Croton zambesicus Root Extract Exert Laxative Effect in Rats

Jude E. Okokon1*, Augustine I. L. Bassey2, Emmanuel E. Nyong3 and Utibe A. Edem1

1Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.
2Department of Pharmacology and Therapeutics, Faculty of Clinical Sciences, University of Uyo, Uyo, Nigeria.
3Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.

Authors’ contributions

This work was carried out in collaboration among all authors. Authors JEO, AILB, EEN and UAE designed the work. Author JEO wrote the protocol and first draft of the manuscript. Authors AILB and EEN reviewed and vetted the first draft. Author UEA performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJOB/2020/v10i230103

ABSTRACT

Background: Croton zambesicus Muell Arg. (Euphorbiaceae) is a medicinal plant used traditionally in the treatment of some ailments.

Aim: The root extract of Croton zambesicus was evaluated for effect on the gastrointestinal tract (GIT).

Materials and Methods: The root extract of C. zambesicus (27-81 mg/kg) was investigated for laxative effect in rats using different experimental models to study its effect on the GIT.

Results: The extract was found to significantly increase the frequency and output of faecal matters in rats in all the experimental models studied.

Conclusion: The findings of this study show that the root extract of C. zambesicus has a significant laxative effect and this supports its use in ethnomedicine for the treatment of gastrointestinal tract disorders.

*Corresponding author: E-mail: judee@ymail;
Keywords: Croton zambesicus; Purgative; GIT.

1. INTRODUCTION

Croton zambesicus Muell Arg. (Euphorbiaceae) (syn C. amabilis Muell. Arg. C.gratissimus Burch) is an ornamental tree grown in villages and towns in Nigeria. It is a Guineo–Congolese species widely spread in tropical Africa. Traditionally, the leaf decoction is used as anti-hypertensive and anti-microbial (urinary infections) [1]. The roots are used as antimarial, febrifuge and antidiabetic by the Ibibios of Niger Delta region of Nigeria [2].

Biological activities reported on the root extract include; antimalarial [2], anticonvulsant, antiulcer [3], anti-inflammatory, analgesic and antipyretic [4], antidiabetic and hypolipidemic activities [5]. Okokon et al., [6] have reported the kidney-protective activity of the root extract of the Croton zambesicus against gentamicin-induced kidney injury. Moroso, immunostimulatory, cytotoxicity against HeLa cell line and antileishmanial activities of the root extract were also reported [7]. Boyom et al., [8] studied the composition of essential oil from the roots of Croton zambesicus and found the oil from the root bark to contain sesquiterpenes and rich in oxygen containing compounds, with spathulenol and linalool as major components. Okokon and Nwafor [2] reported that the root extract contains alkaloids, saponins, terpenes, tannins, phlobatannins, anthraquinones and cardiac glycosides. Furthermore, Gas chromatography–mass spectrometry (GCMS) analysis of hexane fraction of the root of Croton zambesicus revealed the presence of Hexadecanoic acid, 1-Hexadecanol, 2-methyl, Hexadecanoic acid,ethyl ester, Linoleic acid,ethyl ester, 9-Octadecanoic acid (Z), 2-hydroxy-1-hydroxymethyl ethyl ester, Hexadecanoic acid,2-methyl-,methylster, Trachylobane, Androst-4-ene-3,17-dione, Androst-4-en-17-one, 3-hydroxy,-(5β)-, 2,4(1H,3H)-pyrimidinedione,5-nitro,Retinol, Myrcene, γ-Terpine, Linalool, Linalool acetate, Lupeol, α-Humulene, α – Murolene, Stigmast-4-en-3-one, Lanost-7-en-3-one, 9 α, 13 α,14 α,17 α) [7]. We examine in this study the laxative effect of the root extract.

2. MATERIALS AND METHODS

2.1 Plants Collection

The plant material Croton zambesicus (roots) were collected in compounds in Ururu area, Akwa Ibom State, Nigeria in May 2019. The plant was identified and authenticated by researcher of Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria.

2.2 Extraction

The roots were washed and shade-dried for two weeks. The dried plants’ materials were further chopped into small pieces and reduced to powder. The powdered material was macerated in 70% ethanol for 72 h. The liquid ethanol extract obtained by filtration was evaporated to dryness in a rotary evaporator at 40°C. The extract was stored in a refrigerator at 4°C until used for experiment reported in this study.

