Research Article

Evaluation of antidiarrhoeal activity of methanol extract of *Combretum hypopilinum* Diels (Combretaceae) leaves in mice

Mubarak Hussaini Ahmad^{1,2*}, Abdulkadir Umar Zezi¹, Sherifat Bola Anafi¹, Mustapha Mohammed^{3,4}, Rabi Nuhu Danraka¹, Zakariyya Alhassan¹

¹Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria

²School of Pharmacy Technician, Aminu Dabo College of Health Sciences and Technology, Kano State, Nigeria

³Department of Clinical Pharmacy and Pharmacy Practice, Ahmadu Bello University, Zaria, Nigeria

⁴School of Pharmaceutical Sciences, Universiti Sains, Malaysia, 11800, Pulau Penang, Malaysia

Received: 22 April 2020

Revised: 15 May 2020

Accepted: 27 May 2020

Abstract

Background: Medicinal plants have been used in traditional medicine for the management of several diseases. The plant *Combretum hypopilinum* has been used for the treatment of diarrhoea, dysentery and other diseases in Nigeria without scientific validation. **Objective:** This study aims to investigate the antidiarrhoeal potential of the methanol extract of *Combretum hypopilinum* leaves (MECH) in mice. **Material and methods:** Phytochemical screening was conducted using standard methods and the acute toxicity study was conducted according to the Organization of Economic Co-operation and Development (OECD) 423 guideline. The antidiarrhoeal activity of MECH was evaluated using castor oil-induced diarrhoea, castor oil-induced enteropooling and intestinal motility test in mice after oral administration of distilled water (10 ml/kg), MECH (250, 500 and 1,000 mg/kg) and loperamide (5mg/kg). **Results:** Phytochemical screenings of MECH were revealed the presence of flavonoids, glycosides, saponins, tannins, steroids, triterpenes and alkaloids. The oral median lethal dose (LD₅₀) of MECH was estimated to be greater than 5,000 mg/kg. The extract at 500 and 1,000 mg/kg significantly ($p < 0.05$) reduced the number of diarrhoeal stools. In addition, the extract significantly ($p < 0.05$ and $p < 0.01$) decreased the volume of intestinal fluid at 500 and 1,000 mg/kg respectively. There was significant ($p < 0.05$ and $p < 0.001$) decrease in the charcoal movement at 500 and 1,000 mg/kg respectively. **Conclusion:** The methanol extract of *Combretum hypopilinum* leaves contain bioactive compounds that may be responsible for its antidiarrhoeal activity possibly through antisecretory and antimotility effects, thus supporting its folkloric claim.

Keywords: *Combretum hypopilinum*, antidiarrhoeal activity, castor oil, diarrhea, intestinal motility

Introduction

Diarrhoea is a disease affecting gastrointestinal tract (GIT) that increases the passage of intestinal contents, fluidity and frequency of semi-solid or watery faeces three or more times daily (Evi et al., 2018). This disease is among the top health care challenges that cause morbidity and mortality particularly

in developing nations (Mehesare et al., 2017). The highest death rate of diarrhoea occurs in children less than five years of age (Evi et al., 2018).

Despite international organizations continued efforts to combat diarrhoea, it remains one of the highest killer diseases accounting for about 7.1 million deaths annually (Pandey et al., 2012). About 78% of deaths caused by diarrhoea happen in Africa and South East Asia (Tadesse, et al., 2017). The deaths caused by diarrhoea in children is more than a combination of deaths caused by acquired immunodeficiency syndrome (AIDS), malaria and measles (Black et al., 2010). Several synthetic drugs such as diphenoxylate and loperamide are used for the treatment of

*Address for Corresponding Author:

Mubarak Hussaini Ahmad

Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria

School of Pharmacy Technician, Aminu Dabo College of Health Sciences and Technology, Kano State, Nigeria

Email: mubarakhussainiahmad@gmail.com

DOI: <https://doi.org/10.31024/apj.2020.5.2.3>

2456-1436/Copyright © 2020, N.S. Memorial Scientific Research and Education Society. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

diarrhoea but they possess adverse effects which limited their proper use (Pandey et al., 2012). In addition, these drugs are expensive and inaccessible by many people living in rural areas (Wansi et al., 2017).

