Antidiarrheal, antimicrobial, and toxic effects of the aqueous and methanolic leaf and fruit extracts of *Cucumis dipsaceus* (Ehrenb. Ex Spach.)

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**A B S T R A C T**

**Introduction:** *Cucumis dipsaceus* is used to treat diarrhoea, microbial infections, among other diseases across the world; however, there is insufficient empirical data to validate its efficacy, toxicity, and safety. Accordingly, we investigated the antidiarrheal, antimicrobial, and toxic effects of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus*.

**Methods:** Antidiarrheal activities of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* were investigated using the castor oil-induced diarrhoea technique in a Wistar rat model. The disk diffusion and broth microdilution methods were adopted to determine the antimicrobial activities of the studied plant extracts. The acute oral toxicity effects of the studied plant extracts were investigated in Wistar rats according to the Organisation for Economic Co-operation and Development (OECD) guidelines.

**Results:** The aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* significantly (*P* < 0.05) inhibited diarrhoea in a dose-dependent manner in experimental rats. Besides, the studied extracts significantly (*P* < 0.05) inhibited the growth of *Salmonella enteritidis*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Candida albicans* in varying degrees, as depicted by their growth inhibition zones (>6.00 mm) and minimum inhibitory concentrations (MICs <1000 µg/mL). Moreover, the studied extracts did not cause any observable acute oral toxicity effects in the experimental rats across the 14-day experimental period.

**Conclusion:** The aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* present a potential source of safe and efficacious lead compounds for developing antidiarrheal and antimicrobial therapies.

**Implication for health policy/practice/research/medical education:**
This research article valorises *Cucumis dipsaceus* as a potential source of efficacious and safe antidiarrheal and antimicrobial lead compounds.


**Introduction**

Microbial infections of the gastrointestinal tract cause gastric irritation, inflammation, and impaired gastric functioning, manifesting in abdominal discomfort and diarrhoea (1–3). Diarrhoea is among the leading causes of morbidity and mortality worldwide, especially in children, the elderly, and immunocompromised persons (4). Despite the availability of conventional antimicrobial and antidiarrheal drugs, the global public health burden of microbial infections is still high (4). The emergence of antimicrobial-resistant strains of bacteria and fungi has further complicated the successful use of chemotherapy (5).

The presently used antimicrobial agents cause undesirable effects, including constipation, gastric irritation, cardiotoxicity, hepatotoxicity, nephrotoxicity, low efficacy, among other adverse effects (6). Besides, the conventional antidiarrheal drugs are associated with...
constipation, impaired gastric motility, among other life-threatening side effects (7). Besides, these drugs are costly, inaccessible, and unavailable, especially in rural areas of developing countries, where over 80% of the global burden of disease lies, due to inadequate and insufficient healthcare systems and resources (8). Therefore, there is a need for alternative antimicrobial and antidiarrheal therapies, which are efficacious, safe, accessible, and affordable, to avert human suffering, especially in developing nations.

Medicinal plants present a viable source of potent therapies for microbial infections and diarrhoea, among other diseases, due to their rich ethnomedical application history and the diverse array of bioactive principles they contain (9-11). Previously, the use of medicinal plants was based on crude preparations to manage various diseases; however, with the advent of modern technology, various plant-derived drugs have been developed into present-day drugs such as creams, ointments, injections, capsules, and tablets (12). Despite the development of conventional drugs for various diseases, the usage of medicinal plants to treat various diseases is still dominant, due to their easy availability, accessibility, and relative affordability, especially in sub-Saharan Africa (13). The World Health Organization (WHO) report indicates that more than 80% of people, especially in low and medium-income countries, depend on traditional medicine, and over 85% of the medicines are obtained from plants (14).

Various ethnic communities in Kenya utilise plant-based remedies to prevent and treat diseases (15-17). *Cucumis dipsaceus* is widely used to treat bacterial infections, diarrhoea, inflammatory diseases, among other functions, including consumption as food (17). In Kenya, a concoction of boiled roots is used to treat abdominal pains among the Keiyo residents (17). Other ethnomedical reports indicate that the leaf extracts of *C. dipsaceus* have antimicrobial activity (16), and its preparations possess anti-inflammatory, analgesic, diuretic, anti-dysentery, antidiabetic, anti-diarrhoea, among other medicinal properties (18,19). However, despite the extensive utilisation of *C. dipsaceus* in traditional medicine to manage microbial infections and diarrhoeal, especially in Kenya, there is scanty empirical evidence to support the claimed pharmacologic efficacy and safety. Accordingly, we investigated the antimicrobial, anti-diarrhoeal and toxic effects of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* to lay a framework for validating their usage and developing potent, safe, accessible, and affordable therapies.

