

RESEARCH ARTICLE

An *in vitro* study of antibacterial and antifungal activity of *Cynodon dactylon*

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ABSTRACT

Background: Plant-derived antimicrobial agents remain an arena for research to overcome the issues pertaining to microbial resistance and adverse effects associated with synthetic drugs. **Aims and Objectives:** The present study is being performed to evaluate the antibacterial and antifungal activity of *Cynodon dactylon*. **Materials and Methods:** The methanol and n-butanol extracts of *C. dactylon* were prepared. The inoculum was prepared from stock cultures containing nutrient broth (antibacterial activity) and Sabouraud dextrose broth (antifungal activity) and incubated at 37°C (24 h) and at room temperature (48 h) respectively. Antibacterial and antifungal activity of n-butanol and methanol extracts of *C. dactylon* were screened with the aid of agar disc diffusion method on Muller-Hinton agar medium and Sabouraud dextrose agar medium respectively at 1000 µg/ml, 750 µg/ml, and 500 µg/ml concentrations. The measurement of diameter of zone of inhibition was performed and compared with controls-ampicillin for antibacterial activity and amphotericin-B for antifungal activity. **Results:** Methanol extract of *C. dactylon* was observed to have good antibacterial activity with *Salmonella*, *Staphylococcus* spp. being susceptible, and good antifungal activity against *Aspergillus*, *Penicillium* and *Trichoderma viride* at 1000 µg/ml. The n-butanol extract also had good antibacterial activity against *Escherichia coli*, *Pseudomonas* spp., and good antifungal activity against *Aspergillus*, *Penicillium* and *T. viridae* at 1000 µg/ml. **Conclusion:** From this study, we found that *C. dactylon* has promising antibacterial activity against *Salmonella*, *Staphylococcus*, *E. coli*, *Pseudomonas*, and potential antifungal activity against *Aspergillus*, *Penicillium*, *T. viridae*, *Candida* spp.,

KEY WORDS: *Cynodon dactylon*; Antibacterial; Antifungal

INTRODUCTION

With the increasing incidence of chemotherapeutic failure and antibiotic resistance by several microbial agents, antimicrobial evaluation of medicinal plants has become the need of the hour. Plant-derived biomolecules have an added

advantage of being less toxic in comparison to synthetic agents.^[1,2] Also in accordance with WHO, plant-derived drugs have served as a primary healthcare need for an estimate of ~80% of world population.^[3]

Cynodon dactylon (L) belonging to Poaceae family is one of the most commonly occurring weeds which is a hardy, perennial, creepy grass finding a wide distribution around the globe particularly in tropical areas and warm temperature. It has been referred with various regional terminologies such as arugampullu (Tamil), garikoihallu (Kanarese), haritali (Sanskrit), durua (Marathi), garikagoddi (Telugu), durba (Bengali), and dhubkhabbal (Punjabi). The weed is fast growing, drought resistant, very tough and light green with

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a coarse texture and are found in short cylindrical pieces of 2-4 mm in diameter and 3-20 mm long.^[4]

C. dactylon is known to have the following medicinal properties antiseptic, analgesic, anti-inflammatory, wound healing, astringent, antioxidant, immunomodulatory, antidiabetic, and anticancer activities.^[5,6] Research related to the antimicrobial properties of this plant are minimal in the Indian scenario. Therefore, the current research work intends to screen *C. dactylon* for its antibacterial and antifungal activity.

MATERIALS AND METHODS

Collection and Authentication of Plant

C. dactylon was collected from the local area of Thiruvallur district, Chennai and was used for the study following its authentication by a botanist.

Preparation of Plant Extract

The leaves of *C. dactylon* were washed thoroughly with distilled water, followed by drying in sunlight for 48 h.^[7] The leaves were powdered using a sterile motor and pestle. The extract was prepared using Soxhlet apparatus using 100 g of powdered sample and 100 ml of methanol/n-butanol.^[8,9] The solvent extracts were evaporated under controlled pressure.

Procedure for Antibacterial Activity Assay

Escherichia coli, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, were the bacterial species which were utilized for this study with a standard of ampicillin (20 µl/disc).