The use of medicinal plants is a common practice in many African countries for the treatment of diarrhoea (Okpara et al., 2017). These medicinal plants are the only believable, available and affordable treatment options for the treatment of many diseases including diarrhoea in many rural communities (Njume and Goduka, 2012). Several studies were conducted to ascertain the antidiarrhoeal activity of medicinal plants for their effects on gastrointestinal transit time and secretion of water and electrolytes (Chaddha et al., 2013). Therefore, the World Health Organization (WHO) recommends traditional use of medicinal plants for the treatment and prevention of diseases including diarrhoea (Agbon et al., 2013).

The plant *Combretum hypopilinum* Diels (Combretaceae) is a synonym of *Combretum collinum* Fresen sub-specie hypopilinum (Diels). It is a shrub that annually sheds its leaves which is small to medium in size with a lot of branches about 12–17 m tall height. It grows in various soils with semi-arid to moderate rainfall conditions (Idoh et al., 2018). This plant is commonly known in different African languages as *Jar taramniya* or *jar ganye* (Hausa), *buski daneehi* (Fulfulde), *katankara* (Kanuri) and *aro* (Yoruba).

The leaves of *Combretum hypopilinum* have been used in traditional medicine for the treatment of diarrhoea in African region (Stark et al., 2013). However, there is no any scientific investigation conducted on this plant to ascertain its claimed antidiarrhoeal activity based on the literature search. Therefore, this study aimed to investigate the claimed antidiarrhoeal activity of the plant.

Materials and Methods

Collection and identification of the plant

The whole plant of *Combretum hypopilinum* was collected from Galadimawa, Giwa Local Government Area, Kaduna State, Nigeria in September, 2019 and identified at the Herbarium Section of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria where a voucher number (012063) was obtained by comparing with existing specimen.

Drugs and Chemicals

The drugs and chemicals used for this study included: Methanol (Sigma Aldrich Chemical Co. USA), Castor oil (Bell and Sons, Southport PR9 AL, England), gum acacia, medicinal charcoal (Ultracarbon powder-Merck KGaA Darmstadt, Germany), distilled water, loperamide (Imodium[®], Jansen Pharmaceuticals,

Pakistan) and chloroform (Sigma Chemical Co. USA).

Experimental animals

Swiss albino mice (18-24g) of either sex were obtained from the Animal house facility of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria. The animals were housed in well-ventilated cages under standard laboratory conditions in experimental animal room with 12-hour light/12-hour dark cycle. The animals were fed with standard rodent diet and water *ad libitum*. The experimental procedures were approved by Ahmadu Bello University Ethical Committee on Animal Use and Care (approval number: ABUCAUC/2020/40) and was conducted in accordance with the guidelines for the Care and Use of Laboratory Animals as published by the United State National Institute of Health (NIH Publication No. 85-23, revised 1996).

Extraction of plant material

The leaves of *Combretum hypopilinum* were dried in a shaded environment and intermittently weighed until a constant weight was obtained and then size reduced into a powdered form using pestle and mortar. One kilogram (1 kg) of the powdered leaves material was extracted with 70% v/v methanol using soxhlet apparatus. The solvent was removed by placing the extract on a water bath set at 45 °C. The extract was then stored in a tightly labelled container for subsequent experiments. It was subsequently referred to as Methanol Leaves Extract of *Combretum hypopilinum* (MECH). The solution of MECH was freshly prepared with distilled water for each study. The percentage yield of the extract was calculated using the following formula:

$$\text{Percentage yield} = \frac{\text{Weight of the extract}}{\text{Total Weight of the crude leaves}} \times 100$$

Phytochemical Screening

The presence of phytochemicals such as steroids, triterpenes, flavonoids, alkaloids, saponins, tannins, glycosides and anthraquinones in MECH was determined as previously described by Trease and Evans, (2002), and Sofowora, (1993).

Acute toxicity study

The acute toxicity study of MECH in mice was conducted according to the method described by the Organization of Economic Co-operation and Development (OECD) 423 guideline (OECD, 2001). The oral LD₅₀ was determined using nulliparous and non-pregnant female mice. The mice were fasted 3-4 hours before the administration of the extract. In the first phase, a group of three mice each was

administered MECH orally at a dose of 5000 mg/kg. Food but not water was withheld further for one to two hours after administration of the extract. The mice were observed for signs and symptoms of toxicity at least once every 30 minutes for the first four hours and then daily for 14 days, after which the study was terminated.