**Materials and Methods**

**Collection and preparation of plant materials**

Fresh leaves and fruits of *Cucumis dipsaceus* were collected from cultivated regions in the Nkando sub-location, Mbaaria Location, Kiirua division, Buuri sub-County in Meru County Kenya (GPS co-ordinates: 1.12°N; 37.3147°S) in February 2020 during the hot and dry season. The plant was identified by a local herbalist as ‘*Kagerema*’ and selected for the current study based on its ethnomedical information. Voucher specimens were prepared, taxonomically identified, and authenticated at the East Africa Herbarium of the National Museums of Kenya (NMK/BOT/CTX1/8) and archived for future reference. The gathered leaves and fruits were transported to our research laboratories for processing and analysis. The materials were washed gently to remove dirt and were spread on a bench to dry at room temperature (25°C) for four weeks, with occasional grabbing for maximum aeration. The dried leaves and fruits were ground separately into a coarse powder using an electric plant mill, weighed, and packaged in labelled khaki envelopes awaiting extraction.

**Extraction methods**

The cold and hot maceration methods using HPLC-grade methanol and distilled water, respectively, were adopted to obtain the methanolic and aqueous leaf and fruit extracts of *C. dipsaceus*, respectively, following standard procedures described by Harborne (20) and modified by Moriasi et al (21).

**Methanolic extraction procedure**

The cold maceration procedure was followed. Briefly, 400 g of the dry leaf and fruit powders of *C. dipsaceus* were separately soaked in 1 litre of HPLC-grade methanol in 1.5-L conical flasks, shaken, and covered with an aluminium foil in separate setups. The respective mixtures were allowed to extract for 48 hours with regular shaking, after which they were individually filtered through cotton wool rolls into separate clean conical flasks. The resultant filtrates were filtered again through Whatman filter papers (No. 1) into separate round-bottomed flasks and concentrated *in vacuo* using a rotary evaporator at 50°C. The resultant extracts were transferred into well labelled pre-weighed sample bottles and completely dried in a hot air oven set at 35°C. Then, the percentage yields of the respective methanolic leaf and fruit extracts of *C. dipsaceus* were calculated according to the equation (Eq. 1) described by Truong et al (22).

\[
\text{Yield} = \frac{\text{Weight of macerated powder}}{\text{Weight of sample bottle + extract}} \times 100
\]

Eq. (1)

**Aqueous extraction procedure**

The hot maceration method was used for aqueous extraction. Briefly, 400 grams of the respective dried leaf and fruit powders of *C. dipsaceus* were soaked in 2.75 L of distilled water and heated in a water bath at 60°C for two hours. The mixtures were individually filtered.
through rolls of cotton gauze and then through Whatman filter papers (No. 1) into separate flasks. The respective filtrates were subdivided into 200 mL portions and transferred into freeze-drying flasks, which were covered with carbon ice and acetone. The respective flasks, which were labelled appropriately, were then fitted into a freeze dryer and lyophilised for 48 hours. The obtained extracts of the respective plant parts were transferred into clean labelled and pre-weighed sample bottles, weighed and the respective percentage yields determined using the formula in section 2.2.1 (Eq. 1).

**Experimental animals**

In this study, adult, nulliparous, and nonpregnant female Wistar rats aged 8-12 weeks were obtained from the animal breeding facility of our institution. The animals were used as models to investigate the antidiarrheal and acute oral toxicity effects of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus*. They were housed in polypropylene cages supplemented with softwood shavings as bedding material and offered clean drinking water and standard rodent pellets *ad libitum*.

All the experimental animals were acclimatised to laboratory conditions (temperature: 25 ± 2°C; relative humidity: 55%-61%; 12 hours of dark and 12 hrs of light cycle) for 7 days before experimentation. The animals were handled humanely and manipulated according to the guidelines described by the OECD (23), the National Research Council (24), and the Biosafety, Animal Use and Ethics Committee (BAUEC) of our Institution.

**Preparation of experimental dosages**

Following pilot studies, three dose levels of 100, 200, and 400 mg/kg BW were selected to determine antidiarrheal activities of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus*. The extract doses for acute oral toxicity assay (175, 550, and 2000 mg/kg BW) were selected based on the OECD guideline number 425 (23). In all cases, the stock doses of the studied plant extracts were prepared according to the OECD guidelines (23), illustrated by Erhirhie et al (25), and diluted serially to achieve the required doses for administration.

**Determination of the antidiarrheal activity of the studied plant extracts**

The castor-oil-induced diarrhoea method of Rahman et al (26) was followed in this study. Briefly, experimental rats were fasted for 18 hrs and divided randomly into 5 groups (n = 5). Groups I-III were orally administered with the studied plant extracts at doses of 100, 200, and 400 mg/kg BW, respectively. Group IV rats were treated with 3 mg/kg BW of loperamide (the positive control group). Group V rats were administered with 10 mL/kg BW of normal saline and represented the negative control of the experiment. After one hour, all the experimental rats were orally treated with 1 mL of castor oil and kept in separate metabolic cages lined with absorbent papers. The frequency of defecation (average number of wet diarrhoeal droppings) was recorded and used to determine the percentage inhibition of diarrhoea in experimental rats according to the equation (Eq. 2) described by Teferi et al (27).

\[
%\text{Inhibition of diarrhoea} = \frac{\text{DDNC} - \text{DDT}}{\text{DDNC}} \times 100 \tag{2}
\]

Where DDNC: Diarrhoeal droppings in the negative control rats; DDT: Diarrhoeal droppings in the test (Extract-treated or loperamide-treated) rats.

