Antidiarrheal and Antioxidant Activities of the Aerial Parts of Caralluma dalzielii N. E. Brown

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ABSTRACT
Background: Caralluma dalzielii N.E. Brown is commonly referred to as “Karan massalanci” in the Northwest Nigeria where it is used in treating several ailments including pain, diarrhea and stomach issues. Objectives: This study was aimed to investigate the antidiarrheal and antioxidant activities of aqueous aerial parts extract of Caralluma dalzielii. Materials and Methods: The aqueous aerial parts extract of Caralluma dalzielii (CDE) (100, 200 and 400 mg/kg) was evaluated for antidiarrheal properties against gastrointestinal motility, castor oil-induced and prostaglandin-E2 (PGE2) enterpooling models in Wistar rats. Its antioxidant properties were studied by determining the total phenolic content (TPC) using Folin-Ciocalteu reagent, its free radical scavenging activity using 1, 1-Diphenyl-2-Picylhydrazyl (DPPH) and its ferric reducing antioxidant power (FRAP) assays using potassium ferrocyanide ferric chloride methods. Ascorbic acid was used as the reference. Spasmolytic effect was studied in isolated rabbit jejunum preparations in an organ bath experiment using acetylcholine (ACh). Results: CDE significantly (p<0.05) reduced gastrointestinal transit of charcoal meal and the total number of diarrheal feces in the animals. The total phenolic content was determined to be 36.67±3.33 mg GAE/g. IC50 value of 63.44 mg/mL was calculated in DPPH assay. The FRAP value was found to increase as the concentration of the extract increased. CDE inhibited acetylcholine-induced contraction of the rabbit jejunum in a concentration dependent manner with complete inhibition at 20.48 mg/mL of the extract. Conclusion: The extract of Caralluma dalzielii possesses antidiarrheal activities which may be related to its antimitotility, antioxidant and antispasmodic properties.

Key words: Antioxidant, Antispasmodic, Caralluma dalzielii, Gastrointestinal motility, Rabbit jejunum.

INTRODUCTION
Diarrhoea can be described either as the passage of watery stool for not less than three times a day,
[1] or more objectively, as frequency in bowel movements such that a stool production volume or stool production weight of more than 200 mL or 200 g within 24 hr is obtained.
[2] It has been rated as the second leading cause of death amongst children younger than five years of age. Global estimate of death due to diarrhoea is about 2.2 million people per year.
[3] Diarrhoea can be caused by a number of factors such as certain microorganisms, toxins, medications and many other agents that increase gastrointestinal tract (GIT) secretions.
[4] Over the years, human beings have relied on natural products to alleviate and treat their diseases. In the developing countries, over 80% of their healthcare needs are met by traditional sources.
[5] Medicinal plants have also been a source for modern medicines and examples of modern medicines developed from them abound.
[6] They are preferred over modern medicines because of their affordability, cost-effectiveness and fewer side effects. Search for newer drugs with less side effects and cheaper alternatives to modern medicine have continued in every area of health. Medicinal plants that have been employed in treating diarrhoea which have been scientifically validated are many and more are still being evaluated.
[7-10] Caralluma dalzielii (Family: Asclepiadaceae) popularly referred to as Mosque stalk is a shrub that resembles cactus plant. C. dalzielii is widely distributed in the Sahel region of Africa where its height ranges from 0.5 to 1 m. In North-West Nigeria, the plant is used in treating several ailments including diarrhoea, stomach upset, convulsion and infertility.
[11] Snake and scorpion bites, rheumatoid arthritis, diabetes, emesis, leprosy, and severe pains in the epigastrium are other medicinal uses of the plant.
[12-15] The aim of this study was to evaluate the antidiarrheal and antioxidant activities of the aqueous extract of Caralluma dalzielii.
MATERIALS AND METHODS

Preparation of plant extract

The aerial parts of the *Caralluma dalzielii* were collected from Tureta town, Sokoto State, North-West Nigeria. Identification and authentication of the plant were carried out by Mr. Musa Magaji, a taxonomist at the Department of Pharmacognosy and Ethnopharmacology, Usman Danfodiyo University Sokoto (UDUS). A voucher specimen (PGC/UDUS/ASCL/0003) was stored. The plant material was air-dried at room temperature for 4 weeks and pulverized with mortar and pestle. Soxhlet extraction method was employed in extracting 285 g of the powdered plant material in distilled water. The extract realized was dried over water bath at 50°C and the percentage yield was calculated.

