International Journal of Academic Research and Development
ISSN: 2455-4197
Impact Factor: RJIF 5.22
www.academicsjournal.com
Volume 3; Issue 3; May 2018; Page No. 116-121

Antimicrobial activity and phytochemical analysis of aerial parts of *Cynodon dactylon*

Dhanapal Venkatachalam[1], Samuel Thavamani B[2], Muddukrishniah K[3], Suganya Vijayan[4], Vinod KR[5]
[1-4] Department of Pharmacognosy, Sanjo College of Pharmaceutical Studies, Velappara, Palakkad, Kerala, India
[5] Department of Pharmaceutics Sanjo College of Pharmaceutical Studies, Velappara, Palakkad, Kerala, India

Abstract
Objective: The present study was designed to check *in-vitro* efficacy of *Cynodon dactylon* plant extracts against selected bacterial and fungal strains The *Cynodon dactylon* (Family: Poaceae) commonly known as "arugum pillu" (Tamil), popularly known as Bermuda grass grows throughout India**.**
Methodology: Ethanol and aqueous extracts of *Cynodon dactylon* was used for antimicrobial screening. Antibacterial activity was tested against four pathogenic bacterial strains i.e. *Streptococcus aureus* (Gram+) *(MTCC737), Pseudomonas aeuriginosa* (Gram-) *(ATCC27853), Salmonella paratyphi, Enterobacter aergens* while antifungal activity was tested against two fungal strains i.e. *Candida albicans* **(***ATCC2091)***,** *Aspergillus niger*. *(ATCC 6275).* Anti-bacterial activity of *Cynodon dactylon* extract was carried out by conventional disc plate method.
Results: Ethanolic extract of *C.dactylon* showed the largest zone of inhibition (1.1cm) against *Salmomnella paratyphi* at 50µgm/ml and 0.9 cm zone of inhibition against *S.aures, P.aeruginosa and E.aerogens* at 50µgm/ml. Aqueous extract of *C.dactylon* showed 0.8cm zone of inhibitions against *S.paratyphi* and *E.aergens* at 15µgm/ml. Aqueous extract showed 0.7 cm and 0.6cm zone of inhibition against *S.aureus and P.aeruginosa* respectively at 15 µgm/ml. Ethanolic extract has shown more potent antibacterial activity than aqueous extract. Ethanolic extract was found to be more effective against fungus *Candida albicuns* than aqueous extract. The zone of inhibition of ethanolic extract was 2.6 cm and the zone of inhibition of aqueous extract was 1.7cm.Aqueous extract was found to be more effective against fungus *Aspergillus niger* than ethanolic extract. The zone of inhibition of aqueous extract was 3,2cm and the zone of inhibition of ethanolic extract was 2,0 cm.The phytochemical screening demonstrated the presence of different types of compounds like terpenoids, tannins, and flavonoids which may contribute for the anti-microbial action of this plant,
Conclusions: These findings provide scientific evidence of traditional use *Cynodon dactylon* and also indicate the potential of this plant for the development of antimicrobial agents.

Keywords: anti bacterial activity, anti-fungal activity, *Cynodon dactylon*, ethanol extract, aqueous extract