**Determination of the antimicrobial activity of the aqueous and methanolic leaf and fruit extracts of C. dipsaceus**

**Microbial strains**

The antimicrobial activity of the studied plant extracts was investigated using *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 15442), *Salmonella enteritidis* (ATCC 13076), *Bacillus subtilis* (ATCC 6051), and *Candida albicans* (ATCC 10231) based on their clinical relevance, especially in initiating or exacerbating diarrhoea. They were retrieved from the microbiology laboratory of our institution.

**Preparation and standardisation of microbial inocula**

The bacterial strains used in this study (*E. coli, P. aeruginosa, S. enteritidis* and, *B. subtilis*) were sub-cultured in Mueller-Hinton agar for 24 hours according to the antimicrobial susceptibility testing procedures recommended by the Clinical and Laboratory Standards Institute (CLSI) (28). After that, the bacterial colonies were isolated and standardised using normal saline to achieve a turbidity corresponding to 0.5 McFarland scale of about 1-2×10⁶ colony forming units per milliliter (cfu/mL) using a spectrophotometer (530 m). The obtained inocula were used to inoculate the discs in the disc diffusion technique and the broth microdilution assay to determine the minimum inhibitory concentrations (MICs).

Similarly, the fungal strain (*C. albicans*) was sub-cultured in Sabouraud dextrose agar (SDA; Oxoid) for 24 hours and standardised for experimentation as per the previously described method (27). Then, the colonies were isolated and standardized using normal saline to achieve a 0.5 McFarland equivalent of 1-5×10⁵ cfu/mL using a spectrophotometer (530 nm) (28). The prepared inoculum was used for the disc diffusion and broth microdilution experiments, accordingly.

**Preparation of extracts**

The aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* were weighed accurately (1 mg) and dissolved in 10 ml of 1.4 % of dimethyl sulfoxide (DMSO) in clean labelled 15 mL Eppendorf tubes. The respective tubes...
were then vortexed vigorously for 30 minutes to ensure complete dissolution. The stock solutions were then diluted two-fold by applying the serial dilution technique to obtain lower concentrations of 50, 25, 12.5, 6.25, and 3.125 µg/mL, respectively.

The disc diffusion assay
Circular disks measuring 6 mm in diameter were prepared by poking Whatman filter papers (No. 1) and sterilizing in an autoclave at 121°C, for 15 minutes. The discs were then arranged appropriately on the solidified media in Petri dishes (5 per plate). Markings were made at the bottom of each plate to identify the respective discs, type of treatment applied, and the microbe strain under study. Then, using a sterile micropipette, 20 µL of each studied plant extract, at respective concentrations, were aspirated and dispensed carefully onto respective discs. The discs were then gently but firmly pressed onto the media inoculated with 1 mL of the respective microbial strains used in this study to ensure proper contact. The experiments were set up in triplicate, with DMSO as the negative control and ciprofloxacin (for bacterial strains) and nystatin (for the fungal strain) as positive controls. The plates were incubated at 37°C for 24 hours in an incubator, and the inhibition zones of microbial growth were measured using a digital zone reader in millimeters (mm) (28).

Determination of the minimum inhibitory concentration
The broth microdilution method (28) modified by Golus et al (29) was used to determine the MIC of the aqueous and methanolic leaf and fruit extracts of C. dipsaceus, following the disc diffusion assay. Briefly, 10 µL of the respective plant extracts prepared previously (section 2.5.3) were transferred into clean and labeled Eppendorf tubes containing 90 µL of molten Mueller-Hinton broth in triplicate and vortexed gently. Then, 200 µL of the molten agar containing the studied plant extracts were aspirated using a micropipette, dispensed into sterile 96-U-shaped multiwell plates, and micro-diluted serially in two-fold, at 100 µL volumes. The respective positive (ciprofloxacin/nystatin) and negative controls were also included in each microtitre plate using the same procedure. Afterward, 2 µL of respective inocula containing approximately 10^4 cfu were carefully dispensed into each well using a multichannel micropipette and allowed to interact with the treated media for 10 minutes at room temperature. The wells around the multiwell plates were added sterile water to moisture the plates and avoid dehydration during incubation. The plates were then covered in nylon zip-lock bags and incubated at 37°C for 18 hours. After that, 2 µL of freshly prepared resazurin dye was added to each well, and plates were further incubated for 45 minutes at 37°C in an incubator. The plates were carefully examined visually based on the resazurin color changes, and the lowest concentration of the studied extracts and positive control drugs that completely inhibited microbial growth was considered the MIC according to the CLSI guidelines (28).

