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Original Research Article

Inhibition of carbachol-induced gastrointestinal motility mediates the antidiarrheal activity of *Combretum platypetalum* (Welw.) Hutch & Dalziel leaf extract

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Abstract

Purpose: *To evaluate the mechanism of antidiarrheal activity of the leaves of Combretum platypetalum (Welw.) Hutch & Dalziel (Combretaceae) in Swiss albino mice.*
Methods: *The study employed bioactivity-guided fractionation of the extract, which was separated using hexane, ethyl acetate, and butanol. Further fractionation was done with vacuum liquid chromatography (VLC). The antidiarrheal activity of the extracts (100 - 400 mg/kg) and fractions (100 - 200 mg/kg) was evaluated using the charcoal meal antimotility and castor oil-induced antisecretory activity models with 3 mg/kg loperamide standard. The antimotility mechanism of the most active VLC fraction was assessed by measuring gastrointestinal transit (GT) and gastric emptying (GE).*
Results: *Maximum antimotility effect was achieved at 400 mg/kg extract (70.14 %), surpassing loperamide (61.88 %; $p < 0.01$). In the GT test, the control group's charcoal meal traversed a long distance (peristaltic index, PI: 91.48 %), which was significantly reduced ($p < 0.001$) with ethyl acetate fraction treatment at 50, 100, and 200 mg/kg, showing PIs of 23.46 %, 20.18 and 51.74 %, respectively. GT significantly increased ($p < 0.05$) with carbachol and serotonin (10 mg/kg) and 30 mg/kg metoclopramide by 83.5, 69.1 and 68.9 %, respectively, compared to control animals (44.3 ± 5.5 %). Pre-treatment with VLC fraction (200 mg/kg) significantly ($p < 0.05$) inhibited carbachol's action on GE (75.6 vs 27.6 %) but had no significant impact on metoclopramide and serotonin's actions on GE.*
Conclusion: *Combretum platypetalum's antidiarrheal activity is mediated by an antimotility mechanism, specifically through the inhibition of carbachol-induced GT and GE.*

Keywords: *Antimotility, Combretum platypetalum, Gastric emptying, Gastrointestinal transit, Vacuum liquid chromatography*

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INTRODUCTION

Diarrhea is a major global public health issue, particularly affecting children under five, and is the primary contributor to mortality in this age group, surpassing AIDS, measles and malaria combined [1]. It predominantly impacts developing countries, with 78 % of children's deaths from diarrhea occurring in Africa and South-East Asia. Nigeria has the second-highest diarrhea mortality rate across all ages. Factors such as diet intolerance, drugs, infections, and gastrointestinal disorders can trigger diarrhea

through gastric motility upset and fluid imbalance [2]. Antagonists of serotonergic (5-hydroxytryptamine; 5-HT), dopaminergic, and muscarinic pathways play key roles in gut motility, providing important targets for developing antidiarrheal therapies [3].

Antimotility agents often bind non-selectively to muscarinic1-5 (M1-M5) acetylcholine receptor subtypes, causing anticholinergic burden. Non-selective binding to dopaminergic and 5-HT receptors can lead to histamine intolerance and serotonin syndrome [4,5]. These agents are poorly tolerated in ulcerative colitis and can cause bloating, constipation, and drug interactions. More selective plant-based therapies are needed considering the challenges associated with available antimotility agents. Plants play a crucial role in antidiarrheal therapies, especially given the increasing resistance of Salmonella, Campylobacter and Shigella to conventional therapeutic agents such as antibiotics [6]. Many modern drugs are plant-derived, with numerous plants showing potential as antidiarrheal agents [7]. *Combretum platypetalum* (*C. platypetalum*); (Welw.) Hutch & Dalziel, from the Combretaceae family, is a tropical tree found throughout sub-Saharan Africa. It thrives in rainforests, secondary forests, and Savanna regions, and occasionally in swampy areas up to 450 meters above sea level. Various parts of the plant are linked to numerous pharmacological activities; including antioxidant, hypoglycaemic and hypolipidaemic due to its rich phytochemical composition [8]. Despite extensive research on Combretum species, many potential uses of *C. platypetalum* remain unexplored.