Introduction
Herbals have a great potential for producing of new drugs for the benefits of mankind. There are many approaches to search for biologically active principles in plants [1]. Medicinal plants are abundant source of antimicrobial molecules. A wide range of medicinal plants extracts are used to treat several infections as they have potential antimicrobial activity. Some of these bioactive molecules are screened and traded in market as raw material for many herbal industries [2]. Experts turned their concentration back towards obtaining advantages from medicinal plants after observing more side effects of synthetic drugs compared to their benefits [3]. It is estimated that about 35,000 to 70,000 plants species are used as medicinal plants out of 422127 reported worldwide plant species [4]. In the worldwide as well as in the developing countries, the most human died due to infectious bacterial diseases [5]. The bacterial organisms including Gram positive and Gram negative like different species of *Bacillus*, *Staphylococcus*, *Salmonella* and *Pseudomonas* are the main source to cause severe infections in humans. Because these organisms have the ability to survive in harsh condition due to their multiple environmental habitats [6]. the synthetic antibiotics have the following limitation: Firstly, these are costly and are out of range from the patient belonging to developing countries. Secondly, with the passage of time microorganism develop resistance against antibiotics. Therefore, after some time these antibiotics are not effective against the microbes [7, 8]. Furthermore, the antibiotics may be associated with adverse effects on the host, including hypersensitivity, immune suppression, and also allergic reactions. On the other hand, natural products have got incredible success in serving as a guidepost for new antibacterial drug discovery. Moreover, antibiotics obtained in this way have biological friendliness nature [9, 10]. As is well known that the bioactive plant extracts is a promising source of majority of drugs [11]. For example, Quinine (*Cinchona*) and berberine (*Berberis*) are the antibiotics obtained from plants which are highly effective against microbes (*Staphylococcus aureus*, *Escherichia coli)* [12] In India, a vast diversity of bioactive plants grown naturally.
The *Cynodon dactylon* (Family: Poaceae) commonly known as "arugum pillu" (Tamil), popularly known as Bermudagrass grows throughout India. It is a rapid growing perennial grass, native to East Africa, Asia and Australia and southern Europe.

A hardy perennial grass abundant on road sides and paths, and readily takes possession of any uncultivated area. It creeps with culms, rooting at nodes and forming spreading mats on the surface of the soil and flowers nearly throughout the year. The flowers are green or brinjal in colour and the fruit grains are tiny and grayish in colour [13]. It is a weed and has been found to possess various medicinal properties. According to the Ayurvedic Pharmacopoeia, the plant is pungent and bitter in nature with charecterestic fragrance and has cold potency. According to Unani system of medicine, the plant posses sharp and hot taste with good odour. The aerial parts and rhizomes of *Cynodon dactylon* was reported for its cardioprotective action, antibacterial, antimicrobial, antioxidant, wound healing, anti-diabetic, diuretic effects. *Cynodon dactylon* is reported to contain cynodin, hydrocyanic acid and triticin. The plant is traditionally used for jaundice, diuretics, and astringent, to stop bleeding in piles, skin infections in India at West Bengal, Assam, Manipur, and Mizoram parts [14]. C. *dactylon* is used by traditional healers for purifying the blood, anuria, biliousness, conjuctivitis, diarrhoea, gonorrhoea, itches and stomach ache [15]. Traditionally, juice of this plant is commonly consumed as health drink during the early morning in south India for healthy life. It forms an important part of Ayurvedic medicine, the juice of C. *dactylon* was used to treat hysteria, epilepsy and insanity [16]. The present study was designed to check *in-vitro* efficacy of *Cynodon dactylon* plant extracts against selected bacterial and fungal strains and phytochemical evaluation.

Materials and Methods

Plant material

Cynodon dactylon stem were collected during the month of Dec 2017, from Palakkad, Kerala, India and authenticated by Dr. P. Jayraman, Director of plant Anatomy Research Centre, Chennai. The fresh aerial parts were separated and kept for shade drying. Dried specimen was powdered using mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Extraction of Plant material

For preliminary phytochemical analysis, extract was prepared by weighing 1kg of the dried powdered aerial parts were subjected to hot successive continuous extraction with different solvents as per the polarity, petroleum ether, benzene, chloroform, ethanol and finally with aqueous. The extracts were filtered in each step using Whatman filters paper. The filtrate was concentrated using a rotary evaporator at low temperature (40-45ºC) and pressure. The presence or absence of the primary and secondary phytoconstituents was detected by usual prescribed methods [17].

