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Studies of anti-microbial activity using leaf extract of *Cynodon dactylon*

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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 used to investigate the phytochemical constituents of the plant were used to extract the bioactive compounds from the leaf of Cynodon dactylon to screen the antibacterial activity against infectious disease causing bacterial pathogens (Escherichia coli also known as E. coli) using Kirby–Bauer (KB-zone inhibition method). Antimicrobial study of ethanol and aqueous extracts showed antimicrobial activity against the tested pathogens. Antimicrobial activity showed due to presence of bioactive compounds .The zone of inhibition compare with ciprofloxacin with different conc.[0.25mg,0.5mg,1mg.zone of inhibition 0 mm(no see activity), 18.23 mm / ± 0.671, 23.10 / ± 0.743mm.] Ethanolic extraction conc. (0.5mg, 1mg. zone of inhibition 10.59 mm / ± 0.578, 11.33 mm / ± 0.570) , Aqueous extrac tion conc. (0.5mg, 1mg. zone of inhibition 6.84 mm / ± 0.485, 8.64 mm / ± 0.269).

Keywords: *Cynodon dactylon* , Crude extract, Phytochemical, Antimicrobial, Pathogens etc..

INTRODUCTION

Cynodon dactylon is dried whole plant, Fig. 1,belonging to Family-Poaceae. Distinguishing characteristics of this plants are the conspicuous ring of white hairs of the ligule, the fringe of hairs on the keel of the lemma and gray-green appearance of the foliage.

Figure1. Plant of *Cynodon dactylon*

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, Fig. 2. [1-4]

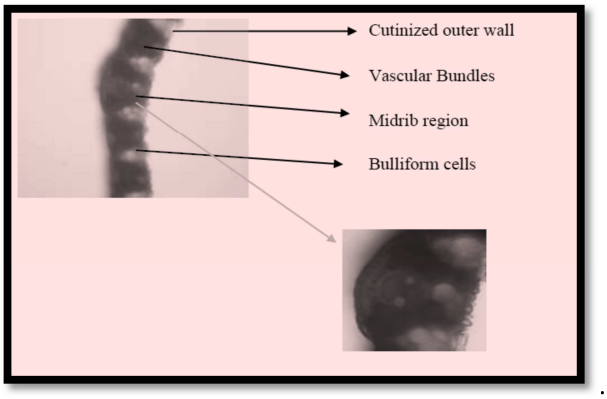


Fig. 2.T.S of the Leaf *Cynodon dactylon* entire view

The leaf contains crude Alkaloid, Carbohydrates, Saponin, Tannins, Flavonoids, Cardiac glycoside. 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).[4-7]

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[8-10] 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. The zone of inhibition compare with ciprofloxacin, Fig. 3.

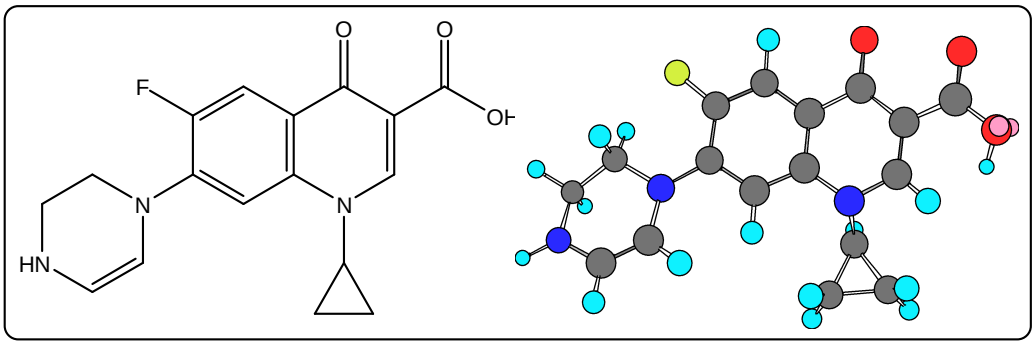


Figure 3. 2D and 3D Structure of Ciprofloxacin

MATERIALS AND METHODS

The leaves were collected from herbal garden of Utthan, Allahabad U.P.(India) in the month of April 2015 and authenticated by, plant taxonomist. Plants were dried in shade and made into fine powder and preserved in clean plastic containers, away from light, heat and moisture until use .The powder was used for extraction of bioactive compounds. The organism *Escherichia coli* was collected from Jeevan Jyoti hospital Allahabad, U. P and incubated for 24hrs.anddiscs of antibiotics (ciprofloxacin) was purchase from market. Ciprofloxacin (0.25mg)(JB11-08)Mfg.by HI media laboratories Pvt. Ltd. Mumbai kept it at -20°C until used.