There are instances of therapeutic failures, adverse effects, or resistance development to antidiarrheal agents [9]. Loperamide and bismuth salicylate have been effective in reducing stool frequency, abdominal cramps, and stool volume. However, loperamide poses a high risk of colonic dilatation in infective colitis, while bismuth salicylate, requiring multiple tablets and having a slow onset, can cause unpleasant side effects like tinnitus and black tongue [10]. Additionally, oral rehydration therapy is limited by a lack of access to clean water and ineffectiveness in severe diarrhea cases [11]. The search for new compounds, particularly plant-based, for managing diarrhea is crucial, with several plants showing potential as antidiarrheal agents.

In ethnomedicine, the decoction, infusion or tincture of the leaves of *C. platypetalum* is used by the people of Southeast Nigeria to control diarrhoeic episodes [12]. It is a common practice to blend various *Combretum* species or to combine them with other common medicinal plants for herbal gastrointestinal remedies. The study, therefore, evaluated the potential and mechanism of antidiarrheal activity of leaves of *C. platypetalum* in experimental animal models.

EXPERIMENTAL

Materials

Combretum platypetalum leaves

The fresh leaves of *C. platypetalum* were sourced from Nsukka, Nigeria in April 2023. Authentication was done by Mr. Felix Nwafor, a taxonomist in charge of the herbarium unit of the Department of Pharmacognosy, University of Nigeria. Voucher specimen of the plant (PCG/UNN/0023) was stored in the herbarium unit of the same Department.

Animals

Swiss albino mice (20 - 25 g and about 6 weeks old) of either sex were adopted for acute toxicity tests and Swiss albino rats (112 - 120 g and 8 - 10 weeks) of either sex were used for the *in vivo* antidiarrheal activity tests. All animals were obtained from the Veterinary Teaching Hospital, University of Nigeria, housed in cages maintained at 25 °C and fed with a standard diet and water when desired. The animals were conditioned for 14 days before the beginning of the study. All protocols were executed adhering to the standards of international animal care and welfare and were assessed and endorsed by the ethical committee of the University of Nigeria (Ref no. FPSRE/UNN/21/0009).

Plant extraction

A coarsely powdered sample (500 g) was cold-macerated in 1 L methanol (95 %v/v) for 48 h with frequent agitation and filtration [13]. The marc was re-extracted for another 24 h twice and also filtered. The combined filtrates were evaporated to a constant weight using a rotary evaporator at reduced temperature and pressure. The dried C. platypetalum methanol extract (CP-ME) obtained was kept at – 20 ºC until further use.

Solvent-solvent partitioning of CP-ME

Ten grams of CP-ME was dispersed in 500 mL of aqueous methanol (10 %v/v) and sonicated for five minutes. The dispersion was partitioned using n-hexane (2 x 500 mL), followed by 3 x 500 mL of ethyl acetate and then 2 x 500 mL of n-butanol respectively [14]. Each combined fraction

was evaporated to dryness using a rotary evaporator at reduced temperature and pressure to obtain n-hexane (CP-HF), ethyl acetate (CP-EF) and n-butanol (CP-BF) fractions. The fractions were stored in a refrigerator at 4 ºC until required for the experiment. The anti-diarrhoeal activity of CP-HF, CP-EF and CP-BF was performed based on the model in which CP-ME showed higher activity.

Vacuum liquid chromatographic separation

Five grams of CP-EF was dissolved in 20 mL of methanol, triturated with 30 g of silica gel and allowed to dry completely [14]. The triturated sample was loaded into a glass column (150 x 1.5 cm) containing silica gel (200 – 600 mesh) and eluted successively with solvent gradients of dichloromethane in ethyl acetate (10:0, 7.5:2.5, 5:5, 2.5:7.5, 0:10) each 500 mL to obtain five sub-fractions (CP-EF1, CP-EF2, CP-EF3, CP-EF4 and CP-EF5). The CP-EF1 to CPEF5 were used for further studies to investigate the anti-motility activity of the sub-fractions.