Antibacterial activity

Materials

Petri dish (sterilized), Nutrient agar medium, Whitman paper discs, incubator, Laminar air flow,

Composition of the medium*:*

Beef extract-0.3gm, Peptone - 0.5gm, Sodium chloride-0.5gm,Bacto agar -0.5gm,Distilled water-100ml

Human pathogenic bacterial species

Out of four human pathogenic bacterial species, one was Gram positive, Streptococcus aureus (MTCC737), and three were Gram negative, Pseudomonas aeuriginosa (ATCC27853), Salmonella paratyphi, Enterobacter aergens and determined the MIC. The bacterial pathogens were obtained through the courtesy of MTCC and Gene Bank, IMTECH, Chandigarh, India

Preparation of sterile disc

Whitman's No.3 filter paper was punched into 6 mm disc form and they sterilized, each sterile disc was incorporated individually with 20 - 60µl of extracts using micropipette. Precautions were taken to prevent the flow of the solvent extract from the discs to the outer surface. The condensed extracts were applied in small quantities on discs and they were allowed to dry in air. After sometimes another doses of extracts were applied on discs. Then they were stored at 4ºC.

In-vitro antibacterial assay

In-vitro antimicrobial bioassay of the ethanolic and aqueous extracts of *Cynodon dactylon* was carried out by conventional disc plate method (Schillinger and Lucke 1989) and determine the minimum inhibitory concentration against gram positive and negative bacteria were determined. The Ethanolic and Aqueous extracts of *Cynodon dactylon* were 5,10,15,20 µgm/ml was applied sterile 6mm diameter paper discs and dried. Each nutrient agar plate was inoculated with a bacterial strains and treated paper discs were incubated at 37*c. The experiment was carried out in triplicates.

Determination of MIC

Minimum inhibitory concentration of the bacterial test pathogens was determined after 36 hours of incubation. The MIC was measured using the scale and record.

Anti-fungal activity

Materials: Petri plates (sterilized), Nutrient agar medium, Cotton buds, incubator, Laminar air flow,

Composition of the medium

Potato Dextrose Agar Medium
Pealed potato - 25gm,
Dextrose- 2 gm,
Agar agar -2 gm,
Distilled water 100ml

Human pathogenic fungal strain

The test fungal strains investigated include *Candida albicans* (ATCC2091), *Aspergillus niger* (ATCC 6275) All the. fungal strains were obtained from National Chemical laboratory (NCL), Pune, India

Preparation of inoculums

Stock cultures were maintained at 4ºC. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to the test tubes of Sahouraud Dextrose broth (SDB),the fungi that were incubated without

agitation for 24 hours at 25ºc. The cultures were diluted with fresh Sahouraud Dextrose broth to achieve optical densities corresponding to 2.0 × 10 spores /ml for fungal strains.

In vitro antifungal assay

In vitro fungal activity was screened by using Potato Dextrose Agar (PDA) obtained from Himedia (Mumbai). The PDA plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5mts.

Determination of MIC

The Minimum Inhibitory Concentration (MIC) of the extracts were determined according to Elizabeth *et al*. (1999)

Results

Preliminary phytochemical analysis

The aerial parts of the powder and various extracts such as petroleum ether extract, benzene extract, chloroform extract, ethanol extract and aqueous extract were subjected to preliminary phytochemical screening for their presence or absence of the constituents and the results were tabulated. (Table -1).

Table 1: Preliminary phytochemical tests for drug powder and various extracts of cynodon dactylon

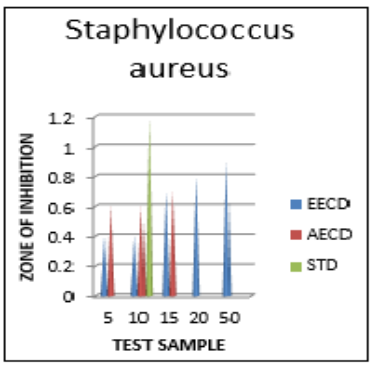
Test	Drug powder	Petroleum ether	Benzene	Chloroform	Ethanol	Aqueous
Sterols	+	+	-	-	-	+
Terpenoids	-	-	-	-	-	+
Carbohydrates	+	+	+	+	+	+
Flavanoids	+	-	-	-	+	+
Proteins	+	+	+	+	+	+
Alkaloids	+	-	-	+	+	+
Glycosides	-	-	-	-	-	-
Saponins	+	-	-	-	+	+
Tannins	+	-	-	-	+	+
Mucliage	-	-	-	-	-	-

