West Afr. J. Pharmacol. Drug Res. Vol. 31 January – December 2016; 8 - 13

ANTISPASMODIC AND ANTIDIARRHOEAL ACTIVITIES OF THE AQUEOUS LEAF EXTRACT OF *CROTON ZAMBESICUS* LINN (EUPHORBIACEAE) IN RATS

Effects of *Croton Zambesicus* Linn (Euphorbiaceae) on the Gastrointestinal Tract

R.O. AYANNIYI [1*], B.B. MAIHA[2], O.O. AYORINDE[1], A. ALLI-OLUWAFUYI [3], M.H. JIMOH AKANBI [3]

[1] Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Ilorin, Nigeria.

[2] Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria.

[3] Department of Pharmacology and Therapeutics, College of Medicine, University of Ilorin, Nigeria.

Abstract

Croton zambesicus Muell Arg. (Euphorbiaceace) is used in the management of various ailments including diarrhoea and dysentery. The present study was carried out to investigate the antispasmodic and antidiarrhoeal activities of aqueous leaf extract of *Croton zambesicus* in albino rats. The effect of the extract on acetylcholine, calcium chloride and angiotensin II-induced contraction of the rat ileum was determined. The antidiarrhoeal activity of the leaf extract was evaluated using castor oil-induced diarrhoea and gastrointestinal motility test in rats. The leaf extract (1.60 and 6.40 mg/ml) significantly inhibited ($P<0.05$ and $P<0.01$) acetylcholine-induced contraction of the rat ileum. Calcium chloride-induced contraction was significantly ($P<0.001$) inhibited at a concentration of (6.40 mg/ml). A ten times lower concentration of the extract (0.64 mg/ml) produced a significant ($P<0.01$) concentration-dependent inhibition of angiotensin II-induced contraction of the rat ileum. The extract (400 and 800 mg/kg) produced a significant ($P<0.05$, $P<0.001$) reduction in frequency of castor oil-induced gastrointestinal motility. In addition, the extract (400 and 800 mg/kg) significantly ($P<0.01$, $P<0.001$) decreased gastrointestinal motility in rats. The results from this study indicate that aqueous leaf extract of *Croton zambesicus* has gastrointestinal relaxant and antidiarrhoeal activities mediated possibly via inhibitory effects on calcium channels, muscarinic and angiotensin type 1 receptors.

Keywords: *Croton zambesicus*; ileum; acetylcholine; calcium chloride; angiotensin II; castor oil

Croton zambesicus Muell Arg. (Euphorbiaceace) est utilisé dans la gestion de diverses affections, y compris la diarrhée et la dysenterie. La présente étude a été menée pour étudier les activités antispasmodiques et antidiarrhéiques d'extrait de feuilles aqueuses de Croton zambesicus chez des rats albinos. L'effet de l'extrait sur l'acétylcholine, le chlorure de calcium et la contraction induite par l'angiotensine II de l'iléon du rat a été déterminé. L'activité antidiarrhéique de l'extrait de feuilles a été évaluée à l'aide d'une diarrhée induite par l'huile de ricin et d'un test de motilité gastro-intestinale chez le rat. L'extrait de feuilles (1,60 et 6,40 mg / ml) a inhibé de manière significative ($P<0,05$ et $P<0,01$) une contraction induite par l'acétylcholine de l'iléon du rat. La contraction induite par le chlorure de calcium était significativement ($P<0,001$) inhibée à une concentration de (6,40 mg / ml). Une concentration dix fois plus faible de l'extrait (0,64 mg / ml) a produit une inhibition significative ($P<0,01$) dépendant de la concentration de la contraction induite par l'angiotensine II de l'iléon du rat. L'extrait (400 et 800 mg / kg) a produit une réduction significative ($P<0,05$, $P<0,001$) de la fréquence de la motilité gastro-intestinale induite par l'huile de ricin. En outre, l'extrait (400 et 800 mg / kg) de manière significative ($P<0,01$, $P<0,001$) a diminué la motilité gastro-intestinale chez les rats. Les résultats de cette étude indiquent que l'extrait de feuilles aqueux de Croton zambesicus a des activités de relaxant gastro-intestinal et antidiarrhéique médiées éventuellement par des effets inhibiteurs sur les canaux calciques, les récepteurs muscariniques et les recepteurs d'angiotensine de type 1.