Investigation of acute toxicity effects of the aqueous and methanolic leaf and fruit extracts of C. dipsaceus
The up-and-down-procedure for acute oral toxicity study described by the OECD/OCDE (23) was followed. Briefly, six experimental rats were randomly selected and divided into two groups, each consisting of three rats (control group and experimental group) for each studied plant extract dose. The rats were labelled on their tails using a red permanent marker pen for easy identification and fasted for four hours before dosing.

An initial extract dose of 175 mg/kg BW of each studied extract was orally administered into respective experimental groups, and normal saline (10 mL/kg BW) was administered to the control group rats. After that, the experimental animals were individually monitored periodically for signs of toxicity by observing the presence or appearance of the mucous membrane, eyes, diarrhoea, skin fur, salivation, sleep, tremors, convulsions, coma, lethargy, sleep, and mortality after 30 minutes, 1 hour, 2 hours, 4 hours, 24 hours, 48 hours, 7 days and 14 days, respectively, as per the OECD guidelines (23), and recorded.

In the absence of toxicity symptoms or mortality, a new set of rats were orally administered with 550 mg/kg BW of the studied plant extracts and monitored in the same manner as for the 175 mg/kg BW-treated group. In the absence of acute oral toxicity signs or mortality, we administered the higher cut-off dose of 2000 mg/kg BW of the extracts to a new set of rats and observed them, accordingly, like in the two lower doses. The median lethal dose (LD₅₀) of the studied plant extracts was estimated to be >2000 mg/kg BW if no signs of toxicity or mortality were observed at the cut-off dose.

Statistical management and data analysis
Quantitative data obtained from the antidiarrheal and antimicrobial experiments were tabulated on an Excel spreadsheet (Microsoft 365) and then exported to GraphPad Prism statistical software version 9.3 for analysis. Descriptive statistics were performed, and the results were expressed as a mean ± standard error of the mean (SEM) of the replicate experiments. One-way or two-way ANOVA was performed, as appropriate, to determine significant differences among means, followed by Tukey’s post hoc test for pairwise comparisons and separations of means. During data analysis, P<0.05, P<0.001, and P<0.0001 were considered statistically significant, accordingly. Acute oral toxicity data were tabulated, and the respective median lethal concentrations of the studied plant extracts (LD₅₀ values) were determined and interpreted according
Antidiarrheal, antimicrobial, and toxic effects of *C. dipsaceus*

The aqueous fruit extract of *C. dipsaceus* significantly inhibited diarrhoea in castor oil-induced diarrhoea in rats in a positive dose-dependent manner (*P* < 0.0001; Figure 1). Notably, at a dose of 400 mg/kg BW of the aqueous fruit extract of *C. dipsaceus*, the percentage inhibition of diarrhoea was not significantly different from that produced by loperamide (the positive control drug) (*P* > 0.0001; Figure 1).

Similarly, the aqueous leaf extract of *C. dipsaceus* significantly inhibited castor oil-induced diarrhoea in Wistar rats in a positive dose-dependent manner (*P* < 0.0001; Figure 2). Loperamide (the positive control drug) produced a significantly higher percentage inhibition of castor oil-induced diarrhoea in experimental rats than the aqueous leaf extract of *C. dipsaceus* (*P* < 0.0001; Figure 2).

The results further revealed significant percentage inhibitions of castor oil-induced diarrhoea in experimental rats by the methanolic fruit extract of *C. dipsaceus* in a dose-dependent manner (*P* < 0.0001; Figure 3). However, the positive control drug (loperamide) showed a significantly higher percentage inhibition of castor oil-induced diarrhoea compared with the inhibitions caused by the methanolic fruit extract of *C. dipsaceus* (*P* < 0.0001; Figure 3).

Moreover, the methanolic leaf extract of *C. dipsaceus* significantly inhibited castor oil-induced defecation in rats in a dose-dependent fashion (*P* < 0.0001; Figure 4). The percentage inhibition of diarrhoea exhibited by loperamide was significantly higher than the inhibition of the methanolic leaf extract of *C. dipsaceus* at all the tested dose levels (*P* < 0.0001; Figure 4).

In this study, the percentage inhibitions of diarrhoea were compared among the studied plant extracts. The comparison results showed that at a dose level of 100 mg/kg BW, the aqueous leaf extract of *C. dipsaceus* exhibited a significantly higher percentage inhibition of diarrhoea than all the other extracts (*P* < 0.05; Figure 5). There was no significant difference between the percentage inhibitions caused by the aqueous fruit and methanolic fruit extracts of *C. dipsaceus* (*P* > 0.05); however, these inhibitions were significantly lower than those exhibited by the aqueous fruit extract.
and methanolic leaf extracts of *C. dipsaceus* (*P*<0.05; Figure 5).