Preparation of Inoculum

Stock cultures were placed on nutrient agar Slant with a 4°C temperature maintenance. Loop full of culture from a stock culture were placed into test tubes with nutrient broth with an incubation period for 24 h at 37°C yielding active cultures for the experiment. The method of the assay was carried out by agar disc diffusion method.

Agar Disc Diffusion Method^[10]

Antibacterial activity of the extracts was determined by disc diffusion method on Muller-Hinton agar (MHA) medium in comparison with standard antibiotic ampicillin (20 µl/disc). For this, MHA medium was placed into the Petri plate and following the solidification of the medium; the bacterial suspension was placed using a sterile swab. Three different dilutions of samples were used in the following concentrations, namely, 1000 µg/ml, 750 µg/ml, and 500 µg/ml. Sterile discs containing three different concentrations of samples and positive control (ampicillin) of 20 µl each were kept in

MHA plates and maintained at 37°C incubation for 24 h. The diameter of inhibition zone was measured to determine the antibacterial activity.

Antifungal Activity Assay

Among the various fungal species, *Candida*, *Aspergillus*, *Trichoderma viride*, *Penicillium* sps. were utilized for the study with amphotericin-B (20 µl/disc) taken as standard.

Preparation of Inoculum

Stock cultures were placed on Sabouraud dextrose agar (SDA) slant with a 4°C temperature maintenance. Loop full of culture from stock culture were placed into test tubes with Sabouraud dextrose broth with an incubation period for 48 h at 37°C yielding active cultures for the experiment. The method of assay was carried out by agar disc diffusion method.

Agar Disc Diffusion Method

With the help of disc diffusion method on SDA medium, the extracts were screened for antifungal activity. For this, SDA medium was placed into the Petri plate and following the solidification of the medium; the fungal suspension was placed using a sterile swab. Samples were diluted for 3 different concentrations, namely, 1000 µg/ml, 750 µg/ml, and 500 µg/ml. Amphotericin-B was taken as positive control. Sterile discs containing three different concentrations of samples and positive control of 20 µl each were kept in SDA plates with a 24 h incubation period at 37°C. The diameter of inhibition zone was estimated to determine the antifungal activity.

RESULTS

The antibacterial activity of different concentrations of *C. dactylon* methanol extract is depicted in Table 1. All the three concentrations of methanol extract of *C. dactylon* showed good efficacy against all 5 bacterial species. However, at 1000 µg/ml it was most effective against *Salmonella* and *S. aureus* sps. exhibiting a 14 mm diameter of zone of inhibition (Figure 1).

The antibacterial activity of different concentrations of *C. dactylon* n-butanol extract is shown in Table 2. n-butanol extract of *C. dactylon* at all 3 concentrations showed good efficacy against *E. coli* and *P. aeruginosa* bacterial species. Figure 2 shows the inhibition zone diameters for different organisms, and it is maximum for *Pseudomonas* and *E. coli* species measuring 25 mm and 23 mm, respectively.

The antifungal activity of different concentrations of methanol extract of *C. dactylon* is described in Table 3. Figure 3 shows the inhibition zone diameters for different organisms. All

the three concentrations of methanol extract of *C. dactylon* showed good efficacy against all selected fungal species. At 1000 µg/ml concentration, it exhibits maximum efficacy

Table 1: Susceptible bacterial organisms to methanol extract with inhibition zone diameters (antibacterial activity)

Organisms	Zone of inhibition (mm)			Antibiotic (1 mg/ml)
	Concentration (µg/ml)			
	1000	750	500	
<i>E. coli</i>	12	11	9	20
<i>Salmonella</i> sps.	14	11	10	25
<i>Pseudomonas</i> sps.	12	11	10	31
<i>S. aureus</i>	14	12	9	22
<i>Bacillus</i> sps.	12	12	10	22

E. coli: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*

Table 2: Antibacterial activity of n-butanol extract with inhibition zone diameters for different organisms

