AQCD) have in vitro anti-inflammatory activity.

Key words: *cynodon ductolon,* phytochemical screening and In-Vitro Anti-inflammatory activity.

INTRODUCTION:

Now there is a need for the new safe, potent, nontoxic or less toxic anti-inflammatory drug. Plant medicines are great importance in the primary healthcare in many developing countries. According to World Health Organization (WHO) still about 80% of the world population rely mainly on plant-based drugs. In Ayurveda, Siddha, and Unani, utilizing a large number of medicinal plants were used for the treatment of human diseases (Valsaraj et al., 1997). The medicinal plants occupied a unique place in human life. It provides more information about the use of plants or plant parts as medicine (Saikia, 2006).

Plant-based drugs used in the traditional medicine have paid great attention because it is easily available, less expensive and also have no side effects (Cathrine and Prabavathi, 2011). Plants have the ability to synthesize a wide verity of phytochemical compounds as secondary metabolites. Many of the phytochemicals have been used to effectively treat the various ailmentsfor mankind. World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species. Most of the medicinal plant parts are used as raw drugs and they possess varied medicinal properties (Mahesh and Sathish, 2008). Plants have a great potential for producing new drugs and used in traditional medicine to treat chronic and even infectious diseases (Panda et al., 2009).The Phytomedicines are more import in the treatment of inflammation. In recent years, there is an increasing awareness about the importance of medicinal plants. Many medicinal plants have shown to exhibit potent anti-inflammatory effect in the treatment of inflammation by using various models.

The aim of present work to carried out extraction, phytochemical analysis and In – Vitro anti-inflammatory activity of aqueous extract of *Cynodon dactylon* using UV Vis. Spectroscopy.

Cynodon dactylon is commonly known as "durva" or dūrvāyugma in India, this grass is used in the Ayurveda system of medicine. In Hinduism, it is considered important in the worship of Lord Ganesha. A clump of 21 shots of this grass is usually offered during the pooja ceremony. It has been a part of Hindu rituals since Vedic times (Kandwal and Sharma, 2011).The rhizomes are reported to act as a diuretic in humans and the grass juice can act as an astringent. It has been observed that *Cynodon dactylon* may be selectively eaten by dogs to swiftly induce vomiting when they have gastrointestinal problems. The effect may be due to irritation caused by bristles on the leaf margin (Pandey and Tripathy, 2014).

Material and methods

Collection, Authentication Plant Drug

The aerial part of selected plant namely *Cynodon dactylon* were identified and collected from local area of Bhopal on the basis of their availability. The plant drug was authenticated by expert botanist of Department of Botany MVM College Bhopal. The collected plant material was cleaned, shade dried, pulverized into moderately coarse powder and stored in airtight container for further use.

Extraction of Plant Drug

The Collected plant drug (Aerial part) was cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant drug was converted into moderately coarse powder in hand grinder. Powdered plant drug was weighed (100 gm) and packed in air tight glass container.

The plant Material (Leaves) was extracted with water for about 24 hrs with randomly shaking. Shaking of the drug during maceration is essential in order to replace the saturated layers around the drug with fresh menstruum.The liquid extract was collected in a tarred conical flask. The solvent removed by evaporating the solvent using hot plate. The dry extract obtained was weighed to calculate the percentage yield (Kokate, 1994).

The percentage yield of each extract was calculated by using following formula:

$$\%\, Yield = \frac{Weight\ of\ Extract}{Weight\ of\ powder\ drug\ Taken}\ x100$$

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994, Harborne, 1973).

Total flavonoid content estimation (Olufunmiso et al., 2011)

In this method, quercetin was used to make the calibration curve. 10 mg of quercetin was dissolve ed in methanol and then diluted to 5,10,15,20 and 25 µg/ml. A calibration curve was made by measuring the absorbance of the dilutions at 420 nm (λmax of quercetin) with a lab science UV-1800 spectrophotometer. Aluminum chloride, 1% and potassium acetate, 1M solutions were prepared. 100 mg of the plant extract was accurately weighed and transferred to 10 ml volumetric flask and made up the volume with methanol. 0.5ml of each extract stock solution, 1.5 ml methanol, 0.1 ml aluminum chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminum chloride with distilled water. Sample and sample blank of all four extracts were prepared and their absorbance was measured at 420 nm. All prepared solutions were filtered through whatmann filter paper before measuring

Evaluation of *in vitro* anti-inflammatory activity (Chandra et al., 2012**)**

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of **AQCA** so that final concentrations become 31.25, 62.5, 125, 250, 500, 1 000 µg/mL. Similar

volume of double-distilled water served as control. Then the mixtures were incubated at (37±2) ^{O}C in a BOD incubator (Labline Technologies) for 15 min and then heated at 70 ^{O}C for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using vehicle as blank and their viscosity was determined by using Ostwald viscometer. Diclofenac sodium at the final concentration of (78.125, 156.25, 312.5, 625, 1250, 2 500 µg/ mL) was used as reference drug and treated similarly for determination of absorbance and viscosity. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ Inhibition} = 100\text{x}(\frac{\text{Vt}}{\text{Vc}} - 1)$$

Where, Vt = absorbance of test sample, Vc = absorbance of control. The extract/drug concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

Results and discussion

The moderately coarse powder of *Cynodon dactylon* (100g) aerial part was extracted by maceration method subjected with water as a solvent. The obtained extract was dried and weighed. The percentage yield of plant was calculated as per standard method. The yields were found to be 11.12 g (11.12% w/w of crude drug) of aqueous extract Table.1

The results obtained from phytochemical screening shows the presence of flavonoids and Diterpenes. Alkaloids, phenols, Saponins, carbohydrates, protein and amino acids were found to be absent as shown in Table. 2

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: Y=0.040X + 0.009, R2=0.999, where X is the quercetin equivalent (QE) and Y is the absorbance. The result is shown in table 4 and fig 1.