Experimental animals

Healthy male Wistar rats (*Rattus norvegicus*) (150-200 g) and New Zealand white rabbits (1.25-1.5 kg) were obtained from the animal facility center of Ahmadu Bello University (ABU) Zaria. The animals were kept under standard environmental condition of 25°C and 12 hr light and dark cycle. The animals had free access to feed and water for 14 days before the study commenced for them to acclimatize to the laboratory conditions. The study was approved by the Animal research ethics committee of Department of Pharmacology and Toxicology Usman Danfodiyo University, Sokoto (PTAC/Cd/CAE/23-19).

Phytochemical screening

Qualitative tests for the presence of saponins, terpenoids, tannins, flavonoids, steroids, glycosides, anthraquinones, alkaloids and fixed oils were carried out.[14]

Antioxidant activity determination

Determination of total phenolic content (TPC)

The TPC was determined using Folin-Ciocalteu method.[15] The Folin-Ciocalteu reagent was added to 20 mg of the extract in 20 ml of 95% methanol and allowed incubate for 5 min. After that, Na$_2$CO$_3$ was mixed with it and incubated at room temperature for 2 hr. The absorbance was then measured at 765 nm on a UV spectrophotometer. A calibration curve prepared with gallic acid as reference standard was used to calculate the TPC. The results were expressed as Gallic acid equivalent (mg GAE/g).

Determination of free radical scavenging activity (FRSA) using 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) Assay

Previously described method,[16] was used to estimate the DPPH FRSA of the extract. Samples (1.0 mL, 1.5 mg) of the extract and the reference antioxidant (ascorbic acid) were prepared separately in varying concentrations of 0.01-0.2 mg/mL in methanol. A concentration of 100 µM of DPPH was prepared and 2 mL of it was mixed with 2 mL of methanol and left in the dark for 30 min. In the same way, 2 mL of the prepared DPPH was also mixed thoroughly with the different concentrations of the extract and ascorbic acid and kept in the dark for the same period of time. After that, the absorbance of mixtures were measured at 517 nm. The percentage radical scavenging activities were calculated thus:

$$\% \text{ Radical scavenging activities} = \left(1 - \frac{A_e}{A_o}\right) \times 100$$

Where $A_e$ = absorbance of DPPH in methanol; $A_o$ = absorbance of the extract or reference sample. The IC$_{50}$ (effective concentration of the extract required to inhibit DPPH radical formation by 50%) values were calculated by obtaining the regression equation from plotting the percentage radical scavenging activities against concentrations.

Determination of ferric reducing antioxidant power assay (FRAP)

The FRAP of the extract was estimated using potassium ferrocyanide ferric chloride method.[17] A volume of 2.5 mL of various concentrations of the extract was mixed with 2.5 mL of potassium ferrocyanide and incubated at 50°C for 20 min. After that, 2.5 mL of 10% trichloroacetic acid was then added to the mixture and centrifuged at 3000 rpm for 20 min. Then, 2.5 mL of the supernatant was mixed with 0.5 mL of freshly prepared 0.1% ferric chloride. Various concentrations of the standard (ascorbic acid), was prepared using the same procedure. Their absorbance were measured at 700 nm. The result was expressed in mg of ascorbic acid (AAE)/mL of the extract.