Castor oil-induced diarrhoea

The method described by Awouters et al. (1978) was adopted. Mice were fasted for 18 hours and randomly divided into five groups containing five animals in each group. Three groups were separately administered MECH (250, 500 and 1000 mg/kg) while distilled water (10 ml/kg) and loperamide (5 mg/kg) were separately administered orally to the negative control and standard groups respectively.

After 60 minutes of treatment, each mouse was orally administered 0.5 ml of castor oil and then placed in separate cages whose floor were lined with white filter paper for an observation period of 4 hours. The parameters observed were the time of onset of diarrhoea, number of wet and dry faeces and the total number of faeces excreted. Reduction in the number of wet faeces in test groups as compared to the negative control group was considered as evidence of antidiarrhoeal activity and expressed as percentage inhibition of diarrhoea as follows:

$$\% \text{ inhibition of diarrhoea} = \frac{\text{No of wet faeces in negative control} - \text{No of wet faeces in a test group}}{\text{Total No of wet faeces in negative control group}} \times 100$$

Castor Oil-Induced Enteropooling

Intestinal fluid accumulation was determined by adopting the method of Robert et al., (1976). Mice were fasted for 18 hours and randomly divided into five groups containing five animals in each group. Three groups were separately administered MECH (250, 500 and 1000 mg/kg) while distilled water (10 ml/kg) and loperamide (5 mg/kg) were separately administered orally to the negative control and standard groups respectively.

After 60 minutes of treatment, 0.5 ml of castor oil was orally administered to each mouse. Then 30 minutes later, the mice were sacrificed. The mice were dissected and their small intestines were immediately removed from pylorus to caecum after ligation at the pyloric sphincter and at the ileo-caecal junction respectively. Each intestine was weighed, and the intestinal content was collected into a graduated syringe and the volume was measured. The empty intestine was reweighed to determine the weight of the intestinal content. Percentage reduction in volume and weight of intestinal content were determined as follows:

$$\% \text{ decrease in the volume of intestinal content} = \frac{A - B}{A} \times 100$$

Where;

A= Volume of intestinal contents in the negative control group

B= Volume of intestinal content in the test group

C= Weight of intestinal content in the negative control group

D= Weight of intestinal content in the test group

Castor oil-induced intestinal transit

An intestinal motility test was carried out according to the method of Di Carlo et al. (1993). The mice were fasted for 18 hours and randomly divided into five groups containing five animals in each group. Three groups were separately administered MECH (250, 500 and 1000 mg/kg) while distilled water (10 ml/kg) and loperamide (5 mg/kg) were separately administered orally to the negative control and standard groups respectively.

After 30 minutes of treatment, the mice were orally administered castor oil (0.5 ml/mouse). Then 30 minutes later, each mouse was administered 0.5 ml charcoal meal (10% activated charcoal suspension in 5% acacia). Then 30 minutes later, the mice were sacrificed. The mice were dissected, their small intestines were immediately removed from pylorus to caecum and placed lengthwise on moist filter paper and measured the distance travelled by charcoal along the length of the intestine using a calibrated ruler. The distance moved by the charcoal relative to the total distance of the small intestine was expressed as a peristaltic index and percentage inhibition was calculated as follows:

$$\% \text{ Peristaltic index of charcoal meal} = \frac{\text{distance travel by charcoal meal (cm)}}{\text{Total length of the small intestine (cm)}} \times 100$$

$$\% \text{ inhibition of charcoal movement} = \frac{A - B}{A} \times 100$$

A = Mean movement of charcoal meal in the negative control group

B = Mean movement of charcoal meal in the test group

Statistical Analysis

The statistical analyses were performed using the IBM SPSS Statistics for Windows, Version of 22.0. Armonk, NY: IBM Corp. Results obtained were presented as the mean $\pm$ standard error of mean (SEM) in tables and figures. The comparisons of means of the different groups were done using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test for multiple comparisons. The results were considered significant at $p \leq 0.05$.

