European Journal of Medicinal Plants
4(10): 1223-1231, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Phytochemical and Anti-diarrhoeal Properties of Methanolic Leaf Extract of *Maerua crassifolia* Forssk

G. C. Akuodor[1*], J. A. Ibrahim[2], J. L. Akpan[1], A. U. Okorie[1] and B. C. Ezeokpo[1]

[1]Department of Pharmacology and Therapeutics, Faculty of Clinical Medicine, Ebonyi State University, Abakaliki, Nigeria.
[2]Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author GCA designed and managed the literature searches. Authors GCA and JLA carried out the antidiarrhoeal study. Author AUO wrote the first draft of the manuscript. Author BCE wrote the protocol and performed the statistical analysis. Author JAI carried out the Phytochemical analysis. All authors read and approved the final manuscript.

Original Research Article

Received 6th May 2014
Accepted 13th June 2014
Published 9th July 2014

ABSTRACT

Maerua crassifolia Forssk. Leaf is used in African traditional medicine for management of gastrointestinal disorders. The anti-diarrhoeal activity of the methanol extract of *Maerua crassifolia* leaf was investigated in rats. The phytochemical screening was also carried out. The methanol extract of *Maerua crassifolia* leaf dose dependently decreased intestinal propulsion of charcoal meal in rats. *Maerua crassifolia* also exerted significant anti-enteropooling effect in rats. A profound anti-diarrhoea activity was observed when the extract was tested in diarrhoeic rats. The frequency of defecation as well as the wetness of the faecal droppings was significantly reduced. Furthermore, the leaf extract produced 100% inhibition of castor oil-induced diarrhoea in rats. Phytochemical screening revealed the presence of alkaloids, saponins, tannins, terpenoids, flavonoids, steroids, resins and cardiac glycosides. The oral LD_{50} obtained was greater than 5000 mg/kg in rats. The study showed that the methanol extract of *Maerua crassifolia* leaf possesses anti-

**Corresponding author: Email: goddyakuodor@yahoo.com;*

diarrhoeal activity and its action may be linked partly to direct inhibitory effect of the extract on the propulsive movement of the gastrointestinal tract smooth muscle.

Keywords: Maerua crassifolia; leaf extract; phytochemical; antidiarrhoea; rats.

1. INTRODUCTION

Diarrhoea, an important health problem worldwide, especially in developing countries, accounts for more than 5-8 million deaths in infants and children under 5 years, each year [1,2]. The incidence of diarrhoeal disease still remains high despite the efforts of many governments and international organisations to curb it. It is therefore important to identify and evaluate available natural sources of drugs as alternative to currently used anti-diarrhoeal drugs, which are not always free from adverse effects [3]. A number of medicinal plants are traditionally endowed with gastrointestinal properties [4,5]

Maerua crassifolia Forssk which belong to the family Capparaceae is mainly found in the Saharan Africa. In Nigeria, the plant is mainly found in Sokoto and some parts of Zamfara and Katsina States. The plant is called 'Jega' in Sokoto, Nigeria and 'agargar' in Niger Republic. The leaf of this plant has long being used for the treatment of malaria [6], toothache and intestinal diseases [7]. However, the plant has not been experimentally tested for its anti-diarrhoeal activity despite the claim by the local population of its use as anti-diarrhoea. Hence, an effort was made to investigate the same with the leaf extract of the plant in experimentally-induced diarrhoeal in rats.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Fresh leaves of *Maerua crassifolia* were collected in the month of March, 2009 from Sokoto, Sokoto State (North West), Nigeria. The plant was identified and authenticated by Dr. (Mrs) Jemilat A. Ibrahim of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria where a voucher specimen (NIPRD/H/6406) was deposited in the herbarium for reference. The international plant number index is Fl. Aegypt.-Arab. P. Cxiii. 1775 (1 Oct. 1775)

2.2 Extraction of Plant Material

The leaves were washed, cut into smaller pieces and dried at room temperature for 7 days and pulverized to a coarse powder. The coarse powder (500g) was macerated in methanol for 24 h. The resultant filtrate was dried on a water bath at reduced temperature to obtain 13.28% w/w of methanol extract. The extract was stored in an airtight container and used for the study.