Anti diarrheal activity evaluation

Gastrointestinal motility test

The animals were fasted for 18 hr and divided into five groups ($n=5$). Groups I-III received oral doses of 100, 200 and 400 mg/kg of the extract. Group IV received loperamide (2.5 mg/kg) (positive control) while group V received distilled water (5 mL/kg) (negative control). A 1 mL of charcoal meal which consisted of 10% charcoal suspension in 5% gum acacia, was administered orally to the rats 30 min after treatment. After an hour, the animals were sacrificed and the distance moved by the charcoal meal from the pylorus towards the caecum was measured. The percentage of the distance moved by charcoal meal to the total length of the intestine (TLI) was calculated.[18] Peristalsis index and percentage inhibition of mobility were calculated as

$$\text{Peristalsis index} = \frac{\text{Distance travelled by charcoal meal} - 100}{\text{Length of intestine} \times 100}$$

$$\% \text{ inhibition of mobility} = \left(\frac{T_L - T_1}{T_L}\right) \times 100$$

Castor oil-induced diarrhoea test

Healthy Wistar rats were fasted for 18 hr and allocated into five groups ($n=5$). Groups I-III were the extract groups and were treated with 100, 200 and 400 mg/kg p.o of the extract. Group IV was the reference drug group treated with loperamide 2.5 mg/kg, p.o while group V received the vehicle (5 mL/kg p.o) and represented the control group. After 1 hr of extract, drug or vehicle pre-treatment, all rats were given 1 mL of castor oil orally to induce diarrhea. All the animals were kept in separate metallic cages with a plain absorbent sheet of paper at the floor and were observed every hour for 4 hr. The total number of diarrhoea feces were noted and the percentage diarrhoea inhibition calculated.[19]

$$\% \text{ diarrhoea inhibition} = \left(\frac{T_w - T_f}{T_w}\right) \times 100$$

where $T_w$ = number of wet feces in vehicle control group

$T_f$ = number of wet feces in test group

Prostaglandin-E$_2$ (PGE2) enteropooling test

Twenty-five healthy rats were fasted for 18 hr and divided into five groups ($n=5$). Groups I-III were the extract groups and received 100, 200 and 400 mg/kg p.o respectively of the aqueous extract of *Caralluma dalzielii*. Group IV represented the standard drug, loperamide (2.5 mg/kg) while the group V was the vehicle control group that received 5mL/kg of distilled water. After 1 hr of treatment, 100 µg/kg of PGE2 was administered orally to all the animals to induce enteropooling. After a period of 30 min, all animals were sacrificed and the whole intestines dissected. The intestinal contents were collected and the total volume measured.[20]
Determination of spasmolytic activities

Isolated rabbit jejunum experiment

The rabbit was decapitated and its jejunum dissected out and placed in Tyrode solution with a controlled temperature of 37°C and aerated. About 2-3 cm of the jejunum was cut out and tied to the isotonic transducer connected with a data acquisition interface (PowerLab) and computer screen for displaying isometric contractions. Isometric concentrations were recorded under a resting tension. The setup was allowed an equilibration period of 30 min. Dose-response relationships were established using standard acetylcholine (Ach) at 1, 2, 4, 8 and 16 µg/mL. To assess the inhibitory effect of the extract on Ach, 8 µg/mL which gave the highest response was used. The dose-responses of extract at 1.28, 2.56, 5.12, 10.24 and 20.48 mg/mL were established against 8 µg/mL of Ach. The response time for each treatment was for a minimum of 2 min before three times washing with Tyrode solution.

RESULTS

Phytochemical analysis

The extract was found to contain saponins, terpenoids, tannins, flavonoids, steroids, glycosides, alkaloids and fixed oils.