The leaf of C. dactyl on cleaned using distilled water and shade-dried at room temperature for 15 days, powdered the leaf mechanically then5gms of dry powder was suspended in 25ml of Solvent (Chloroform, Acetone & Ethanol and water) and kept for 72 hours incubation with continuous shaken on a platform shaker (Lab CompanionTM) at 150 rpm with temperature of 25 ºC to30 ºC.After 72 hrs.the suspension was filtered with Whatman No.1 filter paper. The solvent filtrate was centrifuged at 5000 rpm for 5 min. Obtained extracts were evaporated using Rota Vapor TM (BUCHI). Repeat this process, completely evaporate the Solvent(Chloroform, Acetone & Ethanol and water) [11-14].

Qualitative characteristics were study for the purity test of *C. dactylon* on the basis of Loss on drying, total ash value, Acid insoluble ash, Water soluble extractive value and Moisture Content.

For the phytochemical screening, perform the identification test for different phytochemical of *C. dactylon* leaf extract, like alkaloid (Dragendroff's and Meyer's test), Glycoside (Keller-Killani test method), Flavonoids (Shinoda test), Tannins (Ferric chloride test), Saponin (Hemolytic test and Foam forming test), Carbohydrates (Fehling's test and Benedict's test). [15-16]

ANTIMICROBIAL ACTIVITY OF *CYNODON DACTYLON* [17-20]
MacConkey Agar media was prepared by suspending the measured amount of powder 50 gram in 1 L of purified water and mix thoroughly. Heated with frequent agitation and boil for 1 minute to completely dissolve the powder and sterilized using Autoclave**(**York Scientific New Delhi**)**at 121°C for 15 minutes. MacConkey Agar Plate was prepared by Washing and cleaning the petri plate using liquid soap, dry and sterilized using hot air oven at 121°C for 30 minutes. Then poured the MacConkey Agar media in 4petri plate. Solidified it at room temperature and then incubated at 4°C for further use.

For Inoculation of the plate I have used Laminar Flow (National Scientific Varanasi U.P. Clean with sprit or ethanol by using cotton. UV light was on for the sterilization of Laminar Flow platform and after 20 min offed the UV light Switch. During performing work also start the laminar fan and kept the precaution for prevention of contamination.
Whatman filter paper no. 1 was used to prepare *Cynodon dactylon* discs approximately 6 mm in diameter, which are placed in a petri dish and sterilized using hot air oven.Approximately10 ml of respective solvent of extract of *Cynodon dactylon* was added to the disc (0.5 mg/disc) individually and aseptically. Each disc contained 0.5 mg of extract. Then the disc allowed drying at room temperature. After drying they were used for screening the antibacterial activity. Zone size was recorded on the recording sheet.

RESULTS AND DISCUSSION

Physio-chemical analysis of *c. dactylon* powder is reported in Table 1.

Table 1: Physio-chemical analysis of *c.dactylon* powder

Parameters	Total ash	Acid Insoluble Ash	Water Insoluble Ash	Moisture Content
Results	9.1 %w/w	3.7 % w/w	7.9 % w/w	14.23 % w/w

Table 2:Phytochemical test forleaf of*Cynodon dactylon*

S. N.	Phytochemical. constituents	Test	Result for the respective solvents			
			Acetone Extracts	**Chloroform Extracts**	**Ethanol Extracts**	**Aqueous Extracts**
1.	Alkaloid	Dragendroff's test. Meyer's test.	- -	+ +	- -	- -
2.	Cardiac glycoside	Keller-kiliani	-	-	+	-
3.	Flavonoid	Shinoda test	-	-	+	+
4.	Tannin.	Ferric chloride test	+	-	+	-
5.	Saponin	Hemolytic test Foam test	- -	- -	+ +	+ +
6.	Carbohydrate	Fehling test Benedict test	- -	+ +	+ +	+ +