2.3 Phytochemical Analysis

The phytochemical screening of methanol extract of *Maerua crassifolia* leaf was carried out to determine the presence of the following compounds; alkaloids, tannins, saponins, terpenoides, flavonoids, steroids, resins, cardiac glycosides, phlobatannins and anthraquinones using standard procedures [8,9,10].

2.4 Animals

Wistar albino rats (200-250 g) of both sexes obtained from animal house of Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria, were used for the study. The animals were housed in cages at room temperature and moisture, under naturally illuminated environment of 12:12 h dark/light cycle. They were fed on standard diet and had free access to water. All animal experiments complied with the 'Principles of Laboratory Animal Care' (NIH publication No. 82-23, revised in 1985) [11].

2.5 Acute Toxicity Study of the Extract

The LD_{50} of the leaf extract was tested to determine the safety of the agent according to the guidelines set by OECD (Organization for Economic Cooperation and development) No. 423 [12]. The study was carried out in two phases. In the first phase, nine rats were randomized into three groups of three rats per group and administered 100, 600 and 1000 mg/kg of the extract orally. The animals were observed for the first 4 h and 24 h for signs of toxicity and mortality. The results of this phase informed the choice of doses for the second phase, in which 2000, 3000 and 5000 mg/kg were administered to another set of three rats per group. The rats were also observed for signs of toxicity such as paw licking, salivation, stretching of the entire body, weakness, respiratory distress, coma and death for 72 h.

2.6 Induction of Diarrhoea with Castor Oil

Anti-diarrhoea activity of the extract was evaluated using the castor oil-induced diarrhoea model in rats [13,14]. Thirty rats fasted for 24 h were randomized into five groups of six rats each. Group 1, which served as the negative control was given 20 ml/kg normal saline. Group 2, 3 and 4 were given 100, 200 and 400 mg/kg of the extract while the positive control group received 4 mg/kg loperamide. All administered orally. One hour after the treatment, rats in all the groups were challenged with 1 ml castor oil, orally. The rats in each group were then placed singly in cages with adsorbent paper on their floors. The diarrhoea episodes were observed for 4 h and the cumulative frequency of wet and formed stools was noted. Percentage inhibition of diarrhoea was calculated using the mean stool frequency and anti-diarrhoea activity determine in terms of percentage protection.

2.7 Intestinal Transit Test

The effect of the extract on gastrointestinal motility was evaluated as previously described by Akuodor et al. [15]. Thirty rats were randomly divided into five groups of six rats each and fasted for 24 h prior to the test, but allowed free access to water. Group 1 which served as negative control was treated with 10 ml/kg normal saline. Group 2, 3 and 4 received 100, 200 and 400 mg/kg of methanol leaf extract of *Maerua crassifolia* orally, respectively. Group 5 served as positive control and was treated with 5 mg/kg Atropine (standard drug) orally. Thirty minutes after drug administration, 1 ml of marker- charcoal meal (5% deactivated charcoal suspension in 10% tragacanth) was administered orally to all animals and thirty minutes later, all were sacrificed. The distance travelled by the marker was then measured and expressed as a percentage of the total length of the small intestine (pylorus to caecum).

2.8 Castor Oil-induced Enteropooling

Intestinal fluid accumulation was determined by the method of [16]. Thirty rats were randomly divided into five groups of six each. Group 1 served as negative control and received 20 ml/kg normal saline, while Groups 2, 3 and 4 received 100, 200 and 400 mg/kg of the extract, respectively. Group 5 received 4 mg/kg loperamide. All administered orally. One hour after, 1 ml castor oil was orally administered to all the animals and one hour later, all rats were sacrificed and their small intestines (pylorus to caecum) removed after ligating the ends. Intestinal contents were collected by milking into a graduated tube, and the volumes measured and recorded. Percentage inhibition of enteropooling was determined by calculating the mean volume of intestinal contents and comparing it with values from the negative control group.

2.9 Statistical Analysis

Results were expressed as mean ± S.E.M. The significant difference between mean was determined using one-way analysis of variance (ANOVA). Statistical significance was established at $P<0.05$.

3. RESULTS

3.1 Phytochemical Screening

Phytochemical screening of the extracts revealed the presence of tannins, saponins, flavonoids, steroids, terpenoids, alkaloids, resins and cardiac glycosides while phlobatannins and anthraquinones were not detected (Table 1).