Antioxidant screening

CDE had TPC of 36.67±3.33 mg GAE/g. The in vitro DPPH free radical scavenging activity revealed that the extract had antioxidant activity (Figure 1). This was seen from the IC50 obtained from the regression coefficient (R²). The extract demonstrated antioxidant activity by scavenging DPPH radical with an IC50 of 63.44 mg/mL calculated from y = 0.03058x + 61.91 (where x = 50) and R² of 0.003273 as compared with ascorbic acid with an IC50 of 80.47 mg/mL calculated from y = 1.038x + 28.57 (where x = 50) with R² of 0.03015. The results revealed that CDE and ascorbic acid demonstrated free radical scavenging activity but the activity demonstrated by CDE was higher than that of the ascorbic acid. In the FRAP determination, the reducing ability of the extract expressed as ascorbic acid equivalence was found to be concentration dependent (Figure 2).

Antidiarrheal evaluation

In the gastrointestinal motility test, administration of CDE at all dose levels significantly (p<0.05) reduced the speed of transit of charcoal meal towards the caecum. The inhibition of gastrointestinal motility was dose dependent (Table 1).

At 400 mg/kg, the extract in castor oil-induced diarrhoea produced significant (p<0.05) number of diarrhoea feces in the first and second hours. However, by the third and fourth hours, diarrhoea feces were produced significantly (p<0.05) at all dose levels when compared to the control (Table 2). The extract inhibited the mean number of diarrheal feces in a dose dependent manner. The inhibition at 400 mg/kg was significantly (p<0.05) lower than that of the control group (Figure 3).

The extract failed to inhibit the intestinal content in the enteropooling test. Instead, it dose dependently increase the intestinal content. At 400 mg/kg of the extract, the intestinal content was significantly (p<0.05) higher than that of the control group (Table 3).

Effect of Caralluma dalzielii on isolated rabbit jejunum

Acetylcholine at various concentrations of 1, 2, 4, 8 and 16 µg/mL caused a concentration-dependent contraction of the rabbit jejunum, while the extract (1.28–20.48 mg/mL) produced a dose dependent inhibition of the spontaneous contraction of the jejunum. At 20.48 mg/mL of the extract, the extract completely abolished the contraction produced by 8 µg/mL of Ach (Figure 4).
Table 2: Effect of aqueous extract of *Caralluma dalzielii* on castor oil-induced induced diarrhoea feces per hour.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>1st hr</th>
<th>2nd hr</th>
<th>3rd hr</th>
<th>4th hr</th>
<th>% diarrhoea inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDE</td>
<td>100</td>
<td>3.8±0.86</td>
<td>3.4±0.40</td>
<td>1.4±0.50*</td>
<td>1.4±0.50*</td>
<td>5.66</td>
</tr>
<tr>
<td>CDE</td>
<td>200</td>
<td>2.0±0.89</td>
<td>3.4±0.60</td>
<td>1.8±0.58*</td>
<td>0.8±0.20*</td>
<td>24.53</td>
</tr>
<tr>
<td>CDE</td>
<td>400</td>
<td>1.8±0.37*</td>
<td>2.0±0.31*</td>
<td>0.0±0.00*</td>
<td>1.2±0.37*</td>
<td>52.83</td>
</tr>
<tr>
<td>Loperamide</td>
<td>2.5</td>
<td>1.0±0.63*</td>
<td>0.8±0.80*</td>
<td>0.2±0.20*</td>
<td>0.0±0.00*</td>
<td>81.13</td>
</tr>
<tr>
<td>Distilled water</td>
<td>5 mL/kg</td>
<td>2.6±0.24</td>
<td>2.8±0.58*</td>
<td>2.8±0.73</td>
<td>2.4±0.50</td>
<td>0</td>
</tr>
</tbody>
</table>

CDE= *Caralluma dalzielii* extract; values are presented as mean ± SEM (n=5); *p<0.05 significant when compared to distilled water control group.

Figure 3: Effect of aqueous extract of *Caralluma dalzielii* on mean number of feces in castor oil-induced diarrhoea in rats.