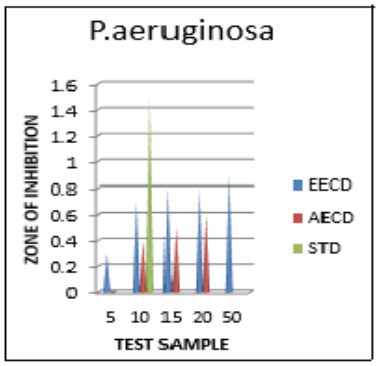
Antibacterial Activity

Table 2: Comparison of Anti-Bacterial Activity of Ethanolic and Aqueous Extracts

S. No		Zone of inhibition(cm)									
		Concentration(µgm/ml)									
		Ethanolic extract					Standard	Aqueous extract			Standard
	Organism	5	10	15	20	50	10µg/disc	5	10	15	10µg/disc
1	*S. aureus*	0.4	0.4	0.7	0.8	0.9	1.2	0.6	0.6	0.7	1.2
2	*P. aeruginosa*	0.3	0.7	0.8	0.8	0.9	1.5	0.4	0.5	0.6	1.5
3	*S.P. typhi*	0.3	0.4	0.6	0.8	1.1	1.8	0.3	0.6	0.8	1.8
4	*Enterobacter aerogens*	0.5	0.6	0.7	0.9	0.9	1.2	0.5	0.6	0.8	1.2

The table 2 illustrated that ethanolic extract of *Cynodon dactylon* aerial parts showed the largest zone of inhibition (1.1cm) against *S. paratyphi* and 0.9 cm zone of inhibition against *S. aureus*, *P. aeruginosa* and *E. aerogens* at 50µgm/ml. Aqueous extract of *Cynodon dactylon* aerial parts showed the largest zone of inhibition (0.8 cm) against *S.paratyphi and E.aerogen*, 0.7 cm and 0.6 cm zone of inhibition against *S.aureus* and *E.aerogens* respectively at 15µgm/ml. Comparison of antibacterial activity of Ethanolic and aqueous extract at 15 µgm/ml concentration, aqueous extracts has shown more potent antibacterial activity against *S.paratyphi* and *E.aerogens*, ethanolic extract has more potent antibacterial activity against *P.aeruginosa*. Both the extracts showed the equal zone of inhibition against *S.aureus.* The phytochemical screening demonstrated that the presence of different types of compounds like terpenoids, tannins, sugars, sterols, alkaloids, and flavonoids which may contribute for the antibacterial action of the *Cynodon dactylon.*





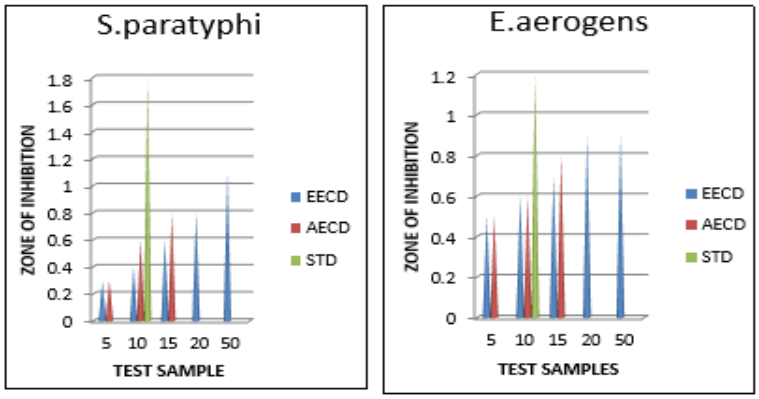


Fig 1-4: Comparison of Anti-Bacterial Activity of Ethanolic and Aqueous Extracts and Standard

EECD-Ethanolic Extract of *Cynodon Dactylon*
AECD-Aqueous Extract of *Cynodon Dactylon*, Std-Standard