Both ethanol and Aqueous extracts showed significant effect to the tested pathogens with the size of inhibition between 8.64 ± 0.269 mm and 11.33 ± 0.570 mm for ethanol extract.. The greatest activity observed was against E.coli (11.33 ± 0.570 mm) for ethanol extract. Ethanol extract conc. is 0.5mg and 1mg that inhibit the zone is (10.59 mm, 11.33 mm) & aqueous extract conc. is 0.5mg and 1mg that inhibit the zone is (6.84 mm, 8.64 mm). Size of Zone of Ethanol & aqueous leaf extract is compare with standard ciprofloxacin 0.5mg and 1mginhibitthezone is (18.23 mm, 23.10 mm), reported in Table 3 and Fig. 4& 5. When the increase the conc. of drug and conc. of Ethanol& Aqueous leaf extract so increases the Zone size, Fig. 6.

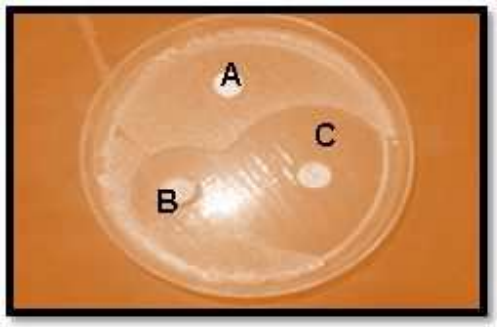
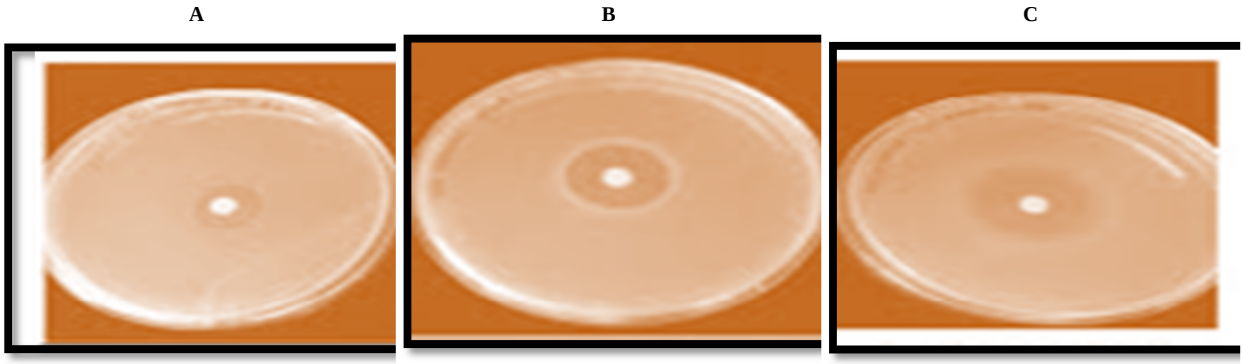
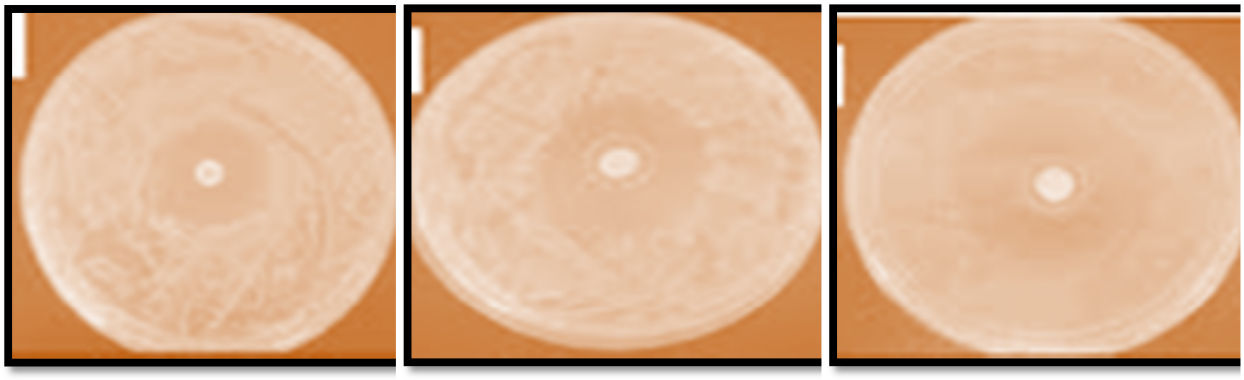


