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Antibacterial Activity of *Cynodon dactylon* leaf Extract

M. Syam Vardhan*[1], K. Narayana[1], B. Saradhi[1], B. Swaroop[1], P. Venkateswara Rao[2]

[1]*Department of Pharmacognosy, St. Mary's Group of Institutions, Guntur-522212, Andhra Pradesh, India.*

[2]*Department of Pharm. Chemistry, St. Mary's Group of Institutions, Guntur-522212, Andhra Pradesh, India.*

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Abstract:

Cynodon dactylon (Leaf) is a type of grass that possesses great medicinal values. Moreover, medicinal plants are the important source of potentially useful structures for the development of novel antimicrobial agents. Historically, plants have provided a source of the development for novel drugs and plant derived drugs which have made large contributions to human health and well-being. Till now very few plants have been scientifically proved by different researchers for their medicinal potential but the therapeutic ability of number of plants are still unknown. In this study, take four different solvents (chloroform, acetone, ethanol and water) in the order to enhance the polarity nature and finally with distilled water. These were used to investigate the phytochemical constituents of the plant to extract the bioactive compounds from the leaf of *Cynodon dactylon* to screen the antimicrobial activity. Antimicrobial study of methanol and aqueous extracts showed antimicrobial activity against the tested pathogens. Antimicrobial activity was reported due to presence of bioactive compounds.

Keywords: *Cynodon dactylol*, Glycosides, Flavanoids, Phytochemical Screening, Antimicrobial Activity.

Introduction:

Cynodon dactylon, also known as *Vilfa stellata*, durva grass, dhoob, bermuda grass, dubo, dog's tooth grass, bahama grass, devil's grass, couch grass, indian doab, arugampul, grama, wiregrass and scutch grass, is a grass that originated in the middle east [1-3]. Distinguishing characteristics of these plants are the conspicuous ring of white hairs of the ligule, the fringe of hairs on the keel of the lemma and graygreen appearance of the foliage. Lamina of the leaf is characterized by nearly square to oval epidermis having irregularly outer wall. The 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 that extend deep into the mesophyll. The leaf contains crude alkaloid, carbohydrates, saponin, tannins, flavonoids, and cardiac glycosides [2]. *E. coli* is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA. *E. coli* is a gram negative, facultative anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (endotherms).

Corresponding Author: M. Syam Vardhan, Department of Pharmacognosy, St. Mary's Group of Institutions, Guntur – 522212, Andhra Pradesh, India. E-mail: syam.mpharm@ gmail.com

Hyperglycemic and hyperlipidemia properties (plant extract), productivity against ischemia (studied in rat heart), CNS depressive activity in rat (ethanol extract of aerial part), improvement in cardiac functions in rat (hydro alcoholic extraction of rhizome), preventive against aluminium induced neurotoxicity and carbofuran induced oxidative stress (aqueous extract), aphrodisiac and male fertility activity were also reported in the species [4-10] but antimicrobial activity is not reported. In present work we study the anti-microbial activity of chloroform, acetone, ethanol and water extracts using zone of inhibition.

Materials and Methods:

Plant material collection:

The plant material of *Cynodon dactylon* was collected from the Chebrolu, Andhra Pradesh, India in January 2018 and identity by Taxonomist.

Drug and Chemicals:

The drug Amoxicillin and Diclofenac sodium were obtained as a gift sample from East African (India) Overseas [Unit I], Pharma City, Selaqui 248011, Uttarakhand, India. All other chemicals/solvents were of analytical/laboratory grade and obtained commercially.

Extraction procedure:

The freshly collected leaf of the plant were shade dried and coarse powdered and passed through 20 the coarse powdered materials were extracted using various solvents like methanol & water for about 8 cycles by soxhletion. The powdered drug to be extracted is packed in a thimble form made of a filter paper and it is placed in the body of soxhlet extractor. The methanol, water was placed in the flask and maintained temperature about 71°C. On heating solvent gets boiled in the flask, and converts it into vapours. These vapours enter into the condenser through the side tube and get condensed into hot liquid which falls on the column of the drug. When the extractor gets filled with solvent, the level of siphon tube also raises up to its top. The solvent containing active constituents of the drug in the siphon tube siphon over and run into the flask thus emptying the body of extractor. The alternation of filling and emptying the body of extractor goes on continuously. The soluble active constituents of the drug remain in the flask with the solvent is repeatedly volatilized. The process of filling and emptying of extractor is repeatedly until the drug is extracted. Plant material is completely dried and kept for aqueous extraction. The crude extracts were concentrated under vacuum. The extracts were stored in desiccators until use.