Results

Percentage yield of methanol extract of *Combretum hypopilinum* leaves

One thousand gram (1,000 g) of the powdered leaves material of *Combretum hypopilinum* yielded 126.24 g of MECH (12.62 %w/w).

Phytochemical Screening

The phytochemical screening of MECH revealed the presence of flavonoids, glycosides, saponins, tannins, steroids, triterpenes and alkaloids. However, anthraquinones were absent. The details of the phytochemical screening results are presented in table 1.

Acute toxicity study

The oral administration of MECH at a dose of 5,000 mg/kg did not produce any visible sign of toxicity and mortality throughout the observation period of 14 days. Therefore, the oral median lethal dose (LD_{50}) of MECH was estimated to be higher than 5,000 mg/kg in mice.

Castor oil-induced diarrhoea

The mice in the group treated with distilled water produced copious diarrhoea at (54.60 ± 13.10) minutes after castor oil administration. The MECH at all the doses used produced a non-

significant ($p>0.05$) and dose-dependent decrease in the time of onset of diarrhoea to (90.20 ± 25.87), (107.80 ± 13.63) and (148.40 ± 20.59) minutes respectively compared to the negative control group. There was significant ($p<0.05$) delay in onset of diarrhoea (158.00 ± 30.42) minutes in the group treated with the standard drug, loperamide (5 mg/kg) compared with the negative control group. The standard drug did not produce significant ($p>0.05$) difference in the onset of diarrhoea compared to all the tested doses of the extract. The extract produced dose-dependent inhibition of the number of wet faeces (46.67%, 56.67% and 60.00%). There was significant ($p<0.05$ and $p<0.01$) decrease in the number of diarrhoea stools in the groups treated with the higher doses (500 and 1,000 mg/kg) of the extract and loperamide (5 mg/kg) respectively compared with the negative control group. The details of the effect of MECH on castor oil-induced diarrhoea are shown in table 2.

Castor oil-induced enteropooling

There was no significant ($p>0.05$) change in the volume of intestinal fluid in the group treated with the lowest dose of the extract (250 mg/kg). However, there was significant ($p<0.05$ and $p<0.01$) reduction (40.00% and 51.67%) in the

Table 1. Phytochemical constituents of methanol leaves extract of *Combretum hypopilinum*

Phytochemical constituents	Results
Flavonoids	+
Cardiac glycosides	+
Saponins	+
Tannins	+
Alkaloids	+
Steroids and triterpenes	+
Anthraquinones	-

Key: + = Present and - = Absent

Table 2. Effect of methanol leaves extract of *Combretum hypopilinum* on Castor oil-induced diarrhoea in mice

Treatments (mg/kg, <i>p.o</i>)	Onset of Diarrhea (min)	Total number of wet Feces	Total number of Feces	% inhibition of diarrhoea
DW (10 ml/kg)	54.60 ± 13.10	6.00 ± 1.05	6.00 ± 1.05	-
MECH (250)	90.20 ± 25.87	3.20 ± 0.80	4.80 ± 1.39	46.67
MECH (500)	107.80 ± 13.63	$2.60 \pm 0.68^*$	3.40 ± 0.68	56.67
MECH (1,000)	148.40 ± 20.59	$2.40 \pm 0.68^*$	4.00 ± 1.14	60.00
LOP (5)	$158.00 \pm 30.42^*$	$1.60 \pm 0.40^{**}$	1.60 ± 0.40	73.33

Data are presented as Mean $\pm$ SEM; * $p<0.05$ and ** $p<0.01$ versus distilled water group (One way ANOVA followed by Bonferroni's post hoc test), min= minutes, DW= distilled water, MECH= Methanol leaves extract of *C. hypopilinum*, LOP= *p.o*= per oral, Loperamide, n=5

Table 3. Effect of methanol leaves extract of *Combretum hypopilinum* on Castor oil-induced enteropooling in mice

Treatments (mg/kg, <i>p.o</i>)	Volume of intestinal content (ml)	Weight of intestinal content (g)	% reduction in volume of intestinal content	% reduction in weight of intestinal content
DW (10ml/kg)	0.60 ± 0.04	0.76 ± 0.07	-	-
MECH (250)	0.56 ± 0.07	0.72 ± 0.07	6.67	5.26
MECH (500)	0.36 ± 0.04*	0.62 ± 0.04	40.00	18.42
MECH (1,000)	0.29 ± 0.06**	0.52 ± 0.09	51.67	31.58
LOP (5)	0.23 ± 0.04***	0.42 ± 0.09*	61.67	44.74