Mots-clés: *Croton zambesicus*; L'iléon; Acétylcholine; chlorure de calcium; Angiotensine II; huile de castor

Corresponding author: **Dr. Rashidat O Ayanniyi,** Department of Pharmacology and Toxicology, University of Ilorin, Nigeria. Email: ayanrash13@yahoo.co.uk Phone no. +2348033908610

Introduction

Traditional medicines of proven quality, safety and efficacy contribute to the goal of ensuring all people have access to health care. The affordability of most traditional medicines make them more acceptable at a time of soaring health care cost and nearly universal austerity (World Health Organization 2013). The regular use of traditional therapies demands scientific evidence for active principles in the therapies so as to improve efficacy and safety (Patwardhan *et al.*, 2005).

Croton zambesicus Muell Arg. (Euphorbiaceace) (syn C. amabilis muell. Arg., C. gratissimus Burch.) is an ornamental tree grown in villages and towns in Nigeria. It is a slender dioecious tree up to 30 feet high, sometimes forming a bole, with scaly bark. The leaves have a pointed apex and are silvery rusty-scaly below. It is commonly called bushveld, and referred to koriba or ichen maser in Hausa, Ajekobale in Yoruba, mfam in Ekoi and Moramora in Kilba (Hutchinson and Daziel, 1972). Croton zambesicus leaf extract is used by traditional medicine practitioners in Nigeria for the management of diarrhoea, dysentery, fever, convulsion, *hypertension and urinary tract infections.*

Previous pharmacological studies revealed the plant possesses hypotensive (Ayanniyi et al., 2014), anticonvulsant (Ayanniyi and Wannang, 2008 a) and neuropharmacological effects (Ayanniyi and Wannang, 2008 b). The ethanolic root extract of the plant has been reported to have anti-inflammatory, analgesic and antipyretic activity (Okokon and Nwafor, 2010). In another study, diterpenes isolated from the plant were reported to have vasorelaxant activity on the rat aorta (Baccelli *et al.*, 2010; Martinsen *et al.*, 2010).

There is dearth of literature on the pharmacological actions of *Croton zambesicus* extract on the gastrointestinal tract. The present study was carried out to evaluate the antispasmodic and antidiarrhoeal activity of aqueous leaf extract of *Croton zambesicus* in experimental animals.

Materials and Methods

Ethics approval. Ethics clearance was obtained from the University of Ilorin Ethics Review Committee. All experiments were carried out in accordance with the Guidelines for laboratory Procedures laid down by the University of Ilorin Ethics Committee on Research as well as the International Animal Care and Use Committee (IACUC) in Nigeria.

Animals. Male Albino rats weighing 120-150 g were obtained from the Animal House of the Department of Biochemistry, University of Ilorin, Ilorin. The animals were allowed to acclimatize for 48 hours in the laboratory prior to the experiments and were fed standard laboratory animal diet and given tap water ad libitum.

Plant material. The leaves of *Croton zambesicus* were collected between April and June, 2014 from Jos, Jos North local government area of Plateau State, Nigeria. The plant was identified by Mr. I.A. Kareem, a taxonomist at the Federal College of Forestry, Jos, Nigeria and further authenticated at the Herbarium Section in the Department of Pharmacognosy, University of Jos, Nigeria. The plant was found to correspond with voucher specimen number UJ/PGPH/HSP/0801.

Preparation of the aqueous leaf extract of *Croton zambesicus* (CZ)

The leaves were removed from the stem, shade dried for 2 weeks and reduced to fine powder using mortar and pestle. The powdered leaves (300 g) were extracted by maceration with distilled water for 24 hours and filtered with Whatman filter paper (No.1). The filtrate was evaporated to dryness on a water bath at 50-55 °C. The leaf extract obtained was dark brown in colour with a percentage yield of 12.2 % w/w. This was stored in a dessicator prior to use.

Effect of *Croton zambesicus* Extract on ACh-Induced Contraction of Rat Ileum

The animals were fasted overnight but allowed free access to water. They were euthanized by cervical dislocation. The abdomen was cut open, the caecum lifted forward and the ileo-ceacal junction identified. The ileum was cut at this point and transferred to a petri dish containing Tyrode solution. The mesentery was removed and the ileum cut into pieces of 1-1.5 cm length. A piece was fixed to a tissue clamp with a thread and placed in a 10 mL organ bath with Tyrode solution at 37 °C and aerated with oxygen. The other end was fixed to an isotonic transducer connected to a data capsule digital recorder 17400 (Ugo basile, Italy). Control responses to ACh (1 µM) were recorded. The ileum was then pretreated with aqueous leaf extract of CZ (0.64 - 6.4 mg/mL) for 3 minutes and corresponding responses to ACh was recorded. This protocol was repeated for atropine (10 µM).