2.3 Animals

Albino wistar rats (120 -135 g) of either sex were used for these experiments. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water ad libitum.

2.4 Effect on Castor Oil-Induced Diarrhoea

This was studied in rats using the method of Nwafor et al. [9]. Animals were fasted for 18 h but allowed free access to water. They were randomized into five groups of six rats each. Group 1(control) received 10% Tween 80 (5 mL/kg) by gavage, Groups 2-4 were treated with C. zambesicus extract (27, 54 or 81 mg/kg, p.o. respectively); Group 5 was treated with atropine (0.1 mg/kg,i.p.). After 1h, each rat received 2 mL of castor oil (p.o) and was then observed for consistency of faecal matter and frequency of defecation for 3h.

2.5 Laxative Activity Test

Laxative effect was evaluated according to a procedure described by Shankara and Sriram [10] with slight modification on rats of either sex. Wistar rats of either sex were fasted for 18 hours. The rats were divided into five groups of six animals each. Group 1 received 10% Tween 80 (5 mL/kg) by gavage, Group 2 received castor oil (2 mL/kg orally), Groups 3-5 received C. zambesicus27, 54 and 81 mg/kg orally respectively. The animals were separated in suitable cages for collection and weighing of the faecal output immediately after
the dosing. Food and water were given to all rats and faecal output was weighed after a period of 6 hours.

2.6 Effect on Small Intestinal Propulsion

The effect of the extract on intestinal propulsion inunaanesthesized rats was tested using charcoal method of Nwafor and Okwuasaba [11]. Animals were fasted for 24h but allowed free access to water only and were further randomized into six groups of six mice each. Group 1(control) received 10% Tween 80 (5 mL/kg) by orogastric gavage; groups 2-4 were treated with C. zambesicus extract (27, 54 or 81 mg/kg, p.o. respectively); Group 5 received Castor oil (2 mg/kg, p.o).After 1h, each rat was administered 1mL charcoal meal (5% activated charcoal suspended in 10% aqueous tragacanth), orally. The animals were killed 30min later by cervical dislocation and bled, and the small intestines were rapidly dissected out and placed on a clean surface. The small intestine of each animal was carefully inspected and the distance traversed by the charcoal meal from the pylorus was measured. The length of the whole small intestine was also measured. The distance traversed by the charcoal meal from the pylorus was expressed as a percentage of the distance from the pylorus to the ileocaecal junction.

2.7 Laxative Activity on Loperamide-Induced Constipation in Rats

The method described by Yadav et al. [12] was used in this study. Wistar rats were fasted for 18 hours and were placed individually in cages lined with clean filter paper. The rats were divided into five groups of five animals each. Group I received 5 mL/kg normal saline orally. Group II received castor oil (2 mg/kg orally).Group III and IV received C. zambesicus root extract; 27, 54 and 81 mg/kg orally respectively. After one hour, all the animals received loperamide (5 mg/kg orally) by gavage. The faecal outputs in all four groups were monitored for 6 hours.

2.8 Data and Statistical Analysis

The results were presented as mean and SEM and comparisons among groups for statistical significant differences were done by analysis of variance (ONE WAY ANOVA) followed by Turkey Kramer’s multiple comparison tests using GraphPad Prism 5.3 application software. The p-values of less than 0.05 were considered as indicative of significance.

3. RESULTS

3.1 Effect of Castor Oil Induced Diarrhoea

The administration of Croton zambesicus root extract to rats was observed to cause dose-dependent increases in mean faecal output of the animal. These increases were significant (p<0.05-0.01) when compared to control. The diarrhoeal effect of castor oil was observed to be increased with the administration of the root extract (Table 1).

| Treatment                  | Dose (mg/kg) | Mean faecal matter |
|----------------------------|--------------|--------------------|
| Control (10% Tween 80)     | 5 mL         | 6.83±0.13          |
| C. zambesicus             | 27           | 11.18±2.84         |
|                            | 54           | 15.93±2.31         |
|                            | 81           | 22.15±2.55         |
| Atropine                  | 0.1          | 1.32±1.12          |

Data are expressed as MEAN ± SEM, Significant at *p < 0.05, **p<0.01, when compared to control. (n=6)

3.2 Effect on Small Intestinal Propulsion Activity in Rats

Administration of root extract of Croton zambesicus caused a dose-dependent and significant (p<0.05-0.01) increase in the small intestinal propulsion activity of rats. The effect of the highest dose of the extract (81 mg/kg) was comparable to that of castor oil (Table 2).