In the present investigation, the in vitro anti-inflammatory effect of AQCA was evaluated against denaturation of egg albumin. The results are summarized in Table 5 and 6.

The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by AQCD throughout the concentration range of 31.25 to 1 000 µg/mL. Diclofenac sodium (at the concentration range of 78.125 to 2 500 µ g/mL) was used as reference drug which also exhibited concentration dependent inhibition of protein denaturation (Table 2); however, the effect of

Diclofenac sodium was found to be less when compared with AQCD. This was further confirmed by comparing their IC50 values. AQCD possessed IC^{50} value 40 µg/mL whereas that of Diclofenac sodium was found to be 625 µg/mL.

Observations

Table 1: Extractive values obtained from aqueous extract of *Cynodon dactylon*

S. No.	Solvent	Time of extraction (Hours)	Color of extract	Yield	% Yield
1	water	24	Light green	11.12 gm	11.12 %

Table 2: Preliminary phytochemical screening of *Cynodon dactylon*

S. No.	Phytoconstituents	Methanolic Extract
1	Alkaloids	-(ve)
2	Saponins	-(ve)
3	Phenols	-(ve)
4	Carbohydrates	-(ve)
5	**Flavonoids**	**+(ve)**
6	Proteins & amino acids	-(ve)
7	**Diterpenes**	**+(ve)**

Total flavonoid content (TFC)

Table 3: Absorbance of standard and aqueous extract of *Cynodon dactylon*

S. N	Concentration of Quercetin (µg/ml)	Mean absorbance
1	5	0.216
2	10	0.425
3	15	0.625
4	20	0.815

5	25	1.021

n=3, values are given in SEM

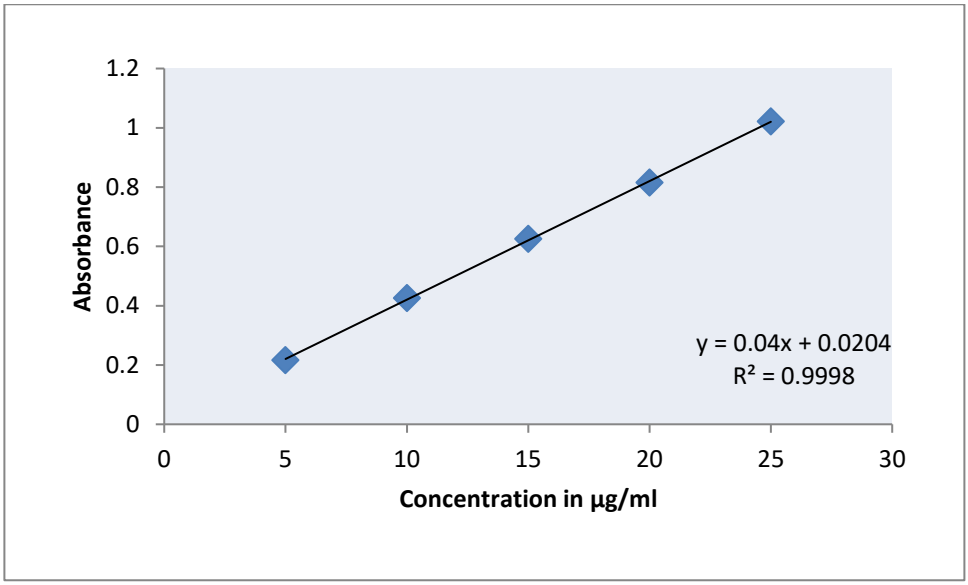

Figure: 1 Standard Calibration curve of quercetin

Table 4: Total Flavonoids content in aqueous extract of *Cynodon dactylon*

S. N.	Extracts 100µg/ml	Flavonoid content Quercetin equivalent mcg/ml
1	**Aqueous extract of *Cynodon dactylon***	0.547

n=3, values are given in SEM

Anti-Inflammatory activity:

Table 5: Effect of AQCD on protein denaturation

Concentration (µg/mL)	% Inhibition
Control	-
31.25	20
62.50	120
125.00	400

250.00	1 320
500.00	2 800
1 000.00	3 700

Table 6: Effect of Diclofenac sodium on protein denaturation

Concentration (µg/mL)	% Inhibition
Control	-
78.125	12.5
156.25	12.5
312.5	25.0
625	50.0
1 250	212.5
2 500	812.5

Conclusion

The major constituents of *Cynodon dactylon* are found to be flavonoids and Diterpenes .Flavonoids are well known natural products known to possess several notable biological properties. In the present study, the in vitro anti-inflammatory activity of *Cynodon dactylon* can be attributed to its flavonoids content. The effect may be due to the synergistic effect rather than single constituent. Therefore, form the results of the present preliminary study it can be concluded that aqueous extract *Cynodon dactylon* possessed marked in vitro anti-inflammatory effect against the denaturation of protein. Further definitive studies are necessary to ascertain the mechanisms and constituents behind its anti-inflammatory actions.

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