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Phytochemical Screening and Antibacterial Activity of *Cynodon dactylon* Extracts against *Escherichia coli*

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Authors' contributions

This work was carried out in collaboration with all authors. Author SN designed the study, laboratory experiments, managed the literature searches, wrote the protocol, wrote the first draft of the manuscript and managed the analysis of the study. Author IN participated in the study design, statistical data entry and analysis, laboratory quality assurance, and wrote the first draft of the manuscript. Author JT participated in study design, drafted and critically supervised and reviewed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the phytochemical constituents and antibacterial potential of *Cynodon dactylon* leaf extracts against *Escherichia coli.*
Study Design: Experimental short-term prospective study.
Place and Duration of Study: The study was conducted in Kampala International University-Western Campus Teaching Hospital (KIU-WCTH), Microbiology Laboratory in Bushenyi District, Western Uganda, between June to December 2017.

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Methodology: The presence of phytochemical constituents was determined using standard method whereas the antibacterial activity of *C. dactylon extracts* against susceptible *E. coli* ATCC 25922 and resistant *E. coli* BAA-2469 was determined by agar well diffusion method.
Results: The *C. dactylon* extract was found to contain flavonoids, saponins, tannins, steroids and glycosides except for the absence of Alkaloids in both extracts. The *C. dactylon* ethanol and aqueous extracts showed antibacterial activity against susceptible *E. coli* ATCC 25922 and resistant *E. coli* BAA-2469. Ethanol extract of *C. dactylon* had a mean zone of inhibition of 24.3±0.6 mm and 20.3±0.6 mm against the susceptible and resistant strains of *E. coli* respectively and aqueous extract exhibited a mean zone of inhibition of 19.3±0.6 mm and 16.3±0.6 against the susceptible and resistant strains of *E. coli* respectively as compared to the standard positive control (ciprofloxacin) which showed a mean zone of inhibition of 47.5±0.9 mm and 43.2±0.3 mm against the susceptible and resistant strains of *E. coli* respectively.
Conclusion: The *C. dactylon* extract was found to contain flavonoids, saponins, tannins, steroids and glycosides except for the absence of Alkaloids in both extracts. The *C. dactylon* ethanol and aqueous extracts showed antibacterial activity against the susceptible *E. coli* ATCC 25922 and resistant *E. coli* BAA-2469.

Keywords: Phytochemical; Cynodon dactylon; E. coli; antibacterial potential.

1. INTRODUCTION

Diarrheal diseases caused by bacterial pathogens are significant causes of morbidity and mortality worldwide, especially in developing countries [1]. Children and young adults are the most affected, particularly in regions with limited resources and poor hygienic measures [2,3]. Infectious diarrhoea due to consumption of vegetables contaminated through insufficiently-treated water, use of fertilisers and foods contaminated with wastes of human or animal origin has been estimated to be responsible for 25-75% of all childhood illnesses in Africa [2,4]. The causes of diarrhoea in endemic areas include a wide variety of bacteria, viruses, and parasites. Bacteria such as *Campylobacter* sp*., Salmonella* sp*., Shigella* sp*.,* and different groups of enteropathogenic *E. coli* are known to cause gastrointestinal diseases worldwide [2].

Antimicrobial drugs play a significant role in decreasing illness and death associated with microbial diseases in food animals and humans [5]. However, there is a global concern on the emergence and spread of antimicrobial resistance (AMR) in both pathogenic and commensal microorganisms; and presents a significant threat to the control of infectious diseases [5,6]. The emergence of AMR is a complicated process involving the interplay of humans, environmental and pathogen-related factors and transmission routes of resistant bacteria as well as resistance genes and the impact of antimicrobial selective pressures in several reservoirs [7,8]. The primary driving force behind the emergence and spread of AMR in pathogenic and commensal bacteria are due to irrational use of antimicrobial drugs in both human and veterinary medicine [9,10].