| Treatment                  | Dose (mg/kg) | Mean faecal matter |
|----------------------------|--------------|--------------------|
| Control (10% Tween 80)     | 5 mL         | 61.2±1.42          |
| C. zambesicus             | 27           | 65.5±3.16          |
|                            | 54           | 71.2±2.45a         |
|                            | 81           | 79.4±4.26b         |
| Castor oil                | 2 mL         | 81.2±0.62c         |

Data are expressed as MEAN ± SEM, Significant at *p < 0.05, **p<0.01, when compared to control. (n=6)

3.3 Laxative Effect

Administration of root extract of Croton zambesicus to rats caused significant (p<0.05-0.001) and dose-dependent increase in faecal output of rats. The effect of the highest dose of
the extract (81 mg/kg) was found to be more than that of castor oil (Table 3).

Table 3. Laxative effect of the root extract

| Treatment        | Dose (mg/kg) | Mean faecal matter |
|------------------|--------------|--------------------|
| Control (10%Tween 80) | 5 mL        | 2.14±1.05          |
| C. zambesicus    | 27 mg/kg     | 3.11±1.22          |
|                  | 54 mg/kg     | 5.98±2.16          |
|                  | 81 mg/kg     | 6.48±2.55          |
| Castor oil       | 2 mL         | 6.15±2.26          |

Data are expressed as MEAN ± SEM, Significant at \( p < 0.05, ^{a}p < 0.001, \) when compared to control. (n=6)

3.4 Effect on Faecal Output of Loperamide-Induced Constipation in Rats

Administration of Croton zambesicus root extract produced a significant \( (p<0.05-0.001) \) and dose-dependent increase in mean faecal output. The effect of the highest dose (81 mg/kg) of the extract was comparable to that of castor oil (Table 4).

Table 4. Effect on faecal output of Loperamide-induced constipation in rats

| Treatment        | Dose (mg/kg) | Mean faecal matter |
|------------------|--------------|--------------------|
| Control (10%Tween 80) | 5 mL        | 1.63±0.68          |
| C. zambesicus    | 27           | 2.61±0.22          |
|                  | 54           | 3.08±0.86          |
|                  | 81           | 3.61±0.94          |
| Castor oil       | 2 mL         | 3.56±0.51          |

Data are expressed as MEAN ± SEM, Significant at \( ^{a}p < 0.01, \) when compared to control. (n=6)

4. DISCUSSION

In this work, root extract of Croton zambesicus was evaluated for laxative activity in rats. The results showed that ethanol root extract of Croton zambesicus produced significant and dose dependent increase in faeces output of rats administered with it. This effect could probably be exerted through the accumulation of water in intestinal loop and the stimulation of gastrointestinal motility. The effect of the root extract was comparable to that of castor oil (standard drug) at highest dose of the extract (81 mg/kg body weight). Castor oil induces diarrhea due to active ingredient, ricinoleic acid, which is liberated as a resulting action of lipases on castor oil. This stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. It also stimulates the release of endogenous prostaglandins [13,14]. Castor oil elicits secretory and motility diarrhea [15]. The observed activities therefore suggest that laxative activity of the root extract may be mediated through this mechanism.

The results of this study further showed that the root extract antagonises the effect of loperamide on the gastrointestinal tract activity. Loperamide, a synthetic opiate, which act by decreasing the transit velocity and increasing the capacity of the intestines to retain their fluids [16]. These results suggest that the root extract must have acted to stimulate Na+, K+ and Cl- secretion. Chatsri et al. [17] had reported that some natural laxatives exert their effects on the colonic epithelium by stimulating Cl- secretion and/or inhibiting Na+ absorption, resulting in an accumulation of fluid and subsequent increased colonic motility.

Secondary metabolites of plants such as terpenoids, sterols, flavonoids, phenolic compounds, tannins and alkaloids [18] have been previously found to be responsible for laxative activities in plants. These constituents that have been reported by Okokon and Nwafor [2] to be present in the root extract of C. zambesicus maybe responsible for the laxative activity of the extract.

5. CONCLUSION

The findings of this study show that the ethanol root extract of C. zambesicus has a significant laxative effect which is due to the activities of its phytochemical constituents and this supports its use in ethnomedicine for the treatment of gastrointestinal tract disorders.

