Trop J Nat Prod Res, August 2020; 4(8):451-454
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Ameliorative Effects of Ethyl acetate Fraction of *Millettia aboensis* Stem Bark on Loperamide-Induced Constipation in Rats

Njoku O.Ugochi, Ogugofor M.Obinna, Ogbodo J.Onyebuchi*

Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria

ARTICLE INFO

Article history:
Received 15 July 2020
Revised 03 August 2020
Accepted 16 August 2020
Published online 28 August 2020

ABSTRACT

*Millettia aboensis*is is one of the plants that have been used for the treatment of different diseases such as gastrointestinal disturbances, ulcer and venereal diseases in Nigeria folklore medicine. This study evaluated the phytochemistry and effects of the ethyl acetate fraction of *Millettia aboensis* stem bark on loperamide-induced constipation in rats. The stem bark of *Millettia aboensis* was first extracted with methanol and further partitioned using aqueous methanol (20%), ethyl acetate and n-hexane. The ethyl acetate fraction was then used for the study. The experimental rats employed for this study were randomly divided into six groups of four rats each. Group 1 (normal control) received distilled water; Group 2 (positive control) received 3 mg/kg b.w. Loperamide, while group 3 received 5 mL/kg b.w. bisacodyl. Groups 4-6 received the graded doses of the fraction (100, 300, and 600 mg/kg b.w). After 7 days of standard drug and *Millettia aboensis* fraction administration, groups 2 to 6 received 3 mg/kg b.w. loperamide. The result of the phytochemical screening of the ethyl acetate fraction of *Millettia aboensis* stem bark revealed the presence of phenols, flavonoids, terpenoids, saponins, alkaloids, glycosides and tannins.The results of water intake; number of faecal pellets output and faecal weight showed significant increase ($p < 0.05$), while the body weight of the rats decreased significantly ($p < 0.05$) when compared with the control groups. The results of the study showed that *Millettia aboensis* ethyl acetate fraction exerted a mild laxative effect against loperamide-induced constipation in rats.

Keywords: *Millettia aboensis*, Phytochemicals, Laxative effect, Constipation, Loperamide.

Introduction

Constipation (dyschezia) is a gastrointestinal disorder characterized by infrequent bowel movements, causing the stool to become hard and dry, and leading to difficulties during defecation, as well as a sensation of incomplete bowel evacuation.[1,2] This occurs when the colon absorbs too much fluid than required as a result of the inability to relax the pelvic muscles to allow for a bowel movement (anismus) or the inability of the pelvic muscles to effectively and correctly coordinate relaxation and contraction (dyssynergia). It could also result from disease conditions or life style.[3,4] Apart from difficulties and pains experienced during bowel movement, other symptoms of constipation include feeling bloated, uncomfortable, and sluggishness.[2,5]However, medicinal plants are the richest source of medicines to humanity.[1] Prehistoric people, especially Africans relied on the healing properties of medicinal plants before the introduction of orthodox medicines.[6] The therapeutic efficiency of these medicinal plants can justifiably be attributed to the phytochemicals in them such as the flavonoids, alkaloids, sterols, terpenoids, phenolic acids, stilbenes, lignans, tannins and saponins, etc.[7] Phytochemicals are known to activate, catalyse and initiate some curative reactions in humans. [8-11] It is also believed that the various phytochemicals could work synergistically to exert their therapeutic effect.[7,11-12]*Milletiaaboensis* is one of the plants that have been reported to possess various medicinal properties in folklore medicine. It belongs to Fabaceae family, and is popularly known as a herbal plant for the treatment of gastrointestinal disorder, ulcer, venereal diseases such as gonorrhoea and syphilis, and malaria.[13] It is known as "Otoroekpo or Uturuekpa" in South eastern Nigeria and commonly found in low land rain forest. Considering the usefulness of this plant in the treatment of gastrointestinal diseases in folklore medicine, there is a need to evaluate the laxative effect of the plant, hence, this study.