Organisms	Zone of inhibition (mm)			Antibiotic (1 mg/ml)
	Concentration (µg/ml)			
	1000	750	500	
<i>E. coli</i>	23	21	16	24
<i>Salmonella</i> spp.	-	-	-	23
<i>Pseudomonas</i> spp.	25	23	9	29
<i>S.aureus</i>	9	-	-	22
<i>Bacillus</i> spp.	-	-	-	22

E. coli: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*

Table 3: Antifungal activity of methanol extract with inhibition zone diameters for different organisms

Organisms	Zone of inhibition (mm)			Antibiotic (1 mg/ml)
	Concentration (µg/ml)			
	1000	750	500	
<i>Aspergillus</i> spp.	17	10	9	47
<i>Candida</i> spp.	9	8	-	20
<i>Penicillium</i> spp.	18	17	15	47
<i>Trichoderma viride</i>	10	9	8	46

T. viride: *Trichoderma viride*

Table 4: Susceptible fungal species to n-butanol extract with inhibition zone diameters (antifungal activity)

Organisms	Zone of inhibition (mm)			Antibiotic (1 mg/ml)
	Concentration (µg/ml)			
	1000	750	500	
<i>Aspergillus</i> spp.	11	9	7	47
<i>Candida</i> spp.	9	8	-	20
<i>Penicillium</i> spp.	10	9	9	46
<i>Trichoderma viride</i>	12	11	8	46

T. viride: *Trichoderma viride*

against *Penicillium* and *Aspergillus* sps. with 18 mm and 17 mm diameters of zone of inhibition, respectively.

The antifungal activity of different concentrations of *C. dactylon* n-butanol extract is represented in Table 4. Figure 4 shows the inhibition zone diameters for different organisms. All the three concentrations of n-butanol extract of *C. dactylon* showed good efficacy against all fungal species. n-butanol extract showed maximum activity against *T. viride* and *Aspergillus* sps. at 1000 µg/ml concentration.

DISCUSSION

In the present study, it was found that methanol extract of *C. dactylon* at all three concentrations, namely, 1000 µg/ml, 750 µg/ml, and 500 µg/ml had wide antibacterial spectrum and being most effective against *Salmonella* and *S. aureus* sps. at a concentration of 1000 µg/ml. In contrast to this n-butanol extract was observed to be efficacious against *E. coli* and *P. aeruginosa* bacterial species at all the three concentrations. When evaluated for the antifungal activity, the methanol extract demonstrated maximum efficacy against *Penicillium* and *Aspergillus* sps. at 1000 µg/ml concentration. While the n-butanol extract showed good activity against *T. viridae* and *Aspergillus* sps at 1000 µg/ml concentration.

The wide antibacterial spectrum observed with all the three concentrations of methanol extract of *C. dactylon* is synonymous with results of similar studies in the past, and it is due to the prevalence of active principles such as polar compounds like saponins which makes it effective against a wide range of bacterial species.^[11,12] The results of methanol extract showing maximum efficacy against *Salmonella* and *S. aureus* sps. at 1000 µg/ml (Figure 1) is partially homologous with the results from another study which showed that it was effective against *S. aureus* and *P. aeruginosa*.^[6] Unlike the wide antibacterial spectrum of activity of methanol extract, the n-butanol extract of *C. dactylon* at all 3 concentrations showed good efficacy against *E. coli* and *P. aeruginosa* bacterial species which is exactly synonymous with the results of another study.^[13] It is known that since the cell wall of gram-negative organisms possesses a thick murine coat, they show more antibiotic resistance than gram-positive organisms.^[14-16] However, in contrast to this finding in our study, we found the n-butanol extract to be more sensitive for gram-negative organisms such as *E. coli* and *P. aeruginosa* which may be attributed to the occurrence of broad spectrum antibiotic compounds such as terpenes, flavonoids, and saponins in the extract of *C. dactylon* leaves which is in accordance with a previous study.^[13] When explored for the antifungal activity of *C. dactylon*, methanol extract showed maximum efficacy against *Penicillium* and *Aspergillus* sps. which were slightly different from the results of a previous study which depicted higher efficacy against *Aspergillus* and *Candida* sps.^[6] The n-butanol extract of *C. dactylon* showed maximum activity against *T. viridae* and *Aspergillus* sps.