In addition, no significant difference was observed between the aqueous and methanolic leaf extracts of *C. dipsaceus* at a dose of 200 mg/kg BW (*P*>0.05; Figure 5). However, the percentage inhibitions of diarrhoea showed that the aqueous and methanolic leaf extracts of *C. dipsaceus*, at a dose level of 200 mg/kg BW, were significantly higher than those of the aqueous and methanolic fruit extracts at the same dose (*P*<0.05; Figure 5). The aqueous fruit extract of the tested plant extracts showed a significantly lower percentage inhibition of diarrhoea than all other extracts, at a dose level of 200 mg/kg BW (*P*<0.05; Figure 5).

At a dose level of 400 mg/kg BW, the percentage inhibitions of defecation exhibited by the aqueous fruit and methanolic leaf extracts of *C. dipsaceus* were not significantly different (*P*>0.05; Figure 5); however, they were significantly lower than those produced by the other extracts (*P*<0.05; Figure 5). The methanolic fruit extract of *C. dipsaceus* produced a significantly higher inhibition of castor oil-induced diarrhoea at a dose level of 400 mg/kg BW than all the other tested plant extracts (*P*<0.05; Figure 5).

**Antimicrobial effects of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus***

The antimicrobial efficacy of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* on *S. enteritidis*, *E. coli*, *P. aeruginosa*, *B. subtilis*, and *C. albicans* was investigated.

The results revealed that the zones of *S. enteritidis* growth inhibition, produced by the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* at concentrations of 3.125, 6.25, and 12.5 µg/mL, respectively, were not significantly different (*P*>0.05; Table 1). However, the inhibition zones were significantly lower than those obtained at concentrations of 25 µg/mL, 50 µg/mL, and 100 µg/mL, respectively, depicting a concentration-dependent increase in inhibition zones (*P*<0.05; Table 1).

Besides, no significant differences in *S. enteritidis* growth inhibition by the studied plant extracts were observed at concentrations of 3.125, 6.25, 12.5, 25, and 50 µg/mL, respectively (*P*>0.05; Table 1). However, at a 100 µg/mL concentration, the aqueous leaf extract of *C. dipsaceus* produced a significantly bigger inhibition zone on *S. enteritidis* than the other extracts (*P*<0.05; Table 1). Notably, the standard drug (10 µg/mL Ciprofloxacin) produced significantly higher inhibition zones of *S. enteritidis* growth than all the tested plant extracts (*P*<0.05; Table 1).

The *E. coli* growth inhibition zones produced by the aqueous leaf and fruit, and methanolic leaf extracts of *C. dipsaceus*, at concentrations of 3.125, 6.25, and 12.5 µg/mL, were not significantly different (*P*>0.05; Table 2). Generally, a significant concentration-dependent increase in *E. coli* growth inhibition by all the studied plant extracts was observed in this study (*P*<0.05; Table 2). In addition, the methanolic fruit extract of *C. dipsaceus* produced significantly bigger *E. coli* growth inhibition zones, at all the tested concentrations except at 10 µg/mL, compared with the other extracts (*P*<0.05; Table 2). Ciprofloxacin
produced significantly bigger E. coli growth inhibition zones than all the tested plant extracts (P < 0.05; Table 2).

Table 1. Antimicrobial effects of the aqueous and methanolic leaf and fruit extracts of C. dipsaceus on S. enteritidis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (µg/mL)</th>
<th>AQL</th>
<th>AOF</th>
<th>MEL</th>
<th>MEF</th>
</tr>
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<td>C. dipsaceus</td>
<td>3.125</td>
<td>6.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td>6.25</td>
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<td></td>
<td>12.5</td>
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<td></td>
<td>25</td>
<td>9.00 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.33 ± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.33 ± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>50</td>
<td>11.33 ± 1.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.67 ± 0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.67 ± 0.33&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>35.67 ± 0.33&lt;sup&gt;e&lt;/sup&gt;</td>
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Values are expressed as mean ± SEM; means with different superscript alphabets within the same column, and those with different subscript alphabets within the same row are significantly different (P < 0.05; two-way ANOVA with Tukey’s post hoc test). AQL: Aqueous leaf extract of C. dipsaceus; AOF: Aqueous fruit extract of C. dipsaceus; MEL: Methanolic leaf extract of C. dipsaceus; MEF: Methanolic fruit extract of C. dipsaceus.

Table 2. Antimicrobial effects of the aqueous and methanolic leaf and fruit extracts of C. dipsaceus on E. coli

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<th>Treatment</th>
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<th>AOF</th>
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<td>100</td>
<td>12.33 ± 0.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>9.67 ± 0.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13.33 ± 0.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13.33 ± 0.33&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>10</td>
<td>40.00 ± 0.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>40.00 ± 0.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>40.67 ± 0.67&lt;sup&gt;e&lt;/sup&gt;</td>
<td>40.67 ± 0.67&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; means with different superscript alphabets within the same column, and those with different subscript alphabets within the same row are significantly different (P < 0.05; two-way ANOVA with Tukey’s post hoc test). AQL: Aqueous leaf extract of C. dipsaceus; AOF: Aqueous fruit extract of C. dipsaceus; MEL: Methanolic leaf extract of C. dipsaceus; MEF: Methanolic fruit extract of C. dipsaceus.