Phytochemical test

All the samples were subjected to phytochemical analysis according to standard methods to detect alkaloids, tannins, flavonoids, saponins, steroids, phenols, glycosides and terpenoids [15].

Acute toxicity test and dosing

An acute toxicity test was conducted following the modified Lorke method as previously described [16]. The gross changes in the mice such as paw licking, loss of appetite, diarrhea, weakness, tremors, hair erection, salivation, stretching of the entire body, sleep, respiratory distress, lacrimation, coma or death were monitored. The therapeutic doses of 0.01 – 0.10 of the calculated LD_{50} were selected as safe doses for administration.

Animal grouping

Swiss rats were randomized into five groups, A - E (n = 5) for antidiarrheal assay of CP-ME. Each rat was placed in a cage with a blotting paper lining the floor. The treatments (0.5 mL normal saline as control; 100, 200 and 400 mg/kg of CP-ME and loperamide 3 mg/kg as standard), were given once orally to rats in groups A - E respectively. For the antidiarrheal assay of CP-HF, CP-EF and CP-BF, rats were randomized into 11 groups (n = 5). Groups A and E served as untreated and standard control respectively. Groups B1, B2 and B3 received 100, 150 and 200 mg/kg of CP-HF respectively; groups C1, C2 and C3 were administered 100, 150 and 200 mg/kg of CP-EF respectively while groups D1, D2 and D3 received 100, 150 and 200 mg/kg of CP-BF respectively.

Castor oil-induced antidiarrheal test

After 30 minutes of treatments, each rat received castor oil (0.3 mL) orally. The observation period was 4 h during which several parameters were recorded. The latency period between castor oil administration and the onset of the first diarrhoeic stool (defined as wet faeces that produce a halo on filter paper), the number of faecal outputs, the count of diarrhoeic stools, and the mass of diarrhoeal stools. Stool consistency was scored on a scale of 1 to 3, where 1 represents normal stool, 2 denotes semi-solid stool, and 3 indicates watery stool [17]. The onset time was determined as the interval from the dosing of castor oil to the emergence of the first diarrhoeic stool.

Charcoal meal test (Antimotility activity)

The rats underwent an 18-hour fasting period with unrestricted access to water before treatment administration. After 1 hour, 0.5 mL of castor oil was dosed orally via gavage. Subsequently, oral dosing of 1 mL of 5 % charcoal suspension occurred 1 hour following the castor oil administration. One hour after the administration of the charcoal, the rats were euthanized through cervical dislocation, followed by the dissection of the small intestine. The distance traveled by the charcoal from the pylorus to the cecum was quantified and expressed as a percentage relative to the entire small intestine length [18]. Lastly, the peristalsis index (PI) and the inhibition ratio were calculated using Eq 1.

$$PI = (L_c/L_i)100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where L_c is mean distance traveled by charcoal meal and L_i is mean length of small intestine.

Castor oil-induced Enteropooling model (Antisecretory activity)

Rats were kept on a fasting schedule for 18 hours before the experiment. Treatments were administered orally 1 h ahead of castor oil administration. One hour later, the rats were sacrificed, and their small intestines were extracted, tied, and weighed. The intestinal contents were drained and measured, followed by re-weighing the empty intestines [19]. The change in weight and volume was quantified. The reduction (%) in intestinal efflux and weight

of intestinal contents was determined using Eq 2 based on the mean volume (MVI) and weight (MWI) of the intestinal contents.