*Corresponding author. E mail: john.ogbodo@unn.edu.ng
Tel: +2348037331402

Citation: Ugochi NO, Obinna OM, Onyebuchi OJ. Ameliorative Effects of Ethyl acetate Fraction of *Millettia aboensis* Stem Bark on Loperamide-Induced Constipation in Rats.Trop J Nat Prod Res. 2020; 4(8):451-454. doi.org/10.26538/tjnpr/v4i8.21

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Materials and Methods

Collection of plant material

The fresh *Millettia aboensis* stem bark was harvested from Obukpa, Nsukka local government area of Enugu State, Nigeria on 28th October 2019. It was identified by Mr. A. Ozioko of the Bioresources Conservation and Development Center, Enugu State. The plant was assigned the voucher number: Inter CEDD/16305: *Milletiaaboensis*.

Animals

Male Wistar rats of between 8 and 12 weeks old and body weights of110-160 g were used for the experiment.The rats were obtained from the Animal House of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. They were acclimatized for seven (7) days and fed on normal rat feed (Pecco feeds, Enugu, Nigeria) and had access to water *ad libitum*.

Extraction of Plant Materials

The stem barks of *Millettia aboensis* were cut into smaller pieces, air-dried under room temperature and pulverized to powder using a miller. The pulverized bark sample weighing 1382.24 g was macerated in 6 L of methanol with thorough shaking at regular intervals for 72 h at room temperature. The extract was filtered and concentrated at 40°C using rotary evaporator. Then, the extract was

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

partitioned into different fractions using aqueous methanol (20%), n-hexane and ethyl-acetate. After the partitioning and concentration of the different fractions, the laxative effect of the different fractions was evaluated to determine the fraction that exerts the best laxative effect. Ethyl acetate fraction was selected for further studies.

Phytochemical Analysis of Ethyl acetate Extract of Millettia aboensisStembark
The screening for phyto-constituents of the *Millettia aboensis* Stem bark ethyl acetate fraction was carried out according to the methods of Trease and Evans,[14]and Harbone.[15] Quantitative analysis was carried out as described by Harbone,[15] and Soni and Sosa.[16]

Acute toxicity study
The acute toxicity study was carried out using the method described by Lorke.[17]

Experimental design
Experimental animals were divided into six (6) groups of four (4) rats each.
Group 1 = Distilled water only (Normal control); Group 2 received 3 mg/kg b.w. Loperamide; Group 3 received 5 mL/kg bisacodyl (positive control); Groups 4-6 received 100, 300, and 600 mg/kg b.wof the fraction, respectively. The administration of the plant fraction and the standard drug was carried out using oral intubation tube. The treatment lasted for seven days (7).The rats were handled according to the guidelines of the National Institute of Health on the case and use of laboratory animals (NIH, 1985). The research (UNN/FBS/EC/1025) was approved by the Ethical Committee of Faculty of Biological Sciences, University of Nigeria Nsukka.
Induction of constipation
Constipation was induced in the animals using the methods described in earlier studies by Wintola*et al.* and Mikhail *et al.*[17,18] This involved the oral administration of 1 mL of loperamide (3 mg/kg body weight in 0.9% sodium chloride daily for 3 days) while the control rats were administered normal saline.

Determination of the dry weight and water content of the faecalpellets
This was determined using a modified method of Izzo*et al.*[19] The excreted faecal pellets of the individual rats were collected daily at 07:30 am throughout the duration of the experiment. The water content was calculated as the difference between the wet and dry weights of the pellet.

Gastrointestinal (GIT) transit
Gastrointestinal (GIT) transit was measured according to the method of Ezieke*et al*.[20]Onday 7 of the experiment, 1 mL of carmine (3 g suspended in 50 mL of 0.5% carboxymethylcellulose) was orally administered to the rats. One hour after the marker was administered, the animals were humanely sacrificed, and the small intestines were quickly removed. The distance over which the carmine had travelled and the total length of the small intestine were measured. The GIT transit was expressed as the percentage of the distance travelled by the carmine relative to the total length of the small intestine.