B. subtilis growth inhibition zones, at concentrations of 50 µg/mL and 100 µg/mL, compared with all the other extracts of similar concentrations in this study (P < 0.05; Table 5). Similarly, the aqueous fruit extract of C. dipsaceus showed significantly higher inhibitions of B. subtilis growth at 12.5 µg/mL and 25 µg/mL than the other extracts at the same concentrations (P < 0.05; Table 5). Ciprofloxacin produced significantly bigger inhibition zones than all other plant extracts (P < 0.05; Table 5).

The antifungal effects of the aqueous and methanolic leaf and fruit extracts of C. dipsaceus on C. albicans were also investigated in this study. The results showed a significant concentration-dependent increase in C. albicans growth inhibition by the aqueous leaf extract of C. dipsaceus (P < 0.05; Table 5). There were no significant differences in C. albicans growth inhibition by the aqueous fruit, and methanolic leaf and fruit extracts of C. dipsaceus were observed in this study (P > 0.05; Table 5). Notably, the zone of inhibition of C. albicans growth produced by the aqueous leaf extract of C. dipsaceus at a 100 µg/mL concentration was not significantly different from that produced by nystatin in this study (P > 0.05; Table 5).

Furthermore, the minimum concentrations of the studied plant extracts and standard drugs that could inhibit...
the growth of the selected microbial strains (Minimum inhibitory concentration: MIC) were determined in this study (Table 6). The aqueous fruit extract of *C. dipsaceus* showed the lowest MIC of 1.563 µg/mL against *P. aeruginosa* compared with other extracts. On the other hand, the aqueous leaf extract of *C. dipsaceus* showed the lowest MIC (3.125 µg/mL) against the fungal strain (*C. albicans*), while the other extracts were inactive (Table 6). In addition, the methanolic leaf extract of *C. dipsaceus* showed a low MIC of 6.25 µg/mL against *E. coli*, whereas the methanolic fruit extract showed lower MIC values (3.125 µg/mL) against *S. enteritidis* and *E. coli* (Table 6).

Acute oral toxicity effects of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus*

The aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* showed no acute oral toxicity effects in the selected doses in the tested animals (Table 2). The acute oral toxicity effects of the extracts were determined by observing any changes in the animals’ behavior, i.e., behavioral changes, changes in the animals’ eating, walking, and sleeping patterns, among others. The extracts were tested orally at concentrations of 1×, 10×, and 50×, respectively, compared to their MICs against the tested microbes. The animals were monitored for a period of 7 days. The results showed no acute oral toxicity effects at the tested doses, indicating that the extracts were safe.
C. dipsaceus showed no observable signs of acute oral toxicity in experimental rats even at the cut-off dose of 2000 mg/kg BW. Therefore, LD<sub>50</sub> values for each of the studied plant extracts were shown to be above 2000 mg/kg BW. Table 7 shows the results of the acute oral toxicity study.

**Discussion**

The standard castor oil-induced diarrhoea method modified by Rahman et al (26) was adopted to determine the antidiarrheal effects of the studied plant extracts. Ricinoleic acid, the major bioactive ingredient of castor oil, induces irritation and inflammation of the intestinal mucosa (26,27). As a result, prostaglandin secretion is evoked, as an immunological response, thereby stimulating and upregulating intestinal motility and secretion, which are characteristic in diarrhoea. Besides, castor oil inhibits sodium and potassium ion absorption while reducing ATPase in the small intestines and the colon, which results in reduced transit times due to high peristaltic activity (26,30,31). Therefore, a drug agent that can inhibit, or reverse the castor oil-induced diarrhoea, is a potential antidiarrheal agent.

The aqueous and methanolic leaf and fruit extracts of C. dipsaceus significantly inhibited the percentage of castor oil-induced diarrhoea in a positive dose-dependent manner, indicating their antidiarrheal efficacy. These findings suggest that the studied plant extracts may have exerted anti-inflammatory effects by inhibiting prostaglandin synthesis, which ameliorated the castor-oil-induced intestinal mucosa's irritation and inflammation (32). Furthermore, the studied plant extracts' phytochemicals may have also maintained or

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Minimum inhibitory concentration (MIC) (µg/mL)</th>
</tr>
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<tr>
<td></td>
<td>S. enteritidis</td>
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<tr>
<td>AQL</td>
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AQL: Aqueous leaf extract of C. dipsaceus; AQF: Aqueous fruit extract of C. dipsaceus; MEF: Methanolic fruit extract of C. dipsaceus; MEL: Methanolic leaf extract of C. dipsaceus; Standard: for bacterial strains, it was Ciprofloxacin (10 µg/mL), while for the fungal strain, it was nystatin (10 µg/mL); ND: Not determined.

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<th>Wellness parameter</th>
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<th>24 h</th>
<th>48 h</th>
<th>7 days</th>
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</table>

N, Normal; A, Absent.