Data are presented as Mean ± SEM; * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ versus distilled water group (One way ANOVA followed by Bonferroni's post hoc test), DW= distilled water, MECH= Methanol leaves extract of *C. hypopilinum*, LOP= Loperamide, *p.o*= per oral, n=5

Figure 1. Effect of methanol leaves extract of *Combretum hypopilinum* on castor oil-induced enteropooling test in mice. Data are presented as Mean ± SEM; * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ versus distilled water group (One way ANOVA followed by Bonferroni's post hoc test), DW= distilled water, MECH= Methanol leaves extract of *C. hypopilinum*, LOP= Loperamide, n=5.

Figure 2. Effect of methanol leaves extract of *Combretum hypopilinum* on castor oil-induced intestinal transit test in mice. Data are presented as Mean ± SEM; * $p < 0.05$ and ** $p < 0.001$, versus distilled water group (One way ANOVA followed by Bonferroni's post hoc test), DW= Distilled Water, MECH= Methanol leaves extract of *C. hypopilinum*, LOP= Loperamide, n=5.

volume of intestinal fluid in the groups treated with the higher doses of the extract (500 and 1,000 mg/kg) respectively, compared with the negative control group. The group treated with loperamide (5 mg/kg), produced significant ($p < 0.001$) reduction (61.67%) in the volume of the intestinal fluid compared with the negative control group. The results of the effect of MECH on castor oil-induced enteropooling are shown in table 3 and Figure 1.

Castor oil-induced intestinal transit

The charcoal travelled 75.46% of the total length of the small intestine in the group treated with distilled water. The group treated with 250 mg/kg of MECH did not show significant ($p > 0.05$) difference in charcoal movement. However, there was significant ($p < 0.05$ and $p < 0.001$) decrease (38.55% and 74.54%) in charcoal movement in groups treated with higher doses of the

extract (500 and 1,000 mg/kg) respectively compared with the negative control. The group treated with loperamide (5 mg/kg) also produced significant ($p < 0.001$) decrease in charcoal movement with a percentage inhibition of 65.66% compared with the negative control. The effect of the extract at the highest dose was higher than that of the standard drug. The details of the effect of MECH on castor oil-induced intestinal transit test are shown in table 4 and figure 2.

Discussion

Several scientific evaluations have been carried out to investigate medicinal plants used traditionally for the treatment of diarrhoea by evaluating their effects on gastrointestinal propulsive movement as well as fluid and electrolyte secretion using animal models of diarrhoea (Mekonnen et al., 2018). The present study investigated the

Table 4. Effect of methanol leaves extract of *Combretum hypopilinum* on castor oil-induced intestinal transit test in mice

Treatments (mg/kg, <i>p.o</i>)	Length of Small Intestine (cm)	Distance travelled by charcoal (cm)	Peristaltic index (%)	% inhibition of charcoal movement
DW 10ml/kg	44.00 ± 1.70	33.20 ± 2.58	75.46	-
MECH (250)	40.60 ± 0.69	24.20 ± 2.89	59.61	27.11
MECH (500)	43.80 ± 1.36	20.40 ± 2.38*	46.58	38.55
MECH (1,000)	42.00 ± 0.84	08.50 ± 1.39**	20.24	74.40
LOP (5)	39.20 ± 1.16	11.40 ± 2.89**	29.08	65.66

Data are presented as Mean ± SEM; * $p < 0.05$ and ** $p < 0.001$, versus distilled water group (One way ANOVA followed by Bonferroni's post hoc test), DW= Distilled Water, MECH= Methanol leaves extract of *C. hypopilinum*, LOP= Loperamide, *p.o*= per oral, $n=5$.

antidiarrhoeal potential of *Combretum hypopilinum*.