Statistical analysis
The results obtained were expressed as mean ± SD and were analyzed using statistical product and service solutions (SPSS) version 16. Tests of statistical significance were carried out using one-way Analysis of Variance (ANOVA). P values < 0.05 were considered statistically significant.

Results and Discussion

Phytochemical evaluation of the plant fraction
The phytochemical analyses of the ethyl acetate fraction of *Millettia aboensis* stem bark extract showed the presence of alkaloids, flavonoids, glycosides, tannins, phenols and terpenoids as presented in Table 1. Flavonoids such as quercetin have been reported to possess laxative effect by promoting gastrointestinal motility and mucinsecretion.[21] Flavonoids are hydroxylated phenolic substances known for several medicinal importance such as antioxidant, antiulcer, among others.[22-24] Tannins are astringent bitter compound found in a variety of plant that binds and precipitate proteins and other organic compounds including amino acids and alkaloids.[25] On the other hand, alkaloids have pharmacological effects such as antibacterial, anticancer, and antiasthmatic activities.[26] Also, the astringent property of tannins present in the plant fraction could reduce the purgative action of the glycosides thereby making the laxative effect of the plant fraction mild.[27]

Acute toxicity study
The acute toxicity profiling of the plant fraction showed that there was no mortality or any significant adverse changes in the behaviour of the rats both in phases 1 and 2,even at thedose of 5000 mg/kg body weight within 24 hours of constant observation. This indicates that the fraction is safe at the doses studied.

Effect of ethyl acetate fraction of Millettia aboensisstems bark on the feed intake, water intake and number of faecal pellets on loperamide-induced constipation inrats
Constipation is a common health problem usually caused by inadequate "roughage" fiber in the diet or as a result of an underlying disease. It causes disruption of the regular digestive routine of diet resulting in discomfort that affects patient quality of life.[1,28]Constipation occurrence increases with age and may develop into a chronic condition requiring the long-term use of laxatives.[23] In this present research, *Millettia aboensis*stem bark fraction ameliorated the loperamide-induced constipation as indicated by the increased water intake, defecation frequency and faecal parameters. The results of the effect of the plant fraction on water intake and number of faecal pellets showed a dose-dependent significant ($p< 0.05$) increase as the dose increased from 100 to 600 mg/kg b.w. compared to the positive control group. The effect of 600 mg/kg b.w. *Millettia aboensis*stem bark fraction was comparable to that of the standard drug, bisacodyl. Constipation is characterized by significant decreases in faecal discharge. [17] In addition, the delay in the release of faecal pellets in the large intestinal lumen can induce absorption of water giving rise to significant decrease in the water content of the discharged pellets.[29,17]

Table 1: Phytochemical constituents of ethyl acetatefraction of *Millettia aboensis* stem Bark

Phytochemical constituents	Qualitative	Quantitative (mg/g)
Alkaloids	+	1.99 ± 0.14
Flavonoids	+	4.69 ± 0.16
Glycosides	+	1.84 ± 0.08
Tannins	+	1.06 ± 0.04
Phenols	+	4.96 ± 0.09
Saponins	+	2.07 ± 0.21
Terpenoids	+	2.20 ± 0.14

n = 3.

Table 2: Acute toxicity profile of ethyl acetate fraction of *Millettia aboensis*stem bark

Phase 1	Dose	Mortality
	10 mg/kg b.w.Extract	0/3
	100 mg/kg b.w.Extract	0/3
	1000 mg/kg b.w.Extract	0/3
Phase 2		
	1600 mg/kg b.w Extract	0/3
	2900 mg/kg b.w Extract	0/3
	1600 mg/kg b.w Extract	0/3

n = 3

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Effect of ethyl acetate fraction of Millettia aboensis stem bark on water content of faecal pellets, weight of faecal pellets and gastrointestinal transit of loperamide-induced constipation inrats