Table1: Details of Plant Extraction

Plant material	Solvent used	Volume of the solvent	Weight of the extract	% Yield
Whole plant (100gm)	Methanol	500ml	7.10gm	7.10
	Water	500ml	2.30gm	2.30

General Procedure for Phyto-chemical Screening Test:

Test for Alkaloids:

A. Mayer's test: To small quantity of extract, mayer's reagent was added. Cream or white coloured precipitate was formed, indicate the presence of alkaloids.

B. Dragandroff's test: To small quantity of extract, dragandroff's reagent was added. Orange brown precipitate was formed which indicate the presence of alkaloids.

C. Wagner's test: To small quantity of extract, wagner's reagent was added. Presence of reddish brown precipitates indicates the presence of alkaloids.

D. Hager's test: To small quantity of extract, hager's reagent was added. Formation of yellow precipitates indicates the presence of alkaloids.

Test for Glycosides:

A. Legal's test: To the hydrolysate 1ml of pyridine, few drops of sodium nitropruside was added. Then it was made alkaline with NaOH. Pink colour was observed which indicates the presence of glycosides.
B. Baljet test: To the extract sodium picrate was added. Yellow orange colour was observed which indicates the presence of glycosides.

Test for Tannins:

A. Ferric chloride test: To the extract add ferric chloride solution. Blue colour is formed for hydrolysable tannins and green colour for condensed tannins.
B. Phenazone test: To the extract, add 0.5gms of sodium acid phosphate and filter. To the filterate add 2% phenazone solution. A blue, black, violet or green precipitate or colour confirmed the presence of tannins.
C. Gelatin test: To the extract add 1% gelatine solution containing 10 % NaCl which leads to the formation of precipitates.

Test for Flavonoids:

A. Shinoda test: To the extract, add few magnesium turnings and add concentrated HCl drop wise. Pink scarlet crimson red or occasionally green to blue colour appears after few minutes indicate the presence of flavonoids.
B. Zinc hydrochloride test: To the extract add a mixture of zinc dust and concentrated HCl. Red colour appears after few minutes indicates the presence of flavonoids.
C. Alkaline reagent test: To the extract, add few drops of sodium hydroxide solution. Intense yellow colour is formed which turns to colourless on addition of few drops of dilute acid indicates the presence of flavonoids.

Test for Steroids:

A. Salkowski test: Few drops of concentrated acid were added to the extract, shaken and on standing lower layer turns to red in colour confirm the presence of steroids.
B. Liedermann burchards test: To the extract few drops of acetic anhydride were added and mixed well. Few ml of concentrated sulphuric acid was added from the sides of the test tube. A reddish brown ring was formed at the junction of two layers.

Test for Saponins:

Foam test: Small amount of extract was shaken with little quantity of water .The foam persisted for10 minutes it confirmed the presence of saponins.

Test of Carbohydrates:

A. Molish test: Molish reagent & conc. sulphuric acid along the sides of the test tube. A reddish violet ring at the junction of two Liquids shows the presence of carbohydrates.
B. Fehling's test: The extract when heated with equal volumes of fehling's A&B solutions gives a brick red precipitate showing the presence of reducing sugar.

Test of Proteins:

A. Ninhydrin test: To the extract ninhydrin reagent was added and boiled. Purple colour was obtained indicates the presence of proteins.
B. Biuret test: To the extract equal volume of 5% sodium hydroxide was added. To these 4 drops of 1% copper sulphate was added. Violet colour obtained indicates presence of proteins.

Table 2: Phytochemical analysis of extracts

Sr. No.	Name of the test	Methanol extract	Aqueous extract
1	Liebermann burchard (for terpenes & steroids)	+	-
2	Salkowski (for terpenes & steroids)	-	-
3	Mayers (for alkaloids)	-	+
4	Molish (for carbohydrates)	-	+
5	Fehlings (for carbohydrates)	-	+
6	Baljets (for cardiac glycosides)	+	+
7	Test for phenolics ($Fecl_3$ test)	+	+

Screening of Anti-Microbial Activity:

Antimicrobial Activity:

The development of drug resistance in human pathogens against commonly used bacterial strains has necessitated a search for new anti-microbial substances from other sources including plants. Plants are known to produce a variety of compounds to protect themselves against a variety of their pathogens and therefore considered as potential source to different classes of anti-bacterial substance. Plants used in traditional medicine contain a wide range of substances that can be used to treat chronic as well as acute infectious diseases. The substances that can either inhibit the growth of micro-organisms or kill them are considered as candidates for developing new drugs for treatment of various infectious diseases. The plants used in traditional system of medicine are well known as remedies for diseases in the rural areas of developing countries. Herbal medicines have been used in developing countries as an alternative to Allopathic medicine. Our extensive review of literature has revealed a variety of medicinal plants possessing anti-inflammatory and hepatoprotective activity which also exhibited good anti-microbial properties. On the basis of these reports the plant extracts were subjected for antimicrobial activity against various microbes.