Table 1. Result of the phytochemical analysis of methanolic leaf extract of *Maerua crassifolia*

Metabolites	Inference
Tannins	+
Saponins	+
Alkaloids	+
Flavonoids	+
Terpenoids	+
Steroids	+
Cardiac glycosides	+
Resins	+
Phlobatannins	-
Anthraquinones	-

Key: + Present; - Absent

3.2 Acute Toxicity Test

There were no lethality or toxic reactions observed at any of the doses of methanolic leaf extract of *Maerua crassifolia* used in the study. All animals were alive, healthy and active during the observation period. The oral median lethal dose of the leaf extract was therefore estimated to be greater than 5000 mg/kg in rats.

3.3 Effect on Castor Oil-induced Diarrhoea

The methanol leaf extract of *Maerua crassifolia* exhibited marked dose-dependent antidiarrhoeal activity in the study. The extract significantly (P<0.05) inhibited both frequency of defecation as well as the wetness of the faecal dropping in rat. Four hundred milligrams per kilogram of the extract produced 100% inhibition of castor oil-induced diarrhoea in rats similar to standard anti-diarrhoeal drug, loperamide (4 mg/kg) (Table 2).

Table 2. Effect of the methanolic leaf extract of *Maerua crassifolia* on castor oil-induced diarrhoea in rats

Treatment	Dose (mg/kg)	Frequency of diarrhoea in 4 h	% Inhibition
Normal saline	20 ml/kg	5.0±0.37	-
M. crassifolia	100	1.33±0.49	74*
	200	0.67±0.21	87*
	400	0.0±0.0	100*
Loperamide	4	0.0±0.0	100*

Results are expressed as mean ± SEM
**significant at P<0.05 when compared to control (n=6)*

3.4 Effect on Intestinal Transit

The methanol extract of *Maerua crassifolia* leaf decreased the propulsive movement of charcoal meal through the gastrointestinal tract (GIT) in a dose-dependent manner. The observation was significantly (P<0.05) different from what was seen in the control group. Four hundred milligrams per kilogram of the extract caused a significant (P<0.05) reduction in distance travelled by the marker. This reduction compares favourably with atropine at 5 mg/kg (Table 3).

Table 3. Effect of the methanolic leaf extract of *Maerua crassifolia* on intestinal motility in rats

Treatment	Dose (mg/kg)	Mean intestinal length (cm)	Mean distance travelled by marker (cm)	% Inhibition
Normal saline	20 ml/kg	41.67±1.45	41.0±1.41	-
M. crassifolia	100	42.67±1.05	15.67±0.67	62*
	200	40.2±11.67	9.0±0.37	78*
	400	39.5±0.62	5.33±0.67	87*
Atropine	5	41.5±1.43	4.17±0.60	90*

Results are expressed as mean ± SEM
**significant at P<0.05 when compared to control (n=6)*

3.5 Anti-enteropooling Effect

A considerable increase was observed in the intestinal fluid volume of the castor oil treated rats when compared to control animals receiving only distilled water. *Maerua crassifolia* exerted a significant (P<0.05) inhibitory effect against the castor oil-induced activity in a dose- dependent manner (Table 4).

Table 4. Effect of the methanolic leaf extract of *Maerua crassifolia* on castor oil-induced enteropooling in rats

Treatment	Dose (mg/kg)	Volume of intestinal content (ml)	% inhibition
Normal saline	20 ml kg	4.33±0.13	-
M.crassifolia	100	1.02±0.08	76*
	200	0.78±0.07	82*
	400	0.63±0.09	85*
Loperamide	4	0.57±0.06	87*

Results are expressed as mean ± SEM
**significant at P<0.05 when compared to control (n=6)*

4. DISCUSSION

The phytochemical screening of the methanolic leaf extract of *Maerua crassifolia* revealed the presence of alkaloids, tannins, saponins, terpenoides, flavonoids, steroids, resins and cardiac glycosides which is an indication that the plant is of high pharmacological importance. These classes of compounds are reported to show important biological activities [17,18] and their presence may be responsible for the anti-diarrhoeal properties observed in the extract. Earlier studies showed that anti-diarhoeal properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids, steroids and terpenoids [15,19,20]. The anti-diarrhoeal activity of the extract may also be by precipitation of proteins in enterocyte and production of protein tannates that lead to reduced secretion and peristaltic movement [21]. The remarkable dose dependent reduction in castor oil-induced diarrhoea in rats is a demonstration of the efficacy of *Maerua crassifolia* as anti-diarrhoeal agent.