Inhibition (%) = ($MVI_{control}$-MVI_{test}/$MVI_{control}$)100 …..... (2)

Gastrointestinal anti-motility mechanism

Forty Swiss rats were randomized to eight groups (n = 5). Groups 1 to 5 received the following oral treatments: normal saline (0.5 mL), VLC fraction CP-EF4 (200 mg/kg), 10 mg/kg each of carbachol and serotonin and 30 mg/kg of metoclopramide (0.1 mL/10 g of rat), respectively. Groups 6, 7 and 8 were pre-treated with VLC fraction CP-EF4 (200 mg/kg) 30 min before receiving 10 mg/kg each of carbachol and serotonin, and 30 mg/kg of metoclopramide (0.1 mL/10 g of rat) respectively. Subsequently, all groups were administered a 0.5 mL/animal carboxyl methylcellulose solution (CMS). After 15 minutes, the rats were sacrificed via slight decapitation. Control rats were sacrificed immediately following CMS administration. The abdominal regions were incised to reveal the stomach and intestines [20]. The stomachs were dissected from the intestines at the pyloric junction to assess gastric emptying (GE), while the intestines were analyzed for the evaluation of gastrointestinal transit (GT).

Measurement of GE

The stomachs were blended in distilled water (5 mL) and subsequently centrifuged at 3,000 rpm for 15 minutes. To 1 mL of the supernatant, 1 mL of 0.025 M NaOH solution was added and thoroughly mixed. The absorbance of the mixture was measured at 560 nm wavelength using a Shimadzu spectrophotometer (Shimadzu, Kyoto). Gastric emptying (GE) was calculated using Equation 3, where A_{test} represents the absorbance of the supernatant from the homogenized stomach, and $A_{control}$ stands for the absorbance of the supernatant from the homogenized stomach of control rats [18].

GE (%) = 100 – (A_{test}/$A_{control}$)100 ……. (3)

Measurement of GT

The assessment of gastrointestinal transit was obtained by measuring the entire small intestine length and the distances traveled by the carboxyl methylcellulose. Gastrointestinal transit was estimated using Eq 4 [20].

GT (%) = (D_p/L_{Ti})100 …… (4)

where D_p is the distance traveled by phenol red and L_{Ti} is the mean total length of the small intestine.

Data analysis

The data were processed using GraphPad Prism v.5.0 and reported as mean ± SEM. Statistical significance was calculated using one-way ANOVA followed by Dunnett's post-hoc test. A $p < 0.05$, < 0.01, or < 0.001 was deemed significant.

RESULTS

Extraction and fractionation yield of CP-ME

The methanol extraction of *C. platypetalum* leaves showed that 500 g of dried coarse sample yielded 73.9 g (14.77 %w/w) of crude extract designated as CP-ME. Subsequent partitioning of 10 g in solvents yielded 1.4125 g (CP-HF, 1.88 %), 2.3325 g (CP-EF, 3.11 %), and 3.3775 g (CP-BF, 4.50 %) of n-hexane, ethyl acetate (EtOAc), and n-butanol soluble fractions respectively relative to 500 mg of powdered sample. The most active fraction, CP-EF, 5.0 g was subjected to further separation using the VLC technique in graded combinations of dichloromethane and EtOAc recovered 0.16, 0.08, 0.05, 0.30 and 0.06 %w/w of VLC fractions CP-EF1 to CP-EF5 respectively.

Phytochemical constituents of *C. platypetalum*

The phytochemical assay of CP-ME revealed the presence of saponins, alkaloids, tannins, flavonoids, glycosides, steroids, and terpenoids. The result also showed that CP-HF is rich in saponins, glycosides, steroids, and terpenoids. The CP-EF is rich in tannins, terpenoids, glycosides, flavonoids, saponins and alkaloids while alkaloids, terpenoids and steroids were not detected in CP-BF. Flavonoids, glycosides and alkaloids were detected in the VLC fraction, CP-EF4.

Acute toxicity of CP-ME

The acute toxicity test of CP-ME showed no adverse reaction at the maximum tested dose of 5000 mg/kg.