Preparation of Test and Standard Solutions:

The stock solution of test compounds was prepared by dissolving the dried extracts at a concentration of 1mg/ml and pure compounds at a concentration of 1mg/ml in DMSO respectively. The stock solution of reference standard (Streptomycin) was prepared at a concentration of 25 µg/ml sterile water. Anti-microbial activity was screened by adding 0.05ml/50µl stock solution to each cup by micro pipette.

Culture medium:

The nutrient agar media used for our anti bacterial studies was prepared by using Beef extract - 0.3%, Sodium chloride - 0.5%, Peptone - 0.5%, Agar - 2.0%, at a pH of 7.2-7.3. The sterilization of the media, water, *etc.*, was carried out by autoclaving at 15lbs/inch, 121°C for 20 minutes. Sterilize the glasswares like syringes, petri dishes, and pipettes for one hour. The sterilized medium was cooled to 40°C and poured into the petridishes with 6 mm thickness. The media was allowed to solidify at room temperature.

Stock Solution:

Initially 10 mg extracts were weighed accurately and dissolved in 10ml methanol to get concentration of 1000 µg/ml.

Principle:

The antimicrobial substance diffuses from the cup through a solidified agar layer in a petridish or a plate to an extent so that the growth of added microorganism is in habited entirely in a circular area or zone around the cavity containing the solution of a known quantity of antimicrobial substance. The antimicrobial activity is expressed as the zone of inhibition in millimetres, which is measured with a zone reader.

Inhibition Zone of Micro-organisms:

Determination of Zone of Inhibition by Cup Plate method:

The anti bacterial activity in terms of zone of inhibition of methanol, and aqueous extracts of *Cynodon dactylon* was determined against 4 different microorganisms and the results were compared with Streptomycin used as standard. All the dilutions for the preparation of test (1000µg/ml) and standard (25µg/ml) drug were done in DMSO. The nutrient agar plates were prepared. The inoculum was spread on the surface using a sterile cotton swab and using sterile Cork borer of 6 mm diameter made cups. Then the cups were filled accordingly. After introducing the sample, standard and control in the cups the petridishes were kept in refrigerator at 4ºC for 2hrs, for diffusion and then incubated at 37ºC for 24 hrs. The antimicrobial activity was measured as a diameter in mm of Inhibitory zones on the agar plates. The experiment was repeated in triplicate and the average value was written. The methanol, chloroform and aqueous extracts of *Cynodon dactylon* plant were screened for anti bacterial activity against a wide spectrum of microorganisms and the activity were compared with appropriate reference standards (streptomycin for both gram-positive and gram-negative organisms). Microorganisms were grown in nutrient agar medium. DMSO was used as control and the drug vehicles for the plant extracts and reference standard respectively.

Figure 1: Formation of Zones in Petriplates

Measuring the Zone of Inhibition:

The presence of definite zone of inhibition (any size) around the cup indicates the anti-microbial activity. The solvent methanol was run simultaneously to assess the activity of test drug which was used as a vehicle. The results were depicted in Table-3.

Table 3: Antimicrobial Activity

Plant material	Dose (mg/ml)	Zone of inhibiton (diameter in mm)		
		Gram(+)ve	Gram(-)ve	fungi
		B.S	E.C	A.N
Methanolic extract	1000	12	12	14
	500	14	16	16
Aqueous extract	500	13	11	18
Standard (Streptomycin)	25	16	18	15
Control (DMSO)	50ml	-	-	-

Conclusion:

The results of this work support the importance of *Cynodon dactylon* in various aspects. The present work confirms that the methanol & aqueous extract of leaf can act as a good source of medicine in natural origin. Methanol & aqueous leaf extract demonstrates antimicrobial activity against gram-positive and gram-negative bacteria. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the

treatment of various gramnegative bacterial infections. From the present study we can draw a conclusion that the traditional use of plant *Cynodon dactylon* for the infectious disease is promising, mainly against bacteria.

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