Phytochemical screening suggests potential biological and harmful effects of medicinal plants. Therefore, it is important to test the presence of phytochemicals responsible for biological activities in medicinal plants (Pandey et al., 2013). The present study revealed the presence of secondary metabolites such as flavonoids, saponins, tannins, alkaloids, steroids and triterpenes in MECH. Therefore, it can be suggested that the presence of these phytochemicals in MECH may be responsible for its antidiarrhoeal activity. Tannins were reported to have spasmolytic and smooth muscle relaxant effect, flavonoids prevent intestinal secretion caused by prostaglandin E_2 , saponins inhibit the release of histamine, terpenoids also inhibit prostaglandin release and phenols inhibit intestinal secretion and motility. These phytochemicals reduce intestinal hypersecretion and hypermotility and subsequently inhibit diarrhoea (Mekonnen et al., 2018).

Acute toxicity study is used to evaluate likely clinical signs that may result following administration of a chemical substance and gives a range of doses to be used in subsequent experiments (Mbiri et al., 2017). From the acute toxicity test conducted in this study, there were no physical signs of toxicity and mortality in mice treated orally with 5,000 mg/kg of MECH throughout the observation period of 14 days. These findings estimated that the LD_{50} of the extract is greater than 5,000 mg/kg and may be relatively safe after acute oral administration in mice. Li et al. (2019) suggested that following acute oral administration of a chemical substance during a 14 days period if there is no death and any signs of toxicity at a dose of 5,000 mg/kg, the substance can be considered as relatively safe.

Diarrhoea is a common disease caused by four pathophysiological mechanisms namely; increased intestinal osmolarity, increased water and electrolytes secretion, decreased water and electrolytes absorption and intestinal hypermotility

that cause decreased intestinal residence time. Therefore, antimotility and antisecretory drugs are majorly used to alleviate the disease (Mekonnen et al., 2018).

Castor oil is metabolized to ricinoleic acid in the duodenum by intestinal lipases. The ricinoleic acid is poorly absorbed in the small intestine. It causes irritation and inflammation of the intestinal mucosa which subsequently results to production and release of autocoids and prostaglandins that stimulate intestinal motility and change electrolyte permeability of the intestinal mucosa and cause increased fluid secretion and diarrhoea (Mehesare et al., 2019). Based on the result of this study, the MECH showed a non-significant and dose-dependent delay in onset of diarrhoea and significant inhibition of diarrhoea stools. The inhibitory effect suggested that the extract may possess its antidiarrhoeal activity possibly by stimulating the re-absorption of water and electrolytes from intestinal lumen as well as antagonizing the effect of prostaglandin which plays a role in the pathophysiology of diarrhoea. Nwabunike et al. (2018) suggested that antidiarrhoeal action of medicinal plants in castor oil-induced diarrhoea is likely due to antisecretory activity.

Castor oil and its active metabolite ricinoleic acid alter electrolytes and water movement across the intestinal mucosa, thereby enhance secretion and fluid accumulation in the intestine (Saheed and Tom, 2016). The fluid accumulation is also as a result of the release of platelet-activating factor, nitric oxide, tachykinins and cAMP (de Oliveira et al., 2017). In this study, the MECH significantly reduced the volume of intestinal fluid. The reduction in the intestinal fluid may likely be due to inhibition of prostaglandin, platelet-activating factor, nitric oxide, tachykinins and cAMP biosynthesis that eventually inhibit fluid hypersecretion, enhanced intestinal fluid and

electrolyte absorption, decreased intestinal motility and overall decrease intraluminal fluid accumulation.

Diarrhoea also develops as a result of a change in motility and accumulation of fluid in the intestine (Rahman et al., 2013). The gastrointestinal motility test model is used to investigate the compounds that possibly possess intestinal ant motility effect (Shoba and Thomas, 2014). Going by the present study; the MECH reduced the intestinal movement of charcoal. This decrease in the charcoal movement suggested its ant motility effect which may prolong small intestinal residence time and promote water and electrolyte absorption.

Loperamide is an antidiarrhoeal drug that decreases intestinal motility by acting at myenteric plexus and increases small intestinal residence time which allows adequate time for water absorption (Emudainohwo et al., 2015). In this study, loperamide also significantly inhibited castor oil-induced diarrhoea, castor oil-induced enteropooling and intestinal movement which shows its antisecretory and ant motility effects. Similarly, the MECH produced antisecretory and ant motility results. Therefore, the extract may possess its antidiarrhoeal activity by similar actions to loperamide.