Anti-diarrhoeal effect of CP-ME in castor oil-induced diarrhoea

The CP-ME elicited a dose-dependent effect in reducing the number of faeces and wet faeces, as well as extending diarrhea onset (Table 1).

Significant delays ($p < 0.01$) in diarrhea onset were observed at 200 and 400 mg/kg dosing compared to the rats treated with normal saline. The 400 mg/kg showed maximum delay, outperforming the standard loperamide dose. All CP-ME doses significantly ($p < 0.001$) reduced the number as well as the weight of wet faeces relative to the negative control. The highest dose demonstrated the greatest inhibition of defecation (52.6 %) and the lowest wet faecal output (28.7 %) among all tested doses and the positive control.

Anti-motility activity of CP-ME in castor oil-induced diarrhoea

The charcoal meal intestinal transit model demonstrated that CP-ME significantly inhibited intestinal transit at all tested doses. The highest anti-motility effect was obtained at the maximum test dose of 400 mg/kg (70.14 %), which is comparable to the effect of loperamide at 3 mg/kg (61.88 %; $p < 0.01$; Table 2).

Anti-motility activity of fractions of CP-ME

In the gastrointestinal transit (GT) test, charcoal meal traveled a longer distance in the normal saline-treated group (PI: 91.48 %), which was significantly $p < 0.001$ inhibited on treatment with CP-EF at 50, 100 and 200 mg/kg p.o. Showing PI of 23.46, 20.18 and 51.74 % respectively. Other fractions (CP-HF and CP-BF) at their highest doses did not elicit any significant anti-motility effect (Table 3).

Table 1: Antidiarrheal activity of CP-ME in castor oil-induced diarrhea

Group	Onset time (min)	No. of faeces	No. of wet faeces	Wet faeces (mg)
A	108.2±12.1	12.6±2.3	10.1±1.2	486.3±38.2
B	139.6±11.7 (29.0)*	10.7±0.6 (15.1)*	8.7±0.8 (13.9)	346.8±25.1 (28.7)
C	178.8±14.3 (65.2)**	7.3±0.9 (42.1)*	6.2±0.9 (38.6)**	249.2±19.3 (48.4)**
D	203.1±19.3 (87.7)**	5.6±0.8 (55.6)**	5.1±0.6 (49.5)*	230.5±11.8 (52.6)*
E	181.5±19.2 (67.7)*	9.4±1.1 (25.4)	5.5±0.5 (45.5)**	227.3±17.4 (53.3)*

*Values in parenthesis represent the inhibition (%); Values are reported as mean ± SEM (n=5); *p < 0.05; **p < 0.01, vs untreated control group; Rats in groups A-E received 0.5 mL normal saline, 100, 200 and 400 mg/kg CP-ME and loperamide respectively*

Table 2: Antimotility activity of CP-ME in castor oil-induced diarrhoea

Group	LSI (cm)	DCCM (cm)	PI (%)	Inhibition (%)
A	61.30±0.94	56.08±0.54	91.48±1.01	-
B	60.25±0.24	42.90±0.52*	71.20±1.24	22.17
C	62.04±0.56	23,85±0.76**	38.44±0.95*	57.98
D	59.82±0.75	16.34±0.47***	27.32±0.82**	70.14
E	59.85±0.43	20.87±0.94***	34.87±0.74***	61.88

*Distance covered by charcoal meal (DCCM); Length of small intestine (LSI); peristaltic index (PI). Values are reported as mean ± SEM (n=5); ***p < 0.001; **p < 0.01; *p < 0.05, vs untreated control group; Rats in groups A-E received 0.5 mL normal saline, 100, 200 and 400 mg/kg CP-ME and loperamide, respectively*

Table 3: Anti-secretory activity of fractions of CP-ME against castor oil-induced diarrhea