ETHICAL APPROVAL

The care and use of animals was conducted in accordance with the National Institute of Health Guide for the use of laboratory animals (NIH, 1996). Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

ACKNOWLEDGEMENTS

The authors are grateful to the management of University of Uyo for providing enabling environment for the successful completion of this work.
COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Adjanohoun EJ, Adjakide V, de Souza S. Contribution to ethnobotanical and floristic studies in Republic of Benin. Agency for Cultural and Technical Cooperation. 1989; 1:245.
2. Okokon JE, Nwafor PA. Antiplasmodial activity of root extract and fractions of Croton zambesicus. J Ethnopharm. 2009; 121:74-78.
3. Okokon JE, Nwafor PA. Antiiulcer and anticonvulsant activities of Croton zambesicus root extract. Pak J Pharmaceut Sci. 2009;22:384-390.
4. Okokon JE, Nwafor PA. Antiinflammatory, analgesic and antipyretic activities of ethanolic root extract of Croton zambesicus. Pak J Pharmaceut Sci. 2010; 23:383-390.
5. Okokon JE, Nwafor PA, Ekpo E, Okokon P, Udobang J. Antidiabetic and hypolipidemic activities of ethanolic root extract of Croton zambesicus on alloxan induced diabetic rats. Asian J Pharmaceut Res. 2011;1:493 – 499.
6. Okokon JE, Nwafor PA, Noah K. Nephroprotective effect of Croton zambesicus root extract against gentimicin-induced kidney injury. Asian Pac J Trop Med. 2011;4:969-972.
7. Okokon JE, Dar A., Choudhary MI. Immunomodulatory, cytotoxic and antileishmanial activities of phytoconstituents of Croton zambesicus Phytopharmacol. 2013;4(1):31 – 40.
8. Boyom FF, Keumdjio F, Dongmo PM, Ngadjui BT, Amvam-Zollo PH, Menut C, Bessiere JM. Essential oil from Croton zambesicus Muell. Arg. growing in Cameroun. Flav Fragr J. 2002;17:215–217.
9. Nwafor PA, Jacks TW, Ekanem AU and Ching FP. Antiiulcerogenic and antidiarrhoeal potentials of Pausinystalia macroceras stem-bark in rats. Nigerian Journal of Natural Products and Medicine,2005; 9: 66-70
10. Shankara S, Sriram N. Laxative effect on methanol extracts of leaves of Basella alba. IntJ Allied Med Sci Clin Res. 2014; 2(4):392-394.
11. Nwafor PA, Okwuasaba FK. Effect of methanolic extract of Cassia nigricans leaves on gastrointestinal tract. Fitoterapia. 2001;72:206-214.
12. Yadav S, Kumar P, Shridhar B, Shukla D, Baiga VP, Mani M. Laxative activity of hydroalcoholic leaves extract of Putranjiva roxburghii. J Drug Delivery and Therapeut. 2018;8(4):142-144.
13. Galvez J, Zarzuelo A, Crespo ME, Lorente MD, Ocente MA, Jimenez J. Antidiarrhoeic activity of Euphorbia hirta extract and isolation of an active flavonoid constituent. Planta Med. 1993;59:333-336.
14. Yoshio K, Kazuko S, Bunsyo M, Kazunori H, Atsushi I, Yasuhiro K. Relationship between antidiarrhoeal effects of Hange-shashin-To and active components. Phytother Res. 1999;13:468-473.
15. Rouf AS, Islam MS, Rahman MT. Evaluation of antidiarrhoeal activity of Rumex maritimus roots. J Ethnopharm. 2003;84:307-310.
16. Vareinshang T, Yadav AK. Antidiarrhoeal activity of Rhus javanica ripen fruit extract in albino mice. Fitoterapia. 2004;75:39-44.
17. Chatsri D, Sutthasinee P, Watchareewan T, Nateetip K. Barakol Extracted from Cassia siamea stimulates chloride secretion in rat colon. J Pharmacol. Expl Therapeut. 2005; 314:732-734.
18. Longanga-Otshudi A, Vercruysse A, Foriers A. Contribution to the ethnobotanical, phytochemical and pharmacological studies of traditionally used medicinal plants in the treatment of dysentery and diarrhea in Lomela area, Democratic Republic of Congo (DRC). J Ethnopharm. 2000;71:411-423.

© 2020 Okokon et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here: http://www.sdiarticle4.com/review-history/61412