The result of the effect of ethyl acetate fraction of *Millettia aboensis* stem bark on body weight gain and faecal properties of constipated rats showed a significant ($p< 0.05$) increase in the water content of the faecal pellets of the groups that received graded doses of the fraction as shown inTable4. The normal control group had a faecal water content of (1.79± 0.10), whereas in the extract-treated groups the faecal water content increased from 1.57 ± 0.09 to 2.97 ± 0.05 as the dose increased from 100 to 600 mg/kg. However, a significant ($p <0.05$) decrease in the water of the faecal pellets in the positive control was observed. Although the feed intake did not differ among the groups, the gain in body weight was higher in the positive control rats when compared to the treated groups. In addition, a significant ($p< 0.05$) decrease in body weight gain was observed in the experimental rats as the dose of the plant fraction increased from 100-600 mg/kg b.w.This could be due to the accumulation of faecal pellets in their bodies, thus giving rise to the extra weight.[30-32] This result was in accordance with the study by Hanauer[27]on the effect of the aqueous extract of *M. aboensis* leaves on Lomotil-induced constipation in rats that showed an increase in the frequency of stooling on the lomotil+AEMA (aqueous extract of *Millettia aboensis*) when compared to the untreated, thereby showing the effectiveness of the extract on treatment of constipation.

Table 3: Effect of ethyl acetate fraction of *Millettia aboensis* stem bark on the feed intake, water intake and number of faecal pellets on loperamide-induced constipated in rats

Group	Feed intake (g)	% Water intake	Number of Faecal Pellets
Normal Control	14.64 ± 0.37^{a}	78.70 ± 2.60^{b}	58.52 ± 3.42^{d}
Positive Control	14.94 ± 0.10^{a}	61.50 ± 2.42^{a}	37.52 ± 3.42^{a}
5 mL/kg b.w.Bisacodyl	14.71 ± 0.44^{a}	82.04 ± 2.16^{b}	63.24 ± 2.75^{e}
100 mg/kg b.w.MAEF	14.97 ± 0.63^{a}	64.60 ± 2.24^{a}	43.76 ± 1.26^{b}
300 mg/kg b.w.MAEF	15.05 ± 0.11^{a}	83.50 ± 1.26^{b}	49.00 ± 1.15^{c}
600 mg/kg b.w.MAEF	15.09 ± 0.16^{a}	84.00 ± 1.83^{b}	60.32 ± 2.22^{e}

Data are mean ± Standard deviation. (n = 4). Values in the same column with different superscript differ significantly ($p < 0.05$). MAEF = *Milletia aboensis*ethylacetate fraction

Table 4: Effect of ethyl acetate fraction of *Millettia aboensis*stem bark on gastrointestinal transit of loperamide induced constipation in rats

Group	Water content of faecal pellets (g)	Weight of faecal pellets (g)	Bodyweight gain (g)	Percentage propulsion
Normal control	1.79 ± 0.10^{c}	7.96 ± 0.2^{d}	12.72 ± 0.83^{c}	61.00 ± 1.83^{b}
Positive control	1.36 ± 0.04^{a}	3.06 ± 0.0^{a}	18.51 ± 0.4^{d}	44.75 ± 2.50^{a}
5 mL/kg b.w Bisacodyl	2.91 ± 0.07^{e}	8.66 ± 0.4^{e}	13.38 ± 0.60^{c}	61.25 ± 3.77^{b}
100 b.w.mg/kg MAEF	1.57 ± 0.09^{b}	3.66 ± 0.2^{b}	13.38 ± 0.52^{c}	59.00 ± 1.41^{b}
300 mg/kgb.w. MAEF	2.27 ± 0.07^{d}	7.00 ± 0.1^{c}	11.91 ± 0.5^{b}	65.25 ± 3.77^{c}
600 mg/kg b. w MAEF	2.97 ± 0.05^{e}	8.34 ± 0.2^{e}	10.60 ± 0.32^{a}	70.00 ± 1.41^{d}