Group	VIC (µL)	Inhibition (%)	WIC (mg)	Inhibition (%)
A	724.8±19.1	-	1147.5±32.9	-
B1	699.6±23.6	3.4	703.8±12.8	39.7
B2	682,1±14.8	5.8	746±23.9	34.9
B3	700.3±43.2	3.3	887,8±34.9	22.7
C1	598.4±18.6	1.7	798.6±29.4	30.4
C2	656.9±19.7	9.4	923.9±31.1	19.5
C3	578.4±23.0	20.2	982.3±28.4	14.4
D1	673.9±13.9	7.0	1005,3±41.3	12.4
D2	692.6±36.9	4.4	924.6±27.4	19.4
D3	703.9±29.6	2.9	938,7±26.4	18.2
E	336.1±22.6**	53.59	688.5±33.4	40.02

*Volume of intestinal contents (VIC); weight of intestinal contents (WIC); Values are reported as mean ± SEM (n=5); ***p < 0.001, **p < 0.01, *p < 0.05 vs untreated control group; A = normal saline-treated group, B1-B3 = groups dosed with 50, 150 and 200 mg/kg n-hexane fraction; C1-C3 = groups dosed with 50, 150 and 200 mg/kg ethyl acetate fraction; D1-D3 = groups dosed with 50, 150 and 200 mg/kg n-butanol fraction; E = loperamide-treated group*

Gastrointestinal anti-motility mechanism of CP-EF4

The gastrointestinal anti-motility effects of CP-EF4 were evaluated by the impacts of CP-EF4 and carbachol, metoclopramide and serotonin interactions on gastric emptying (GE) in rats as presented in Figure 1. The GE was significantly ($p < 0.05$) enhanced by carbachol (75.6 ± 5.1 %), serotonin (80.2 ± 8.3 %), and metoclopramide (92.5 ± 5.7 %) relative to control rats (48.1 ± 3.6 %). Similarly, GT was significantly ($p < 0.05$) enhanced by 10 mg/kg carbachol, 10 mg/kg serotonin and 30 mg/kg metoclopramide by 83.5 ± 7.6, 69.1 ± 5.2 and 68.9 ± 9.4 % respectively, relative to control animals (44.3 ± 5.5 %). Pre-treatment with CP-EF4 significantly neutralized the carbachol effect on GE (75.6 ± 5.1 vs. 27.6 ± 4.3 %), and also significantly ($p < 0.05$) blocked carbachol action on GT, without any influence on GE and GT.

DISCUSSION

Combretum platypetalum extract is used in folk medicine in the management of swelling, mumps, lower backache, headache, fever and tonic, eye problems, blindness, malaria, cough, sexually transmitted disease, helminthiasis, post-partum bleeding and diarrhea [6-8]. Its antioxidant, hypoglycaemic and hypolipidemic activities have been documented [7]. Despite these, and several other claims of its ethno-pharmacological relevance across Africa, extensive studies on the anti-diarrhoeal activities are yet to be determined. In this study, the anti-diarrhoeal activity encompassing anti-motility and anti-secretory activities of *C. platypetalum* extract, fractions and VLC sub-fractions were investigated using several rat models.

Available antidiarrheal drugs are limited by bronchospasm, vomiting, and fever [9]. The WHO's Diarrhoeal Disease Control Program promotes studies with natural products, especially traditional medicinal plants, for managing diarrhea globally. Numerous plants with antidiarrheal properties have been documented. These plants contain secondary metabolites responsible for their pharmacological activity, which can be extracted using various solvents [21]. In this study, *C. platypetalum*'s methanol extract was separated with hexane, EtOAc, and butanol. The CP-EF soluble fraction was further separated using solvent combinations, reflecting the polarity differences of the solvents used [15]. Hydro-alcoholic co-solvents like 80-96% methanol provide a high extraction yield but lack a universal standard solvent [22].