EGR: Experimental group rats (Administered with 175 mg/kg BW/ 550 mg/kg BW/2000 mg/kg BW of the aqueous/methanolic leaf/fruit extracts of C. dipsaceus); CGR: Control group rats (Administered with 10 ml/kg BW of normal saline only); n=3 animals per step.
restored intestinal electrolyte homeostasis to prevent diarrhoea (26,27,29,31).

Various phytochemicals, including phenols, flavonoids, saponins, and glycosides, exhibit antidiarrheal efficacy by reducing gastric motility and secretion to avert diarrhoea (29-31). Besides, plant extracts contain various mineral elements, including zinc (Zn), sodium (Na), potassium (K), magnesium (Mg), among others, which are essential in regulating electrolyte and osmotic homeostasis in the gut and other body organs (10). Insufficiency or impaired homeostasis of these mineral elements causes electrolyte imbalance, which may manifest in diarrhoea, among other health complications. These mineral elements and bioactive phytochemicals were probably responsible for the antidiarrheal efficacy of the studied plant extracts, as observed in the present study.

The positive control drug used in this study, loperamide, exerts its antidiarrheal efficacy by regulating electrolyte balance and secretions in the gastrointestinal tract (32,33). Perhaps, the mechanism of antidiarrheal activity of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* may be like that of loperamide. Additionally, the studied plant extracts may possess anticholinergic effects, which are associated with reduced gastric motility and secretions, through a mechanism like that of atropine, which antagonises the muscarinic acetylcholine receptors of the central nervous system (33). Nevertheless, our study lays a framework for elucidating and characterising specific antidiarrheal compounds and their specific mode(s) of pharmacologic action(s) through focused and extensive empirical studies.

Microbial infections are key etiologic agents for inflammation (34). Various pathogenic microbial species produce metabolites, and other components, including the lipopolysaccharide, endotoxins, and cell capsule carbohydrates, which damage the integrity of the gut and evoke immunological responses, with devastating sequelae (35). Besides, a balanced gut microbiota with normal flora has been shown to play a crucial role in immunomodulation and promote gut health by synthesising and enhancing proper digestion and absorption of key nutrients in the body (36,37). However, colonisation by pathogenic microbes deters the normal gut microbiota population and functioning, resulting in gastrointestinal dysfunction, indigestion, diarrhoea, and other distressing symptoms.

The disk diffusion and broth microdilution techniques were used to determine the antimicrobial effects of the studied plant extracts on *E. coli, S. enteritidis, B. subtilis, P. aeruginosa,* and *C. albicans,* as stipulated in the CLSI guidelines (27). The obtained zones of microbial growth inhibition from the disk diffusion assay and the MICs from the broth microdilution assay were considered indicators of the antimicrobial efficacy. Research indicates that plant extracts, which produce microbial growth inhibition zones of 6-9 mm possess weak antimicrobial activity; those with inhibition zones of 9-12 mm possess moderate activity; those exhibiting inhibition zones of 13-16 mm have high antimicrobial activity; those with inhibition zones of 16-19 mm have very high antimicrobial activity, and those producing inhibition zones measuring >20 mm have remarkable antibiotic activity (38). Based on this appraisal criteria, the studied plant extracts demonstrated mixed antimicrobial effects against selected microbial strains in a concentration and extract-type dependent manner.

All the studied plant extracts were inactive at concentrations of ≤ 12.5 µg/mL and moderately active at concentrations of 25-100 µg/mL against *S. enteritidis.* Similarly, the aqueous leaf and fruit and methanolic leaf extracts of *C. dipsaceus* were not active against *E. coli* at concentrations of ≤12.5 µg/mL, and weak to moderate antimicrobial effects against *E. coli* were recorded at concentrations of 25-100 µg/mL. However, the methanolic fruit extract of *C. dipsaceus* exhibited weak to high antimicrobial activity against *E. coli* and demonstrated superior efficacy than the other extracts. Besides, the aqueous leaf extract of *C. dipsaceus* showed weak to moderate activity against *P. aeruginosa* (38, 39).

The aqueous fruit and methanolic leaf extracts of *C. dipsaceus* demonstrated weak to high antimicrobial activities against *P. aeruginosa.* Moderate to very high antimicrobial activity against *P. aeruginosa* was exhibited by the methanolic fruit extract of *C. dipsaceus,* which proved to be more potent than the other extracts (38,39). When *B. subtilis* was exposed to the tested plant extracts, the aqueous and methanolic leaf extracts of *C. dipsaceus* exerted weak to high antimicrobial effects, while the aqueous and methanolic fruit extracts showed weak to very high antimicrobial efficacy, in a concentration-dependent fashion (38,39).

The positive control drug (ciprofloxacin) demonstrated remarkable antibacterial efficacy in all the experimented bacterial strains, as demonstrated by ≥20 mm microbial growth inhibition zones. Moreover, only the aqueous leaf extract of *C. dipsaceus* demonstrated antifungal activity against *C. albicans* (weak to very high) in this study, and nystatin (the positive control drug) showed very high activity (38,39). The varied antimicrobial activity of the studied plant extracts is attributable to the presence of various antimicrobial phytochemicals, in varying concentrations, in the studied plant extracts (40).