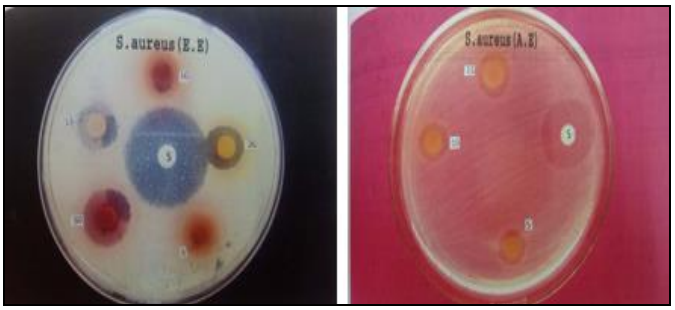


Fig 5-6: Anti-bacterial activity of Ethanolic and Aqueous extracts of *Cynodon dactylon* against *S.aureus*

Fig 7-8: Anti-bacterial activity of Ethanolic and Aqueous extracts of *Cynodon dactylon* against *P.aeruginosa*

Fig 9-10: Anti-bacterial activity of Ethanolic and Aqueous extracts of *Cynodon dactylon* against *S.paratyphi*

Fig 11-12: Anti-bacterial activity of Ethanolic and Aqueous extracts of *Cynodon dactylon* against *E.aerogens*

Anti-fungal Activity

Table 3: Comparision of Antifungal Activity of Various Extracts

Sl. No.	Organisms	Zone of Inhibition Ethanolic	Aqueous
1	*Candida albicans*	2.6	1.7
2	*Aspergillus niger*	2.0	3.2

The above results clearly demonstrates that ethanolic extract had more potent antifungal activity against *Candida albicans* than aqueous extract of *Cynodon dactylon*. Aqueous extract had more potent antifungal activity against *Aspergillus niger* than ethanolic extract of *Cynodon dactylon.* The phytochemical screening demonstrated that the presence of different types of compounds like terpenoids, tannins, sugars, sterols, alkaloids, and flavonoids which may contribute for the antifungal action of the *Cynodon dactylon.*

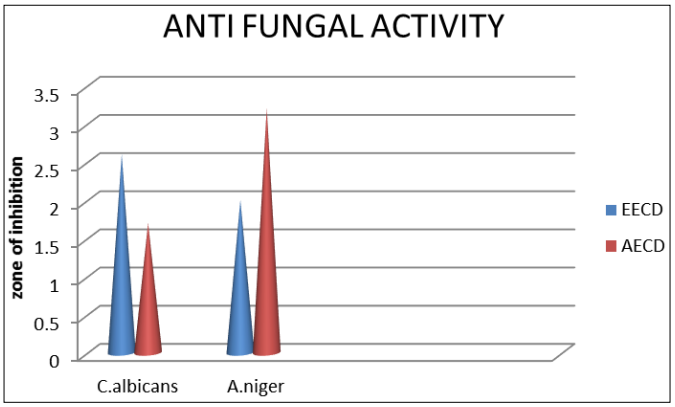


Fig 13: Comparision of Antifungal activity of ethanolic and aqueous extracts of *Cynodon dactylon*

Fig 14-15: Comparison ofAntifungal activity of ethanolic and aqueous extracts of *Cynodon dactylon*

Discussion

In recent times there has been considerable significance in the use of plant material as an unconventional method to control pathogenic microorganism (Aqil *et al.*, 2005) and many components of plants products have been shown to be particularly targeted against resistant pathogenic bacteria (Nostro *et al*., 2006). The appearance of multi drug resistant strain of many pathogens is a severe threat and makes chemotherapy more difficult. Furthermore, the current price of most of the chemotherapeutic agents is intolerable to the public particularly in developing countries like India (Gopalakrishna Sarala *et al*., 2010). Therefore attempts must be directed towards the development of effective natural, non-toxic drug for treatment. Therefore the present work was carried out to explore the antimicrobial property of *Cynodon dactylon.* The ethanolic and aqueous extract of *C. dactylon* aerial parts showed the activity against four tested microorganisms but the activity was very significant against *S. paratyphi*. The plant based products have been effectively proven for their utilization as source for antimicrobial compounds. The present study shows that the aqueous and ethanolic extracst of *C. dactylon* shows significant activity against. *P.aeroginosa, S, aureus* and *E. aerogens,* which may be due to their phytochemical or secondary metabolites. Ethanolic extract was more effective against fungus *Candida albicans* than aqueous extract. Aqueous extract was more effective against fungus *Aspergillus niger* than ethanolic extract.