The search for alternative antimicrobial drugs of plant origin is due to the fact that they contain multiple biochemical compounds to which microbes cannot develop resistance simultaneously [9]. The indiscriminate and irrational use of antibiotics has led to the evolution of new resistant strains of bacterial pathogens hence the need to search for alternative and effective antimicrobial agents. Although the drug resistance development by microbes cannot be stopped, appropriate use of more efficient antibiotics including natural plant products may reduce the mortality and health care costs [10,11]. Therefore, the study aimed to determine the phytochemical constituents and antibacterial activity of *C. dactylon* ethanol and aqueous extracts against the susceptible *E. coli* ATCC 25922 and resistant *E. coli* BAA-2469.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

The leaves of *C. dactylon* were collected from Bushenyi District, South Western Uganda. Plant identification using plant shoots with leaves and flowers was carried out at the Department of Botany, Mbarara University of Science and Technology located at Latitude: 0° 37' 0.59" N, Longitude: 30° 39' 14.39" E in Mbarara District, Western Uganda. Voucher specimen (SN 001) was collected by plucking from the mature plants and deposited at the Kampala International University-Western Campus (KIU-WC) Herbarium.

2.1.1 Preparation and extraction of plant material

The Sorted leaves were cleaned with distilled water and air dried under room temperature (30±2°C) in the pharmacy laboratory, KIU-WC. Dried leaves were pulverized using a blender. Powdered sample material was packaged in clean dry polythene bags and stored at room temperature up to extraction time.

Fig. 1. The picture of *Cynodon dactylon*

2.1.1.1 Ethanol extraction

A portion of 100 g of the *C. dactylon* fine leaf powder were dissolved in 250 ml of 70% ethanol kept inside a well-sealed container for 4 days and then filtered using Whatman No 1 (10 mm diameter circles) filter paper. The filtrates were evaporated in the oven at 40°C and the crude extracts kept in well-sealed containers under refrigeration at 4°C [12].

2.1.1.2 Aqueous extraction

The aqueous extract of the *C. dactylon* was prepared by soaking 100 g of dried fine leaf powder in 200 ml of sterile distilled water and kept inside a well-sealed container for 4 days. The extract was filtered using Whatman filter paper No 1 (10 mm diameter circles) and the crude extracts kept under refrigeration at 4°C.

2.2 Phytochemical Screening of the *C. dactylon* Extracts

The extracts of *C. dactylon* were analyzed separately for the presence of alkaloids, flavonoids, saponins, tannins, steroids and glycosides as follows. Chemical tests were carried out on the aqueous and ethanol extracts using standard procedures to identify the constituents as previously described [13-18].

2.3 Preparation of Bacterial Inoculum

The bacterial inoculum was prepared by suspending a loopful of a pure culture in sterile normal saline and the turbidity adjusted to match 0.5 McFarland standards; that is, about $1.5x10^8$ CFU/ml. The test strains of susceptible *E. coli* ATCC 25922 and resistant *E. coli* BAA-2469 were obtained from the Department of Microbiology and Immunology, KIU-WC.

2.3.1 Screening for antibacterial activity of *C. dactylon* extracts

The antibacterial activity of *C. dactylon* extracts were screened against susceptible *E. coli* ATCC 25922 and resistant *E. coli* BAA-2469 by the agar well diffusion method using Mueller-Hinton agar (Oxoid, UK) with slight modifications [19]. A total of 4 mm diameter wells were punched into the agar using sterile borer and filled with 40µl of the plant extract reconstituted with Dimethyl sulfoxide (DMSO) to various concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml). The plates were then incubated at 37°C for 18 to 24 hrs. Ciprofloxacin was used as positive control in the assay, while DMSO was the negative control. The antibacterial activity was expressed as the zone of inhibition in millimeters, which is measured with a zone reader (Vernier caliper and ruler).

2.4 Data Analysis

The data obtained from measured inhibition zone diameters were entered and analyzed using Statistical Package for the Social Sciences (SPSS) version-20. Descriptive statistics were computed to obtain mean and standard deviations of inhibition zone diameters exhibited by the plant extract and controls. The antibacterial activity was reported in terms of diameters of the zones of inhibition (mm). The data was presented as mean ± standard deviation (SD). One way ANOVA and Tukey's Post hoc analysis were used to establish statistical difference in the antibacterial activity of extracts and control. Statistical significance was considered at 95% level of confidence.

3. RESULTS

The results for phytochemical analysis showed that ethanol extract had more secondary metabolites than the aqueous extract. The phytochemical screening (qualitative) of *C. dactylon* leaf extracts showed the presence of

Table 1. Qualitative analysis of the phytochemical constituents of *C. dactylon* leaf extract

SN	Phytochemical constituents	Tests	Ethanol extract	Aqueous extract
1	Alkaloids	Dragendroff's test.	-	-
2	Flavonoids	Shinoda test	+	+
3	Saponins	Frothing test	+	+
4	Tannins	Ferric chloride test	+	-
5	Steroids	Salkwoski's test	+	ND
6	Glycosides	Fehling's test	+	-

+: Present; -: Absent; ND: Not Done

flavonoids, saponins, tannins, steroids and glycosides but without Alkaloids in both extracts (above Table 1).