Data are mean ± Standard deviation. (n = 4). Values in the same column but with different superscript differ significantly ($p < 0.05$). MAEF = *Milletia aboensis*ethylacetate fraction

Conclusion

The presence of phytoconstituents like glycosides, flavonoids, terpenoids, and alkaloids could be responsible for the laxative effect of *Milletiaaboensis*ethylacetate fraction. In addition, the tannins present in the plant fraction could have modulated the laxative effect of the plant by reducing the purgative effect of the plant fraction and thereby ensuring that laxative effect is mild.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors humbly acknowledge Dr. Offormata of Energy Research Centre, University of Nigeria Nsukka and Mr. Alfred Ozioko for their assistance.

References

1. Chatoor DE. Constipation and evacuation disorders. Best Prac Res Clin Gastroenterol. 2009; 23(4):517-530.
2. Cirillo C and Capasso R. Constipation and botanical medicines: an overview. Phytother Res. 2015; 29:1488 - 1493.
3. Drossman DA. Functional Gastrointestinal Disorders: History, Pathophysiology, Clinical Features, and Rome IV. Gastroenterol. 2016; 150:1262 – 1279.

4. Leung L, Riutta T, Kotecha J, Rosser W. Chronic constipation: an evidence-based review. J Am Board Fam Med. 2011; 24(4):436 - 451.
5. Mostafa SM, Bhandari S, Ritchie G, Gratton N, Weastone R. Constipation and its implication in the critically ill patient. Br J Anaesthesiol. 2003; 91:815 - 819.
6. Ncube NS, Afolayan AJ, Okoh AJ. Assessment techniques of antimicrobial properties of natural compounds of plants origin: current methods and future trends. Afr J Biotechnol. 2008; 7(12):1797-1806.
7. Egba IS, Ogbodo JO, Ogbodo PO, Obike CA. Toxicological Evaluation of Two Named Herbal Remedies Sold Across Orumba South Local Government Area of Anambra State, South-Eastern Nigeria. Asian J Biochem. 2018;1(1):1–6.
8. Afolabi OA, Aderoju AH, Alagbonsi AI. Effects of methanolic extract of *Moringaoleifera* leaves on semen and biochemical parameters in cryptorcid rats. Afr J Trad Comp Altern Med. 2013; 10(5):230-235.
9. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol. 2005; 4(7):685-688.
10. Kaiko GE, Ryu SH, Koues OI, Collins PL, Solnica-Krezel L, Pearce JE, Pearce EL, Oltz ME, Stappenbeck ST. The colonic crypt protects stem cells from microbiota-derived metabolites. Cell. 2016; 165(7):1708-1720.
11. Okwuonu CG, Chukwuonye IJ, Adejumo OA, Agaba EI, Ojogwu LI. Prevalence of chronic kidney disease and its risk factors among adults in a semi-urban community of South-East Nigeria. Niger Postgrad Med J. 2017; 24:81-87.
12. Madziga HA, Sanni S, Sandabe UK. Phytochemical and elemental analysis of *Acalphawilkesiana* leaf. J Am Sci. 2010; 69(11):510-514.
13. Mosihuzzaman M. Herbal medicine in health care. An overview. Nat Prod Crop Protect. 2012; 29:913-920.
14. Ugwueze ME, AdonuCC, Attama AA. Evaluation of the hepatoprotective activity of root extracts of *Millettia aboensis* on carbon tetrachloride [CCl_4] induced hepatotoxicity in rats. Int J Adv Res. 2013; 1(5):65 - 70.
15. Trease GE and Evans WC. Trease and Evans Pharmacognosy. 4thEdn. W. B. Saunders, USA, 2002. 820 – 835 p.