Table 3: Effect of aqueous extract of *Caralluma dalzielii* on PGE2-induced enteropooling in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Volume of intestinal content</th>
<th>% inhibition of intestinal fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDE</td>
<td>100</td>
<td>3.14 ± 0.37</td>
<td>3.09</td>
</tr>
<tr>
<td>CDE</td>
<td>200</td>
<td>3.70 ± 0.27</td>
<td>-14.2</td>
</tr>
<tr>
<td>CDE</td>
<td>400</td>
<td>4.87 ± 0.57</td>
<td>-50.31</td>
</tr>
<tr>
<td>Loperamide</td>
<td>2.5</td>
<td>2.68 ± 0.31</td>
<td>17.28</td>
</tr>
<tr>
<td>Distilled water</td>
<td>5 mL/kg</td>
<td>3.24 ± 0.27</td>
<td>0</td>
</tr>
</tbody>
</table>

CDE= *Caralluma dalzielii* extract; values are presented as mean ± SEM (n=5); *p<0.05 significant when compared to distilled water control group.

Figure 4: The inhibitory effect of the extract of *Caralluma dalzielii* on acetylcholine contraction of rabbit jejunum.

DISCUSSION

The study evaluated the antioxidant, antidiarrheal and antispasmodic activities of CDE using different experimental models in Wistar rats. The antioxidant activities were evaluated by determining the TPC, the DPPH scavenging activity and the FRAP of the plant. The antidiarrheal activity was tested against gastrointestinal motility, castor oil-induced and prostaglandin-E2 (PGE2) enteropooling models in Wistar rats. Isolated rabbit jejunum was used to investigate the antispasmodic activity of the extract. The data obtained showed that the extract possessed antioxidant, antidiarrheal and antispasmodic activities.

Free radicals are regarded as one of the major pre-disposing factors in the development of diseases including diarrhoea.[22] Plant polyphenols being the potential source of natural antioxidants have played important role in alleviating such diseases. The presence of phenolic hydroxyl groups in these compounds, confers the antioxidant activity.[23] The phenolic compounds scavenge free radicals by releasing hydrogen atoms and electrons to exhibit their antioxidant capacity.[24] Increase in phenolic content an extract leads to a corresponding increase in its antioxidant activity.[25] Furthermore, water being polar solvent, has the capacity to extract the phenolic compounds.[26] The total phenolic content found in our extract was comparable to previous reports of other plants with antioxidant properties.[27,28] Therefore, the antioxidant activity demonstrated by the aqueous extract of *Caralluma dalzielii* may be attributed to its phenolic content.

To further ascertain the antioxidant (FRSA) activity of the plant, the DPPH assay was determined. The DPPH assay is regarded as an indication of the capacity of plant extract to scavenge DPPH free radicals by converting unpaired electrons to paired ones.[29] The IC₅₀ of the extract was lower than that of ascorbic acid showing that the extract possesses a stronger *in vitro* antioxidant activity. To assess the extract’s reducing power, FRAP assay was determined. FRAP assay assesses the reducing ability of a substance (an antioxidant) reacting with ferric tripyridyltriazine complex to produce ferrous tripyridyltriazine which is coloured.[30] Our extract increased the FRAP in a concentration-dependent manner. This property suggests that the extract of *Caralluma dalzielii* may act as a free radical scavenger that can transform reactive free radical species into stable non-radical products.

In the management of diarrhoea, antimotility and antisecretory agents are regarded as the major agents used to alleviate the pathophysiologic conditions responsible for diarrhoea development.[31] Drugs which are capable of relaxing intestinal smooth muscle are usually employed as antidiarrheal agents. This is because they inhibit the intestinal hyper motility evident in diarrhea disease.[32] In the antidiarrheal study, pre-treatment with the extract of *Caralluma dalzielii* caused a reduction in the gastrointestinal motility suggesting that the extract possesses an antidiarrheal activity. To further verify the antidiarrheal activity of the plant, its effect on castor oil-induced diarrhea was investigated.
Castor oil induces diarrhea owing to its active metabolite known as ricinoleic acid. Ricinoleic acid stimulates peristaltic activity in the small intestines and cause changes in the permeability of intestinal mucosa to electrolytes. This mechanism cause diarrhea and also leads to the secretion of endogenous prostaglandin. Our extract dose dependently inhibited the mean number of feces produced by the animals. However, the inhibition produced by the standard drug, loperamide, was more than that produced by the extract. Castor oil-induced diarrhea have been shown in previous studies to be inhibited by many plant extracts such as Crataegus azarolus, Bixa orellana, Vitex doniana, and Entada africana.