Figure 4: Zone Inhibition of ciprofloxacin. A. 0.25mg (No Zone Inhibition) B. 0.5mg (19.11mm) C. 1mg (23.12 mm)





D **E** **F**

Figure 5: Zone Inhibition of A. Aqueous Extract 0.5mg (7 mm) B. Ethanol Extract 0.5mg (11.38 mm) C. Ciprofloxacin 0.5mg (19.11 mm) D. Aqueous Extract 1.0 mg (9 mm)E. Ethanolic Extract 1.0 mg(12.10 mm) F. Ciprofloxacin 1.0 mg (24 mm)

Table 3. Zone of Inhibition of drug/ Extract of *C. dactylon*

S.No.	DRUG/ Extract of *C. dactylon* Concentration(mg)	Zone of Inhibition (mm) E.Coli	Mean ± S.D.
1.	**Ciprofloxacin**		
	0.25mg	0.00 (No zone)	18.23 ± 0.671
	0.5mg	18.10 mm	
	0.5mg	17.48 mm	
	0.5mg	19.11 mm	
	1.0 mg	22.18 mm	23.10 ± 0.743
	1.0 mg	23.12 mm	
	1.0 mg	24.00 mm	
2.	**Aqueous Extract**		6.84 ± 0.485
	0.5mg	7.35 mm	
	0.5mg	6.19 mm	
	0.5mg	7.00 mm	
	1mg	8.35 mm	8.64 ± 0.269
	1mg	9.00 mm	
	1mg	8.58 mm	
3.	**Ethanolic Extract**		10.59± 0.578
	0.5mg	10.41 mm	
	0.5mg	11.38 mm	
	0.5mg	10.00 mm	
	1mg	11.18 mm	11.33 ± 0.570
	1mg	10.37 mm	
	1mg	12.10 mm	

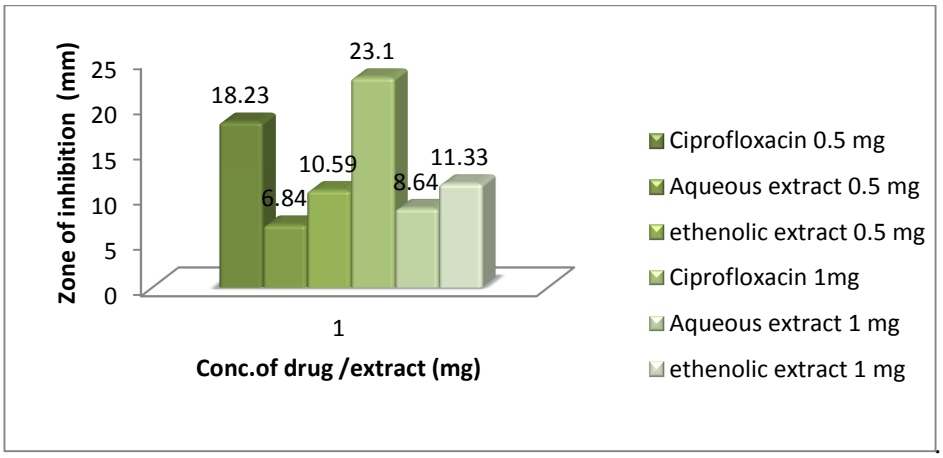


Figure 6.Conc. (mg) v/s zone inhibition (mm) response chart

CONCLUSION

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