The ethanol extract showed a slightly higher zone of inhibition against susceptible *E. coli* ATCC 25922 (24.3±0.6 mm) as compared to resistant *E. coli* BAA-2469 (20.3±0.6 mm), this was statistically significant (*p = .001*). Similarly, the aqueous extract exhibited a significantly (*p = .001*) higher antibacterial against susceptible *E. coli* ATCC 25922 (19.3±0.6 mm) as compared to resistant *E. coli* BAA-2469 (16.3±0.6 mm). The ethanol extract showed a higher antibacterial activity against resistant *E. coli* (20.3±0.6 mm) compared to the aqueous extracts (16.3±0.6 mm). This was statistically significant (*p = .001*) as shown in Table 2.

The positive control (Ciprofloxacin) exhibited the largest zone of inhibition against susceptible *E. coli* (47.5±0.9 mm) and resistant *E. coli* (43.2±0.3 mm). This was statistically different (*p = .001*) from that exhibited by both the ethanol and aqueous extract of *C. dactylon.*

4. DISCUSSION

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 basis for the development of drugs [19]. In this study, the findings of the phytochemical analysis of *C. dactylon* ethanol and aqueous extracts showed the presence of phytochemical constituents (i.e. flavonoids, saponins, tannins, steroids, and glycosides) except for the absence of Alkaloids in both extracts (Table 1). The findings of this study are in accordance with other previous studies [19-21]. The important phytochemical constituents such as, flavonoids, luteolin carotenoids, glycosides, phytosterols, saponins and volatile oils were reported from *C. dactylon* [22]. The age of the plant, percentage humidity of the harvested material, situation and time of harvest, and the method of extraction are possible sources of variation for the chemical composition, and bioactivity of the extracts [21,23].

There is a growing interest in traditional remedies utilising plant products among rural communities in developing countries for management of various diseases, in the absence of an efficient primary health care system [10,24,25]. In the present study, *C. dactylon* ethanol and aqueous extracts showed antibacterial activity against the susceptible *E. coli* ATCC 25922 and resistant *E. coli* BAA-2469 (Table 2). The results of this study are in accordance with other previous studies on other medicinal plants [24-26]. The observed antibacterial activity could be attributable to the phytochemical constituents of *C. dactylon*. The secondary metabolites (Phytochemical constituents) of plants are known to act by different mechanisms and exert antimicrobial activity [19]. Tannins bind to proline-rich proteins and interfere with the protein synthesis [27].

Table 2. Antibacterial activity of *C. dactylon* ethanol and aqueous extracts

Extracts	Mean inhibition zone diameters ±SD (mm)	
	Susceptible *E. coli* ATCC 25922	**Resistant *E. coli* BAA-2469**
Ethanol	24.3±0.6	20.3±0.6
Aqueous	19.3±0.6	16.3±0.6
Ciprofloxacin, 5µg	47.5±0.9	43.2±0.3
DMSO	0	0
P-value	*P = .001**	*P = .001**

**Statistically significant (p < 0.05), DMSO- Dimethyl sulfoxide*

Flavonoids activity is probably due to their ability to complex with extracellular soluble proteins and bacterial cell walls [28]. Antimicrobial activity of saponins is due to its ability to cause leakage of proteins and specific enzymes from the cell [29]. Steroids associate with membrane lipids and exert its action by causing leakages from liposomes [30,31]. Therefore, the traditional use of plant *C. dactylon* for the management of infectious diseases caused by bacteria is promising.

5. CONCLUSION

The *C. dactylon* ethanol extract was found to contain flavonoids, saponins, tannins, steroids and glycosides whereas the aqueous extract was found to contain flavonoids, and saponins except for the absence of Alkaloids in both extracts. This study revealed that ethanol and aqueous extracts of *C. dactylon* have antibacterial activity against *E. coli*-ATCC 25922 and *E. coli* BAA-2469. Further research studies to isolate, identify, characterize and elucidate the structure of the bioactive compounds of *C. dactylon* extracts to explore more in molecular level approach against various bacterial diseases.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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