Understanding the phytochemical composition in plants can explain the mechanism of their bioactivity. Identifying bioactive plants is the first step in discovering lead compounds [15,23]. CP-EF contains moderately polar phytochemicals such as alkaloids, flavonoids, saponins, and tannins, while CP-HF includes non-polar constituents such as steroids and terpenoids. The qualitative phytochemical tests showed that *C. platypetalum* is rich in these compounds. The anti-diarrhoeal activity mediated via anti-motility and anti-secretory activities of *C. platypetalum* could be due to the plant's high concentration of alkaloids, terpenoids, flavonoids, steroids, tannins, glycosides, saponins and phenols suggesting their roles in the anti-diarrheal activity of the plant [6,8].

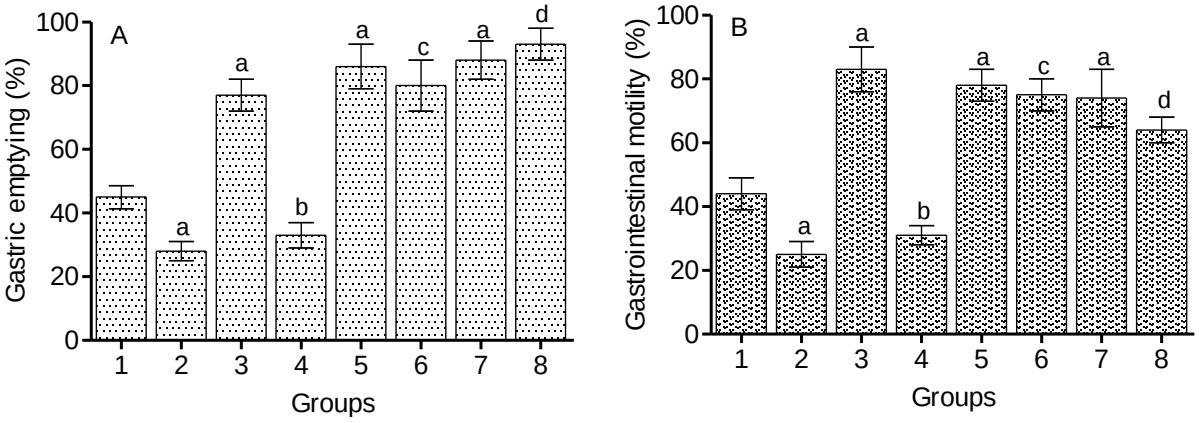

Figure 1: Effect of CP-EF4 on (A) GE and (B) GT. *[a]P < 0.05 vs group 1; [b]P < 0.05 vs carbachol; [c,d]P > 0.05 (not significant) vs serotonin and metoclopramide, respectively; normal saline (group 1), CP-EF4 only, 200 mg/kg (group 2), carbachol, 10 mg/kg (group 3), CP-EF4 + carbachol (group 4), serotonin, 10 mg/kg (group 5), CP-EFv4 + serotonin (group 6), metoclopramide, 30 mg/kg (group 7), CP-EF4 + metoclopramide (group 8)*

Natural products from medicinal plants are crucial for developing new pharmaceutical compounds [13]. However, their safety, including potential interactions with prescribed drugs, is a significant concern. The numerous active compounds in plant medicines increase the likelihood of such interactions [16]. These compounds may act synergistically, enhancing therapeutic effects [14]. Therefore, toxicological evaluation of extracts is necessary before pharmacological testing. The acute toxicity of CP-ME was assessed to determine its safety and optimal dose for pharmacological screening. The result showed an LD_{50} > 5000 mg/kg, indicating a high safety margin for the methanol extract.