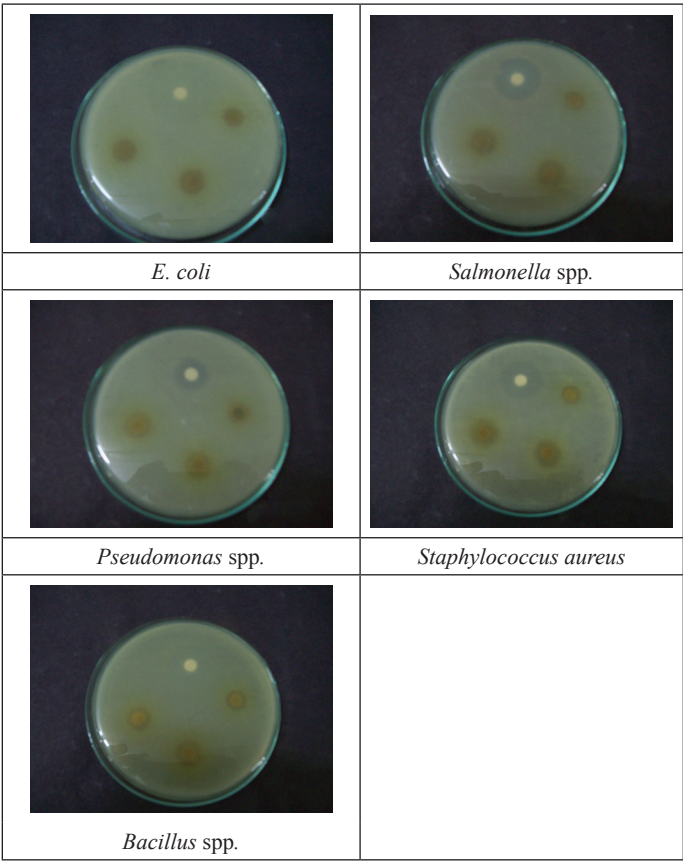


Figure 1: Antibiotic sensitivity tests of methanol extract of *Cynodon dactylon*

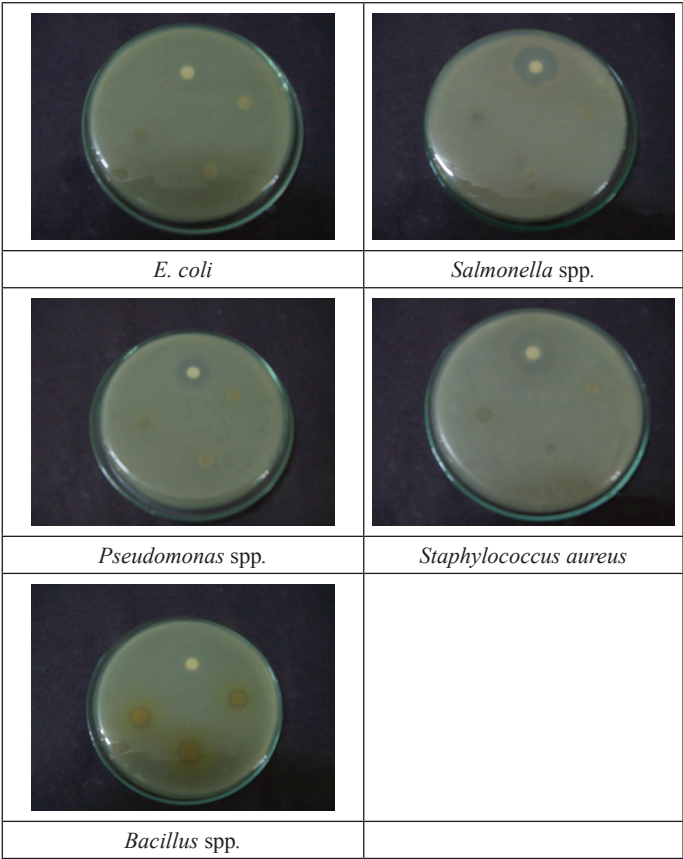


Figure 2: Antibiotic sensitivity tests of n-butanol extract of *Cynodon dactylon*

