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POTENTIAL ANTIBACTERIAL AND ANTINFUNGAL ACTIVITY OF AQUEOUS EXTRACT OF CYNODON DACTYLON

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ABSTRACT

The use of medicinal plants in the world and especially in India, contributes significantly to primary health care. Whole plant of the *Cynodon dactylon* is traditionally used to treat painful and inflammatory condition. We have undertaken this study since no detailed scientific study was available regarding the antifungal and antibacterial activity of the aqueous extract of the whole plant. In this study the aqueous extract of *Cynodon dactylon* was used to determine the antimicrobial activity against *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis* and *Candida albicans*. The antimicrobial activity was determined using the agar well diffusion method. The diameter of the clear zone of inhibition surrounding the well was measured. The aqueous extract of *Cynodon dactylon* had antimicrobial activity against all the test organisms except *Candida albicans.* Phytotoconstituents present included Saponins, Tannins, steroids and Flavonoids. It can be concluded that aqueous extract of whole plant of *Cynodon dactylon* may be considered as an antibacterial agent and can be used to source antibiotic substances for possible treatment of bacterial infections.

INTRODUCTION: Even though pharmacological industries have produced a number of new antibiotics in the last three decades, the resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [1]. Plant materials continue to play an important role in the maintenance of human health since antiquity. Over 50% of all modern chemical drugs originated from natural plant sources. These plant products are the major source of drug development in pharmaceutical industry [2]. Several plants are now being used in part or as a whole to treat many diseases. Active components of these plants are now being investigated, extracted and developed into drugs with little or no negative effects or contra-indication [3]. Rural dwellers in most parts of the world do not depend on the orthodox medicine for the cure of diseases and aliments. This is because most of the

modern equipments are expensive and service delivery too expensive to afford.

As a result of this, a larger section has resulted to the use of traditional medicines, which are believed to be less expensive, and of little or no side effects. One of such plant considered of great importance is Cynodon dactylon Pers. (Family: Graminae, Durba in Bengali, Dhub in Hindi, Bermuda grass in English), a creeping grass found in warm climates all over the world between 450 south and north altitude. The Cynodon dactylon is available throughout the year; the material is used by the domestic animals as food and for pooja in all parts of India.

The juice of the plant is astringent and is applied externally to fresh cuts and wounds. It is also useful in treatment of catarrhal ophthalmic, dropsy, hysteria, epilepsy, insanity, chronic diarrhea and dysentery. The plant is folk remedy for anasarca, calculus, cancer, carbuncles, cough, hypertension, snakebites, stones, gout, fever, skin diseases and rheumatic infections [4, 5, 6, 7].

Further, it has also antiestrogenic, antimicrobial, anathematic, antihistaminic, antiemetic, antipyretic, antidiabetic and antioxidant activities [8, 9, 10]. Previous studies [11] showed that butanolic, ethanolic and methanolic extracts of *Cynodon dactylon* leaves showed a wide range of antibacterial activity. No detailed scientific data is available regarding the efficacy of aqueous extract of whole plant of *Cynodon dactylon*. In our laboratory, we have previously investigated the antihyperlipidemic, wound healing and estrous cycle effects of aqueous extract *Cynodon dactylon*. The present study was undertaken to further analyze the antibacterial and antifungal activity of *Cynodon dactylon*.

MATERIALS AND METHODS:

Plant material: The whole plant with the roots of *Cynodon dactylon* was collected from the campus of Kasturba Medical College, Manipal University, Mangalore, India. It was Identified and authenticated by a plant taxonomist. The collected plant was washed thoroughly in tap water and dried in room temperature for 15 days. The dried 20 g plant were powdered and soaked separately in 100 ml water and chloroform by keeping it in a shaker for 3 days. The extracts were filtered through cheesecloth and the extracts were reduced to 10% of its original volume. The organic solvent filtrates were concentrated in vacuum using a rotary evaporator, while aqueous extract was dried using water bath. The extract preparation for the present experiment was done in MCOPS (Manipal college of pharmaceutical sciences), Manipal university, Manipal.

Microorganisms used for the tests: The antibacterial activity of the extract was determined by individually testing on Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923), Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, Escherichia coli ATCC 25922 and clinical isolates *Klebsiella pneumoniae* and *Proteus mirabilis*) and antifungal activity on *Candida albicans*.

Phytochemical screening: Chemical tests were carried out on aqueous extracts of *Cynodon dactylon* using standard procedures to identify the constituents as described by Sofowora [12] Trease and Evans [13] and Harborne [14].

Alkaloids: About 0.2 g of the extracts was warned with 2% H_2SO_4 for two minutes. It was filtered and few drops of Dragendroff's reagent were added. Orange red precipitate indicates the presence of alkaloids.

Tannins: Small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins

Steroids: 2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of $H_2 S0_4$.The color changed from violet to blue or green in some samples indicating the presence of steroids.