The lack of death at oral treatment in over 5000 mg/kg obtained suggests that methano extract of *Maerua crassifolia* leaf is practically non-toxic agent. It is therefore safe acutely for oral use in the ethno-therapeutic management of diarrhoea. The high safety profile obtained may have been responsible for its wide spread use in different ethno-therapeutic interventions. The leaf extract showed significant activity in reducing the frequency of castor oil-induced diarrhoea, which is comparable to that of the standard antidiarrhoeal drug, loperamide. Loperamide is a commonly used opioid anti-diarrhoeal agent which acts by increasing colonic phasic segmenting activities through inhibition of presynaptic cholinergic nerves in the submucosal and myetheric plexuses. These effects result in increased colonic transit time and faecal water absorption thus reducing the frequency of defaecation [22].

The anti-muscarinic drug, atropine and different doses of the extract decreased the propulsive movement in the charcoal meal study, the former being more potent than the leaf extract at the doses used. The significant inhibition of the castor oil-induced enteropooling in rats suggests that the methanol extract of *Maerua crassifolia* leaf produced relief in diarrhoea by spasmolytic activity *In vivo* and anti-enteropooling effects. The presence of tannins in the leaf extract may also be responsible for the anti-diarrhoeal activity. Clearly perceptible and plausible is the fact that many plants contain tannins and tanic acid, which denature proteins by forming a complex (protein tannate). The complex formed coats the intestinal mucosa and makes it more resistant while simultaneously diminishing gastric secretions [23,24]. Thus, *Maerua crassifolia* seemed to have satisfied Geiger's criteria for the classification of a drug as an anti-diarrhoeal agent [25,26].

5. CONCLUSION

The results of this investigation have shown that *Maerua crassifolia* has potential therapeutic option in the effective management of diarrhoea, thus justifying its widespread use by the local population for this purpose. Further studies are to be carried out to fractionate and purify the extract in order to find out the molecules responsible for the anti-diarrhoeal activity observed.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

ACKNOWLEDGEMENTS

The authors are grateful to Mrs Maryam Idris- Usman for the collection of the plant material from Sokoto, Sokoto State, Nigeria. We are also grateful to Obinna Okwuosa and Ifediniru for their technical assistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mujumdar AM, Misar AV, Upadhye AS. Antidiarrhoeal activity of ethanol extract of the bark of *Dalbergia lanceolaria*. Journal of Ethnopharmacology. 2005;102(2):213-216.
2. Rao NV, Prakash KC, Kumar SMS. Pharmacological investigation of *Cardiospermum halicacabum* (Linn) in different animal models of diarrhoea. Indian Journal of Pharmacology. 2006;38:346-9.
3. Hardman JG, Limberd LE. The pharmacological basis of therapeutics. In: Goodman and Gilman's (Eds), 10th edition. MacGraw Hill, New York. 1996;914-931.
4. Kouitcheu MLB, Penlap BV, Kouam J, Ngadjui BT, Fomum ZT, Etoa FX. Evaluation of antidiarrhoeal activity of the stem bark of *Cylicodiscus gabunensis* (*Mimosaceae*). Afr Journal of Biotechnol. 2006;5(11):1062-1066.
5. Tijani AY, Okhale SE, Salawu TA, Onigbanjo HO, Obianodo LA, Akingbasote JA, et al. Antidiarrhoeal and antibacterial properties of crude aqueous stem bark extract and fractions of *Parkia biglobosa* (Jacq.) R. Br. Ex G. Don. Afr Journal of Pharm Pharmacol. 2009;3(7):347-353.
6. Akuodor GC, Essiet GA, Ajoku GA, Ezeunala MN, Chilaka KC. Antimalarial potency of the methanol leaf extract of *Maerua crassifolia* Forssk (*Capparaceae*). Asian Pacific Journal of Tropical Disease. 2014;4(1):35-39.