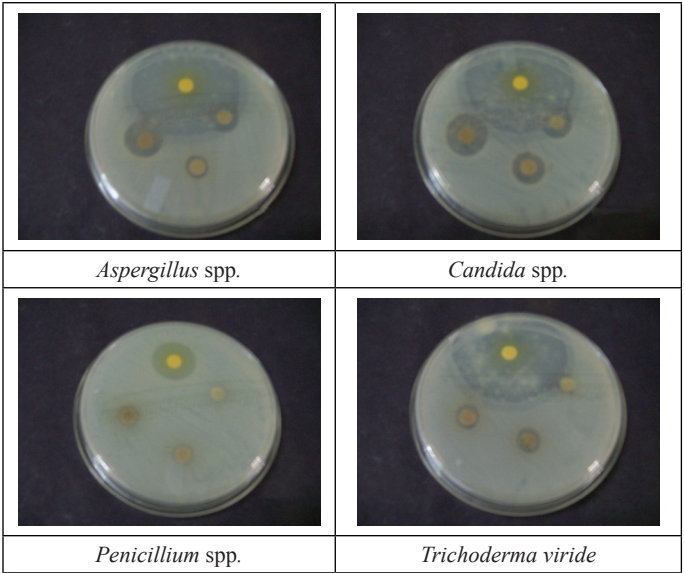


Figure 3: Antifungal sensitivity tests of methanol extract of *Cynodon dactylon*

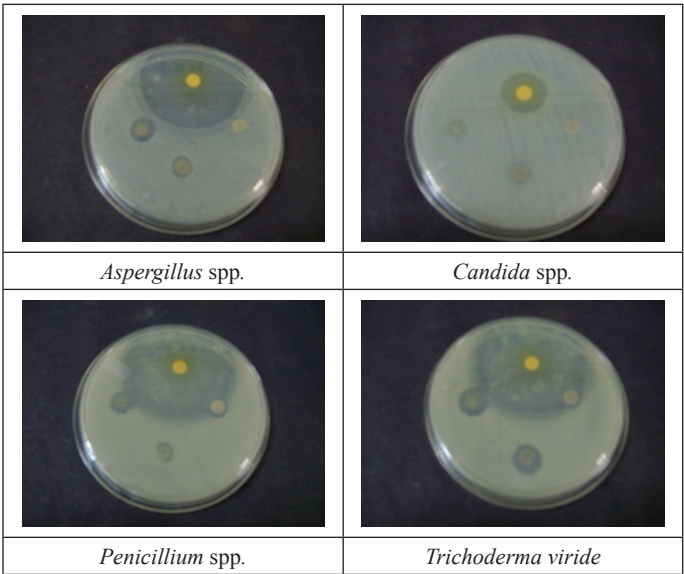


Figure 4: Antifungal sensitivity tests of n-butanol extract of *Cynodon dactylon*

at 1000 µg/ml concentration. Previous studies on ethanol extract of *C. dactylon* have demonstrated antifungal activity attributed due to the presence of triterpenoid saponin.^[17] However, to the best of our knowledge with an extensive literature review, the screening of n-butanol extract of this plant for its anti-fungal activity is lacking.

The results of our study suggested that leaves of *C. dactylon* possess significant antibacterial and antifungal activities. However, it necessitates further extensive molecular and cellular level investigations to evaluate the therapeutic effect of phytochemicals present in *C. dactylon* and to identify its mechanism of action, following which it can serve as a valuable therapeutic option for bacterial and fungal infections.

CONCLUSION

Methanol and n-butanol extracts of *C. dactylon* leaves have potential antibacterial and antifungal activity, particularly against *Salmonella*, *Staphylococcus*, *E. coli*, *Pseudomonas*, and *Aspergillus*, *Penicillium*, *T. viridae*, *Candida* sps. respectively and may serve to play a vital role in ethnomedical practice.

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