Conclusion

It is concluded that this study would lead to the establishment of some valuable compound that has to be used to formulate new different and more potent antimicrobial drugs of natural origin. Further studies are needed to identify the biologically active compounds and to evaluate the efficiency of the compound against pathogenic microorganisms associated with various human diseases.

Acknowledgement

The authors are thankful to the Director and Principal of Sanjo College of Pharmaceutical studies, Vellapara, Palakkad for providing facilities to carry out the present research work.

References

1. Parekh J, Chandren S. In vitro antimicrobial activities of extracts of *Launaea procumbens* Roxb (Labiateae). Afr J Biomed Res. 2006; 9:89-93.
2. Uniyal SK, Singh KN, Jomwal P, Lal B. Traditional use of medicinal plants among the tribal communities of Chota Bengal,Western Himalaya. J Ethnobiol Ethnomed. 2006; 2:1-14.
3. Renisheya Joy Jeba, Malar T, *et al*. Antibacterial activity of ethanolic extracts of selected medicinal plants against human pathogens, Asian Pacific Journal of Tropical Biomedicine, 2011, S76-S78.
4. Riaz Bushra, *et al*. Neuropharmacological screening of the iyengaria stellata revealed its memory. Boosting, anxiolytic and antidepressant effects, International research journal of pharmacy. 2012; 3(10):90.
5. Yamin Bibi, Sobia Nisa, Fayyaz M, *et al*. Antibacterial activity of some selected medicinal plants of Pakistan BMC Complementary and Alternative Medicine, 2011.
6. Nathan C. Antibiotics at the crossroads. Nature. 2004; 431:899-902.
7. Ahameethunisa AR, Hoper W. Antibacterial activity of Artemisia nilagirica leaf extract against clinical and phytopathogenic bacteria. BMC Complementry and Alternative Medicines. 2010; 10:6-10.
8. Walsh FM, Amyes SGB. Microbiology and drug resistance mechanisms of fully resistant pathogens. Curr Opi Micro. 2004; 7:439-444.
9. Alder JD. Daptomycin, a new drug class for the treatment of Gram-positive infections. Drugs Today. 2005; 41(2):81.
10. Walsh C. Where will new antibiotics come from?. Nat Reviews Micro. 2003; 1:65-70
11. Koehn FE, Carter GT. The evolving role of natural products in drug discovery. Nat Rev Drug Discov. 2005; 4:206-220.
12. Bibi Y, Nisa S, Waheed A, Zia M, Sarwar S, Ahmed S, *et al*. Evaluation of *Viburnum foetens*for anticancer and antibacterial potential and phytochemical analysis. Afri J Biotechnol. 2010; 9:5611-5615.
13. Maridass M, De Britto AJ. Origins of Plant Derived Medicines. Ethnobot Leaflets. 2008; 12:373-387.
14. Bhandari MM. Flora of the Indian desert. 2nd ed. New Delhi: Dhriti Printers, 1990.
15. Bharati D, Sonawane SA, Amrutkar MP, Undale VR, Wankhade AM, Bhosale AV. Pharmacologyonline. 2009;

2:1-11.
16. Chellaiah Muthu, Marriappan Ayyanar, Nagappan Raja, Savarimuthu Ignacimuthu. Medicinal plants used by traditional healers in Kancheepuram District of TamilNadu. Indian J. of Ethanobio and Ethnomed. 2006; 2(43):1-10.
17. Khandelwal KR. Practical Pharmacognosy-Techniques and Experiments. Pune: Nirali Prakashan, 2002.