Conclusion

The findings obtained in this study suggest that the MECH contain bioactive compounds that may be responsible for antidiarrhoeal potential possibly due to antisecretory and ant motility effects. This provides scientific evidence for the use of *Combretum hypopilinum* in the treatment of diarrhoea in traditional medicine. However, further investigation should be carried out to evaluate its possible mechanism of antidiarrhoeal activity.

Conflict of interest

The authors declare that no conflict of interest exists.

Acknowledgements

The authors are thankful to the technical staff of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria particularly Mallam Mu'azu Mahmud for their technical support during the conduct of this research.

Funding Statement

This research did not receive any funding from any specific funding body agencies in the public, commercial, or not-for-profit sectors.

References

Agbon AN, Kwaneshie HO, Hamman WO. 2013. Antidiarrheal activity of aqueous fruit extract of *Phoenix dactylifera* (DATE PALM) in Wistar rats. *British Journal of Pharmacology and Toxicology*, 4(3): 121-127.

Awouters F, Niemegeers CJE, Lenaerts FM, Janssen PAJ. 1978.

Delay of castor oil diarrhoea in rats: a new way to evaluate inhibitors of prostaglandin biosynthesis. *Journal of Pharmacy and Pharmacology*, 30(1): 41-45.

- Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, Bassani DG, Eisele T. 2010. Global, regional, and national causes of child mortality in 2008: a systematic analysis. *The lancet*, 375(9730): 1969-1987.
- Chaddha V. A. R. U. N, Kushwah AS, Shrivastava VA. I. B. H. A. V. 2013. An Importance of herbal drugs as antidiarrheal: a review. *International Journal of Research in Applied, Natural and Social Sciences*, 1(7): 25-28.
- de Oliveira FR, Quirino ZG. M, Diniz FFM., Marinho AF, Tavares JF, Batista LM. 2017. *Maytenus erythroxylon* Reissek (Celastraceae) ethanol extract presents antidiarrheal activity via ant motility and antisecretory mechanisms. *World Journal of Gastroenterology*, 23(24): 4381-4389.
- Di Carlo G, Autore G, Izzo AA, Maiolino P, Mascolo N, Viola P, Capasso F. 1993. Inhibition of intestinal motility and secretion by flavonoids in mice and Rats: structure-activity relationships. *Journal of Pharmacy and Pharmacology*, 45(12): 1054-1059.
- Emudainohwo JO, Goodies EM, Daniel EE, Erhirhie EO. 2015. An investigation into the anti-diarrhoeal effects of aqueous and ethanol stem bark extracts of *Alchornea cordifolia* in Wistar rats. *Journal of Pharmacognosy and Phytochemistry*, 4(1): 183-187.
- Evi BE, Charity IO, Fabian A, Josephine O, Philip O, Victoria OI, Buniyamin A. 2018. Evaluation of the antidiarrhoeal and ex-vivo intestinal activity of *Hymenocardia acida* Tul.(Phyllanthaceae) methanol leaves extract and chloroform fraction using animal models. *Nigerian Journal of Pharmaceutical and Applied Science Research*, 7(1): 34-43.
- Idoh K, Dosseh K, Kpatcha T, Agbonon A, Gbeassor M. 2018. Protective effect of *Combretum hypopilinum* diels: Root bark extract against CC14-Induced hepatotoxicity in wistar rats. *Pharmacognosy Research*, 10(3): 325-331.
- Li Y, Kandhare AD, Mukherjee AA., Bodhankar SL. 2019. Acute and sub-chronic oral toxicity studies of hesperidin isolated from orange peel extract in Sprague Dawley rats. *Regulatory Toxicology and Pharmacology*, 105(1), 77-85.
- Mbiri JW, Musila MN, Ngai DN, Njagi SM, Mbinda WM, Ngugi MP. 2017. Acute and sub-chronic oral toxicity study of methanolic extract of *Caesalpinia volkensii* (harms). *Journal of Drug Metabolism and Toxicology*, 8(1): 222-229.