Phlobatanins: The extract (0.5 g) was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl. Red precipitate shows the presence of phlobatanins.

Flavonoids: Extract of about 0.2 g was dissolved in NaOH and HCl was added. A yellow solution that turns colorless indicates the presence of flavonoids.

Saponins: About 0.2 g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing of the extracts shows the presence of Saponins

Antimicrobial activity determination [15, 16, 17]: The antimicrobial activity was determined using the agar well diffusion method. Overnight cultures were grown at 37°C in Muller-Hinton Broth (MHB) and diluted to contain 105 cfu/ml. Petri dishes containing 20 ml of Muller-Hinton Agar (MHA) (Hi Media), were used. The bacterial culture was spread over the surface of the MHA plate. A total of 4 mm diameter wells were punched into the agar and filled with 20µl of the plant extract of various concentrations (400 mg/ml, 200 mg/ml, 100 mg/ml and 50 mg/ml). The plates were then incubated at 37^{o}C for 18 hrs.

Culture Media: The media used for antifungal test was Sabouraud's dextrose agar/broth of Hi media Pvt. Bombay, India. The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 hours and the suspensions were checked to provide approximately 105 CFU/ml. The clinical fungal test organisms used for study are *Candida albicans* ATCC were procured from National Chemical Laboratory (NCL), Pune, Maharashtra, India.

Determination of antifungal activity: The agar well diffusion method [18] was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabourauds dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks (hydro alcohol, and hexane). Standard antibiotic (Fucanazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37oC for 72 hours. The diameters of zone of inhibition observed were measured.

RESULTS AND DISCUSSION: The presence of antibacterial substances in the higher plants is well established [19, 20]. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of unani and ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug. As shown in **Table 1**, the phytochemical analysis of aqueous extract showed the presence of tannins, steroids, flavonoids, saponins, and absence of alkaloids and phlobatanins.

TABLE 1: PHYTOCHEMICAL CONSTITUIENTS OF AQUEOUS EXTRACT OF CYNODON DACTYLON

Phytochemical	Positive (+) / Negative (-)
Alkaloids	-
Tannins	+
Steroids	+
Phlobatanin	-
Flavonoids	+
Saponins	+

Phytochemical constituents are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms, insects and other herbivores [21]. The primary phytochemical analysis revealed that the extracts contained some phytoconstituents such as saponins, steroids, tannins flavonoids, which could be responsible for the observed antimicrobial property. These bioactive compounds are known to act by different mechanism and exert antimicrobial action.

Tannins bind to proline rich proteins and interfere with the protein synthesis [22]. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [23].

Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell [24]. Steroids have been reported to have antibacterial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes [25, 26].

The diameter of the clear zone of inhibition surrounding the well was measured as shown in **Table 2**, showed that the aqueous extract of Cynodon dactylon had antimicrobial activity against all the test organisms except *Candida albicans*.

TABLE 2: ZONES OF INHIBITION OF AQUEOUS EXTRACT OF CYNODON DACTYLON AGAINST VARIOUS ORGANISMS

Organism	Zone of inhibition (mm)			
	Extract 50mg/ml	Extract 100mg/ml	Extract 200mg/m	Extract 400mg/m
E. coli	8	9	10	14
Staph. aureus	5	7	8	10
Ps. Aeruginosa	5	6	10	14
Kl. Pneumonia	4	6	9	10
Pr. mirabilis	4	6	9	12
C. albicans	-	-	-	

The test organisms used in this study are associated with various forms of human infections. From a clinical point of view, *Klebsiella pneumoniae* is the most important member of the Klebsiella genus of Entero-bacteriaceae and it is emerging as an important cause of neonatal nosocomial infection [27]. *E. coli* causes septicemias and can infect the gall bladder, meninges, surgical wounds, skin lesions and the lungs, especially in debilitate and immunodeficient patients [28]. Proteus mirabilis causes wound infections and urinary tract infections in the elderly and young males often following catheterization or cystoscopy, and it is a secondary invader of ulcers and pressure sores [29, 30].

The demonstration of activity against both gram-negative and gram-positive bacteria is an indication that the plant can be a source of bioactive substances that could be of broad spectrum of activity. The fact that the plant was active against both clinical and laboratory isolates is also an indication that it can be a source of very potent antibiotic substances that can be used against drug resistant microorganisms prevalent in hospital environments. In the present study even though the aqueous extract proved to be a potent antibacterial agent but it showed the negative results for the antifungal activity against *Candida albicans*.

CONCLUSION: Aqueous extract demonstrated a broad-spectrum of activity against both gram-positive and gram-negative bacteria but negative results for antifungal activity. The broad-spectrum antibacterial activities of the plant extract, possibly due to the identified phytochemical constituents, further confirm its use as a health remedy in folklore medicine. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacteria including gonorrhea, pneumonia, eye infections. Isolation, identification and purification of these phytoconstituents and determination of their respective antibacterial potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agents should be the future direction for investigation. 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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