The anti-diarrhoeal activity of *C. platypetalum* was evaluated using three assay models in rats. Diarrhea, characterized by the regular passage of liquid or semisolid faeces, involves declined fluid absorption, increased intestinal motility, and increased secretions [17]. This leads to electrolyte loss, dehydration, and potentially death. The castor oil-induced diarrhea model was applied to assess the test extract's antidiarrhoeal activity [19]. Castor oil results in an imbalance in the small intestine's secretory and absorptive processes, leading to irritation and inflammation. Ricinoleic acid, the active constituent, stimulates fluid and electrolyte secretion, mimicking the pathophysiologic processes of human diarrhea [18]. The study confirmed that CP-ME elicited a dose-dependent response, extending diarrhea onset in rats. The highest activity was observed at 400 mg/kg of CP-ME, 200 mg/kg of CP-EF, and 200 mg/kg of CP-EF4. Lower doses did not significantly prolong diarrhea onset, possibly due to insufficient test extract. Higher doses are needed for optimal antimotility and antisecretory effects. *C. platypetalum* also significantly decreased the average weight and number of wet faeces in the castor oil-induced diarrhea model, indicating recovery [16,18].

Decreased stool frequency and volume were linked to antisecretory and antimotility mechanisms, reducing intestinal muscle tone and peristalsis, and slowing fecal movement through the GI tract [18]. Gastrointestinal motility decreased significantly with all test doses of CP-ME. Studies confirm increased peristalsis in diarrhea cases [19]. Antidiarrheal agents, like loperamide, reduce GI peristalsis by activating μ receptors, inhibiting acetylcholine release, enhancing colonic segmentation, and slowing peristalsis, thus enhancing transit time [18]. All plant extract doses showed significant peristaltic movement reduction. The other model in this study, the castor oil-induced enteropooling model, assessed the inhibition of gastrointestinal secretions after castor oil administration [16]. Results showed that CP-ME, CP-EF, and their fractions reduced the intestinal contents' weight and volume in a dose-dependent manner when compared to the negative control. The highest effect was observed with 400 mg/kg of CP-ME, comparable to the standard. The ricinoleic acid content of castor oil induces gastrointestinal secretions through the activation of the nitric oxide pathway and prostaglandin synthesis [17]. This proposed mechanism, which could not be established is the blockade of the nitric oxide pathway, halting GI secretion.

To further understand the anti-motility mechanism, the effects of carbachol, metoclopramide, and serotonin on muscarinic, dopaminergic, and 5-HT pathways were evaluated. The study found that CP-EF4's ability to alter the propulsive effects of carbachol suggests that the muscarinic pathway may be a target for *C. platypetalum*'s inhibitory effects. However, this study did not further explore the possibility that *C. platypetalum*'s antimotility mechanism was due to its blockade of the muscarinic receptor beyond its ability to suppress both GE and GT induced by carbachol. Phytochemical screening results support various antidiarrheal mechanisms. Studies suggest that flavonoids and tannins increase the reabsorption of colonic water and electrolyte. Tannins enhance the resistance of intestinal mucosa by hindering secretion, normalizing the transport of water, and reducing intestinal transit. Terpenoids and saponins can inhibit autacoids like prostaglandins and histamines while Phenolics and alkaloids also suppress intestinal motility [18]. Flavonoids display diverse biological activities, including enzyme inhibition (prostaglandin synthase, cyclooxygenase, and lipoxygenase), contributing to their antidiarrheal effects.

CONCLUSION

Combretum platypetalum possesses significant antidiarrheal activity through an antimotility mechanism. This activity is mediated by inhibiting carbachol-induced gastrointestinal transit and gastric emptying. Consequently, the study offers scientific validation for the use of *C. platypetalum* in ethnomedicine and furnishes empirical data supporting natural product-based management of diarrhea.

DECLARATIONS

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Ethical approval

Animal study was approved by the Committee on Animal Ethics of the Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka (Ref no. FPSRE/UNN/21/0009).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Concept and design- Christopher Ezugwu and Christopher Ugwoke; Data acquisition- Cecilia Idoko; Data analysis/interpretation- Charles Nnadi and Edith Diovu; Drafting manuscript- Edith Diovu; critical revision of manuscript- Christopher Ezugwu, Charles Nnadi and Christopher Ugwoke; statistical analysis- Cecilia Idoko; final approval of draft manuscript- all authors.

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