- Mehesare SS, Waghmare SP, Thorat MG, Hajare SW, Itankar PR, Siddiqui MF, Ali SS. 2017. Evaluation of antidiarrhoeal activity of polyherbal preparation. *Journal of Pharmacognosy and Phytochemistry*, 6(6): 723-725.
- Mehesare SS, Waghmare SP, Thorat MG, Hajare SW, Itankar PR, Ali SS. 2019. Evaluation of antidiarrhoeal activity of extract of unripe fruit of *Aegle marmelos*. *Journal of Pharmacognosy and Phytochemistry*, 8(4): 2390-2392.
- Mekonnen B, Asrie AB, Wubneh ZB. 2018. Antidiarrheal Activity of 80% Methanolic Leaves Extract of *Justicia schimperiana*. *Evidence-Based Complementary and Alternative Medicine*, 2018.
- Njume C, Goduka NI. 2012. Treatment of diarrhoea in rural African communities: an overview of measures to maximise the medicinal potentials of indigenous plants. *International Journal of Environmental Research and Public Health*, 9(11): 3911-3933.
- Nwabunike IO, Okoli CO, Ezeugwe P, Ezike AC, Onyeto CA, Mbaaji FN, Onwuka AM. 2018. Antidiarrhoeal potentials of *Schwenckia Americana* L. (Solanaceae): a comparative study of the aerial part and root extracts. *International Journal of Pharmacy and Pharmaceutical Sciences*, 10(1): 195-201.
- OECD Guidelines for the Testing of Chemicals (No. 423). Acute Oral Toxicity-Acute Toxic Class Method. (Adopted on 17 December, 2001).
- Okpara JO, Ambali FS, Ayo JO, Ayo YO, Suleiman MM. 2017. Antidiarrhoeal and Antimotility Activity of the Maize Silk (*Stigmas maydis*) Aqueous Extract. *International Journal of Science and Applied Research*, 2(1): 1-4.
- Pandey P, Mehta A, Hajra S. 2012. Antidiarrhoeal activity of ethanolic extracts of *Ruta graveolens* leaves and stem. *Asian Journal of Pharmaceutical and Clinical Research*, 5(4): 65-68.
- Pandey P, Mehta R, Upadhyay R. 2013. Physico-chemical and preliminary Phytochemical *Psoralea corylifolia*. *Archieve of Applied Science Research*, 5(2): 261-265.
- Rahman MK, Barua S, Islam MF, Islam MR, Sayeed MA, Parvin MS, Islam ME. 2013. Studies on the anti-diarrheal properties of leaves extract of *Desmodium puchellum*. *Asian Pacific Journal of Tropical Biomedicine*, 3(8): 639-643.
- Robert A, Nezamis JE, Lancaster C, Hanchar AJ, Klepper MS. 1976. Enteropooling assay: a test for diarrhea produced by prostaglandins. *Prostaglandins*, 11(5): 809-828.
- Saheed S, Tom AAO. 2016. Research Article Antimicrobial and Antidiarrheal Activities of *Pelargonium luridum* (Andrews) Sweet Root Extracts. *Journal of Pharmacology*, 7: 202-210.
- Shoba FG, Thomas M. 2014. Evaluation of anti-diarrhoeal effect of four medicinal plants on castor oil-induced gastrointestinal motility in mice. *Advances in Applied Science Research*, 5:153-156.
- Sofowora A, 1993. *Medicinal Plants and Traditional Medicine in Africa*. 2nd Edition., Spectrum Books Ltd., Ibadan, Nigeria, ISBN-13: 9782462195, Pages: 289.
- Stark T, Mtui D, Balemba O. 2013. Ethnopharmacological survey of plants used in the treatment of gastrointestinal pain, inflammation and diarrhoea in Africa: future perspectives for integration into modern medicine. *Animals*, 3(1): 158-227.
- Tadesse E, Engidawork E, Nedi T, Mengistu G. 2017. Evaluation of the anti-diarrheal activity of the aqueous stem extract of *Lantana camara* Linn (Verbenaceae) in mice. *BioMed Central Complementary and Alternative Medicine*, 17(1): 190.
- Trease GE, Evans WC. 2002. *Trease and Evans Pharmacognosy*, 15th edition. W.R Saunders, London.
- Wansi SL, Deumeni CRM, Laure S, Kamani P, Sama LF, Tchoumba LM. 2017. Antidiarrhoeal activity of aqueous and methanolic *Alchornea laxiflora* (Euphorbiaceae) leaves extracts in rats. *Journal of Medicinal Plants*, 5(1): 205-211.