The MIC of each of the studied plant extracts against the selected microbial strains was determined using the broth microdilution assay technique described by the CLSI (27). MIC is a commonly determined value used to appraise the degree of antimicrobial agent’s efficacy. A previous research showed that plant extracts and chemical substances with MIC values below 1000 µg/mL are potential sources of efficacious antibiotics (41). Furthermore, low MIC values indicate high antimicrobial
efficacy and a greater propensity for higher potency in vivo.

In the present study, the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* showed very low MIC values against the selected bacterial strains, indicating their high antimicrobial efficacy and potency. In addition, the low MIC value recorded for aqueous leaf extract of *C. dipsaceus* against *C. albicans* indicates its high antifungal efficacy. The antimicrobial effects of the studied plant extracts of *C. dipsaceus* reported in this study are attributable to the presence of various antimicrobial-associated bioactive secondary metabolites.

Previous studies indicate that various phytochemical compounds present in the aerial parts of *C. dipsaceus*, especially flavonoids, tannins, terpenoids, and phenols, among others, have antimicrobial effects (39-41). Therefore, it is suggested that these phytochemicals were responsible for the antimicrobial efficacy of the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus*, as observed in this study. Furthermore, the differences in antimicrobial effects against various strains could be due to the differences in concentration and type of phytochemicals present in the extracts, which may have different modes of bioactivity against various microbes (39).

Medicinal plants have a long history of usage in traditional medicine due to their easy accessibility, affordability, and presumed safety and potency (11,43). However, due to insufficient empirical data, various safety concerns regarding their safety and pharmacologic efficacy have been raised (43). For instance, there are no specific dosages or guidelines for herbal preparations, storage, labelling, marketing, and specific indications, like those of pharmaceutical drugs (43). Additionally, there is scanty empirical information on the herbal drug-herbal drug and herbal drug-conventional drug interactions and associated effects, which raise safety concerns (43,44). Therefore, it is imperative to investigate the safety and toxicity profiles of ethnomedically used plants to appraise their safety and offer empirical data to guide further research and development of therapies based on medicinal plants (44).

Despite the long-lasting utilisation of *C. dipsaceus* to manage various diseases in Kenyan traditional medicine, there is no sufficient data on its safety and toxicity profile. Therefore, the current study investigated the acute oral toxicity effects of *C. dipsaceus*. The Organisation for Economic Co-operation Development (OECD) guidelines for acute oral toxicity (23) were adopted in this study. The aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* did not elicit any observable acute oral toxicity effects; hence they were deemed safe according to the OECD guidelines (23). The safety of the studied plant extracts is attributable to the absence or low concentrations of toxic amalgams.

In Kenya, the leaves of *C. dipsaceus* are consumed as vegetables and as medicines (18). Besides, the leaves and fruits are used to treat wounds, stomach-aches, diarrhoea, poisoning, snakebite envenomation, diabetes mellitus, among other diseases, and as food in Kenya (15-19,35,45). Therefore, it is suggestive that the diverse applications of *C. dipsaceus* are due to its safety and health benefits.

Based on the toxicity results reported in this study, the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* demonstrate a higher prospect of offering efficacious and safe antimicrobial and antidiarrheal therapies upon further experimentation. Therefore, extensive in vivo studies, including clinical setups, should be considered upon establishing their safety and toxicity profiles. Nevertheless, this study partly validates the ethnomedical usage of *C. dipsaceus* in managing microbial infections and diarrhoea.

**Conclusions and recommendations**

The aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* have significant antidiarrheal efficacy and varying degrees of antimicrobial activity against *S. enteritidis, E. coli, B. subtilis, P. aeruginosa. C. albicans* was only susceptible to the aqueous leaf extract of the plant. Additionally, the aqueous and methanolic leaf and fruit extracts of *C. dipsaceus* do not cause acute oral toxicity effects in experimental rats. Further empirical studies to elucidate the specific antidiarrheal and antimicrobial compounds, and their specific mode(s) of bioactivity are recommended. Moreover, extensive screening and toxicological investigations of the tested plant extracts using other experimental models and approaches are recommended to establish their pharmacologic potential and safety profiles.

**Acknowledgments**

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**Authors' contributions**

PK, TM, JM, and GM conceived the research idea. PK performed the study, analysed the data, interpreted the findings, and wrote the manuscript under close guidance by GM. JM, and TM supervised the entire study. All authors reviewed and approved the final manuscript for publication.

**Conflict of interests**

The authors declare that there is no conflict of interest whatsoever regarding this study.

**Ethical considerations**

This research was approved by the Biosafety, Animal Use,
Data Availability
All the data used to support the findings of this study are included in the article. Additional data is available from the authors upon request.

References