In an attempt to suggest other possible mechanism of activity of the plant extract, the PE enteropooling test was carried out. PGE2 produces diarrhoea by inhibiting glucose absorption and causing accumulation of fluid in the intestinal lumen. This implies that drugs with potential inhibitory activity against prostaglandins could be suitable for preventing the enteropooling effect of PGE2. Our extract, however failed to inhibit the hypersecretion and enteropooling in the gastrointestinal tract causing a dose dependent increase in the accumulation of intestinal fluids. This suggests that the extract may not be acting in a similar manner as loperamide whose antidiarrheal mechanism includes inhibiting enteropooling caused by PGE2. The antidiarrheal activity of the extract may therefore be due to its antinociceptive activity but not antisecretory effect. To confirm its antinociceptive activity, the antiinflammatory activity was carried out using rabbit jejunum.

The extract concentration-dependently inhibited the acetylcholine-induced contractions of the isolated rabbit jejunum. Acetylcholine is an agonist that cause intestinal smooth muscle contraction by activating the muscarinic M1 receptors. It induces smooth muscle contraction via inositol phosphate (IP3) pathway, which mediates Ca2+ release from sarcoplasmic reticulum. The extract from the aerial parts of Caralluma dalzielii possesses antispasmodic activity involving cholinergic mechanism. Phytochemical constituents of the extract were determined to identify the possible constituents that could be responsible for the antioxidant, antidiarrheal and antispasmodic activities seen with the plant extract. The presence of saponins, terpenoids, alkaloids, tannins and flavonoids detected in the plant extract could be responsible for these activities. In a similar experiment, the antidiarrheal activities of some medicinal plants were found to be due to the presence of tannins, alkaloids, saponins and flavonoids contained in them.

CONCLUSION
The present study showed that CDE possesses antidiarrheal, antioxidant and spasmylocic activities. The results obtained also showed that the antidiarrheal properties of CDE may be due to its antioxidant and spasmylocic properties. This study thus provides the pharmacological evidence for its use in the traditional setting for treating gastrointestinal disorders. Further studies are required to isolate and characterize active compounds in the plant.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

ABBREVIATIONS
ACh: acetylcholine; CDE: extract of Caralluma dalzielii; DPPH: 1,1-Diphenyl-2-Picrylhydrazyl; FRAP: ferric reducing antioxidant power; FRSA: free radical scavenging activity; GIT: gastrointestinal tract; PGE2: prostaglandin-E2; TLI: total length of the intestine; TPC: total phenolic content.

REFERENCES
The anti diarrheal and antioxidant activities of the aqueous extract of the aerial parts of *Caralluma dalzielii* N. E. Brown were studied by determining its effect on gastrointestinal motility, castor oil-induced diarrhea, and PGE2 enteropooling models in Wistar rats. The antioxidant parameters, TPC, FRSA using DPPH and FRAP assays were determined. Spasmolytic property was studied in isolated rabbit jejunal preparations. The extract reduced gastrointestinal transit of charcoal meal and the total number of diarrhoeal feaces in the animals. The TPC was 36.67±3.33 mg GAE/g. IC<sub>50</sub> of extract was 63.44 mg/mL while that of ascorbic acid was 80.47 mg/mL in DPPH assay. The FRAP value increased in a concentration dependent manner. The extract possesses antidiarrheal activities which may be related to its antimotility, antioxidant and antispasmodic properties.
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