16. Harbone JB. Phytochemicals methods: a guide to modern techniques of plant analysis. Chapman and Hall Ltd., London, 1998. 49 – 188 p.
17. Soni A and Sosa S. "Phytochemical Analysis and Free Radical Scavenging Potential of Herbal and Medicinal Plant Extracts." J Pharm Phytochem. 2013; 2(4):22-29.
18. Wintola OA, Sunmonu TO, Afolayan AJ. The effect of *Aloe ferox* mill in treatment of loperamide-induced constipation in Wistar rats. BMC Gastroenterol. 2010; 10:95.
19. Mikhail ON, Taoheed AA, Rukayat OJ, Mutiu IK. Ameliorative effect of *Lecaniodiscus cupanioides* (Sapindaceae) Aqueous Root Extract in Loperamide-induced Constipated Rats. Trop J Pharm Res. 2015; 14(6):1057-1062.
20. Izzo AA, Nicoletti M, Giannattasio B, Capasso F. Antidiarrheal Activity of *Terminalia Sericea* Burch. ex DC Extracts. Nat Drugs Dig Tract, Capasso, F Mascolo, N (Eds), EMSI, Rome. 1992. 225-256 p.
21. Ezike AC, Akah PA, Okoli CO, Ufere IK, Ezeudu E, Okoye CF, Ashara C, Igbokwe IN. "Studies on Gastrointestinal Effects of *Desmodiumvelutinum*: A Traditional Remedy for Diarrhea." Am J Pharm Toxicol. 2014; 9(2):114-124.
22. Kim JE, Lee MR, Park JJ, Choi JY, Song BR, Son HJ, Choi YW, Kim KM, Hong JT, Hwang DY. Quercetin have been reported to possess laxative effect by promoting gastrointestinal motility and mucin secretion in loperamide-induced constipation of SD rats through regulation of the mAChRs downstream signal. Pharm Biol. 2018; 56(1):309-317.
23. Njoku UO, Uzoagulu CH, Ogugofor MO. Protective effect of *Cnidoscolus aconitifoilus* leaves against diclofenac-induced gastric mucosal damage. Pak J Pharm Sci. 2020; 33(2):651-657.
24. Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview J NutrSci, 2016; 5:e47-e55.
25. Njoku UO, Nwodo OFC,Ogugofor MO. Cardioprotective potential of methanol extract of *Costusafer* leaf on carbontetrachloride-induced cardiotoxicity in albino rats. Asian J Pharm Res Health Care 2017; 9(2):51-58.
26. Petridis GK. Tannins: Types, Foods Containing and Nutrition. Nova Science publishers, New York, 2010. 231 – 247 p.
27. Gaikwad SB, Mohan GK, Rani MS. Phytochemicals for diabetes management. Pharm Crop. 2014; 5(1):11 - 28.
28. Hanauer SB. The role of loperamide in gastrointestinal disorders. *Reviews in* GastroenterolDisord. 2008; 8(1):15 – 20.
29. Lee HY, Kim JH, Jeung HW. Effects of *Ficuscarica* Paste on Loperamide-Induced Constipation in rats. Food ChemToxicol. 2012; 50:895 – 902.
30. Méité S, Bahi C, Yéo D, Datté JY, Djaman JA, N'guessan DJ. Laxative activities of *Mareyamicrantha* (Benth.) Müll. Arg. (Euphorbiaceae) leaf aqueous extract in rats. BMC CompAltern Med. 2010;10:7.
31. Eor JY, Tan PL, Lim SM, Choi DH, Yoon SM, Yang SY, Kim, HS. Laxative effect of probiotic chocolate on loperamide-induced constipation in rats. Food Res Int. 2019; 116:1173-1182.
32. Marjorie C. Plant products as antimicrobial agents. ClinMicrobiol Rev. 1996; 12:564 - 582.
33. Niwa T, Nakao M, Hoshi S, Yamada K, Inagaki K, Nishida M, Nabeshima T. Effect of dietary fiber on morphine-induced constipation in rats. Biotech Biochem. 2002; 66(6):1233 - 1240.