7. Rahman MA, Mossa JS, Al-Said MS, Al-Yahya MA. Medicinal plant diversity in the flora of Saudi Arabia 1: A report on seven plant families. Fitoterapia. 2004;75:145-161.
8. Mukherjee PR. Quality Control of herbal drugs, an approach to evaluation of botanicals. 13th Edn. Busines Horizones Publisher, New Delhi. 2006;419-459.
9. Oloyode OI. Chemical profile of unripe pulp of *Carica papaya*. Pak J Nutr. 2005;4:379-381.
10. Sumitra C, Jigna P, Nehal K. Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegate* L. Bark Afr J Biomed Res. 2006;9:53-56.
11. NIH. Guide for the care and use of laboratory animals (Revised). NIH Publication No. 82-83; 1995.
12. OECD Guidelines for the Testing of Chemicals. Testing no. 423 Acute oral toxicity Acute Toxicity Class Method. 2010;1(4):1-14
13. Akuodor GC, Ibrahim M, Usman-Idris M, Megwas UA, Akpan JL, Chilaka KC, et al. Evaluation of the antidiarrhoeal activity of methanol leaf extract of Bombax buonopozense in rats. Ibnosina J Med Biomed Sciences. 2011;3(1):15-20.
14. Capasso R, Borrelli F, Cascio MG, aviello G, Huben K, Zjawiony JK, et al. Inhibitory effects of salvinorin a, from Salva divinorum on Ileiti-induced hypermotility: Cross-talk between kappa-opiod and cannabnoid CB (1) receptors. Br Journal of Pharmacol. 2008;18:622-8.
15. Akuodor GC, Mbah CC, Essien AD, Akpan JL, Ezeokpo BC, Iwuanyanwu TC, et al. Ulcer-protective and antidiarrhoeal effects of the aqueous stem bark extract of *Bridelia ferruginea* in rodents. Pharmacologia. 2012;3(11):591-597.
16. Robert A, Nezamis JE, Lancaster C, Hanchar AJ, Klepper MS. Enteropooling assay; a test for diarrhoea produced by prostaglandins. Prostaglandins. 1976;11:809-828.
17. Ghoghari AM, Rajan.i M. Densitometric determination of hecogenin from *Agave americana* leaf using HPTL. Chromatography. 2006;64:133-116.
18. Panda S, Kar A. *Annona squamosa* seed extract in regulation of hyperthyroidism and lipid-Peroxidation in mice: Possible involvement of quercetin. Pytomedicine 2007;14:799-805.
19. Longanga-Otshudi A, Vercruysse A, Foriers A. Contribution to the Ethnobotanical, Phytochemical and Pharmacological studies of raditionally used Medicinal Plants in the Treatment of Dysentary and Diarrhoea in Lomela area, Democratic Republic of Congo (DRC). Journal of Ethnopharmacology. 2000;71(3):411-423.
20. Al- Rehaily AJ, El-Tahir KEH, Mossa JS, Rafatulla S. Pharmacological Studies of various extract from hexane extract of *Ticlea nobilis* in rodents. Nat Prod Sci. 2001;7:76-82.
21. Yu LL, Liao JF, Chen CF. Anti-diarroeal effect of water extract of *Evodiae fructus* in mice. J Ethnopharmacol. 2000;73(1-2):39-45.
22. Cammilleri M. Chronic diarrhoea: A review of pathophysiology and management for the clinical gastroenterologist. Clin Gastroenterol Hepatol. 2004;2:198.
23. Tripathi KD. Essentials of medical pharmacology. Jay pee Brothers. Medical publishers, New Delhi; 1994.
24. Idris-Usman MS, Akuodor GC, Idris HS, Akpan JL, Ugwu TC, Anyalewechi NA, et al. Gastrointestinal properties of the ethanolic leaf extract of *Maerua crassifolia*. African Network for Drugs and Diagnostics Innovation Book of Abstract. 2010;63.

25. Aniagu SO, Binda LG, Nwinyi FC, Orisadipe A, Amos S, Wambebe C, et al. Anti-diarrhoeal and ulcer-protective effects of the aqueous root extract of Guiera senegalensis in rodents. J Ethnopharmacol. 2005;97:549-554.

26. Akah PA. Purgative potentials of *Euphorbia heterophylla*. Fitoterapia. 1988;60:45-48.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=575&id=13&aid=5260