Effect of *Croton zambesicus* Extract on $CaCl_2$-Induced Contraction of Rat Ileum

The rat ileum was isolated and mounted as described above. The tissue was bathed for 15 minutes in Ca^{2+} free Tyrode solution (Gilani *et al.*, 2010). Two cumulative concentration-response curves for $CaCl_2$ (1-30 mM) were obtained. The ileum was pretreated with CZ (0.64 - 6.4 mg/mL) for 3 minutes, and then a third cumulative concentration-response curve for

$CaCl_2$ was obtained. This protocol was repeated for nifedipine (2.5 µM).

Effect of *Croton zambesicus* Extract on Angiotensin II (Ang II)-Induced Contraction of Rat Ileum

A piece of ileum about 1.5 cm was fixed to a tissue clamp with a thread and placed in 10 mL organ bath containing Tyrode solution at 37 °C and aerated with oxygen. The tissue was allowed to stabilize for 20 minutes. A cumulative concentration-response for Ang II (0.01 nM-1 µM) was obtained. The tissue was washed three times every 5 minutes and allowed to equilibrate for 15 minutes to allow full recovery of receptors. The tissue was then pretreated for three minutes with CZ and another cumulative concentration-response curve for Ang II was obtained. This protocol was repeated for CZ concentrations of (0.08-6.4 mg/mL) and completed with a cumulative concentration curve of Ang II alone.

Effect of *Croton zambesicus* Extract on Castor Oil-Induced Diarrhoea in Rats

The antidiarrhoeal activity of the aqueous leaf extract of *Croton zambesicus* was evaluated using the castor oil-induced model in rats (Capasso *et al.*, 2008).

Thirty rats were randomly grouped into six groups, each with five rats and fasted for 18 hours prior to the experiment. The first group was injected with saline; three groups were treated with CZ (100-800 mg/kg). The fifth group was injected atropine 6 mg/kg *i.p.*

One hour after this treatment, the rats in all the six groups received a single oral dose of 1 ml Castor oil. Each rat was placed in cages with adsorbent paper on the floor. The cumulative frequencies of wet and dry stools were observed over a four-hour period in all the groups. The percentage inhibition of diarrhoea was calculated and the antidiarrhoeal activity of the extract determined in terms of percentage protection (Chitme *et al.* 2004; Tijani *et al.* 2009).

Effect of *Croton zambesicus* Extract on Gastrointestinal Motility Test in Rats

Thirty rats were randomly grouped into six groups of five rats each. They were fasted for 18 hours prior to the experiment. The first group was injected with saline; three groups were injected with CZ (100-800 mg/kg). The fifth group was injected atropine 6 mg/kg *i.p.*

One hour after treatment, all animal in the six groups received 1 mL of the marker (10 % activated Charcoal suspension in 5 % Acacia gum) orally.

The rats in all the groups were euthanized after 30 minutes by cervical dislocation and the abdomen was cut open. The distance traveled by the marker from the pylorus to the caecum was measured using a thread and the length of the thread was determined with a ruler. This length was expressed as a percentage of the total length of the small intestine. (Chitme *et al.*, 2004; Tijani *et al.*, 2009; Dosso *et al.*, 2012).

Intestinal transit % =

$$\frac{\text{Length traveled by charcoal marker}}{\text{Total Length of small intestine}} \times 100$$

Percentage of transit inhibition = $(T_c - T_t / T_D) \times 100$

T_D = total length of intestine

T_t = charcoal distance of test group

Statistical Analysis

Data obtained were expressed as mean ± standard error of mean (SEM). Graphs were plotted using Microsoft Excel. Statistical analysis of difference between control and treated groups was carried out using Student's t-test and one-way analysis of variance (ANOVA) followed by Bonferroni post-hoc test. Statistical significance was taken at $P < 0.05$.

Results

Effect of *Croton. zambesicus* Extract on Acetylcholine (ACh)-Induced Contraction of Rat Ileum

The aqueous leaf extract 0.64 mg/mL produced a transient increase in contraction of the ileum. Higher concentrations of 1.6 and 6.4 mg/mL significantly inhibited ($P<0.05$, $P<0.01$) ACh-induced contraction of the rat ileum with an IC_{50} of 2.3±0.05 mg/mL. This effect was similar to that produced by atropine 10 µM (Fig 1a).

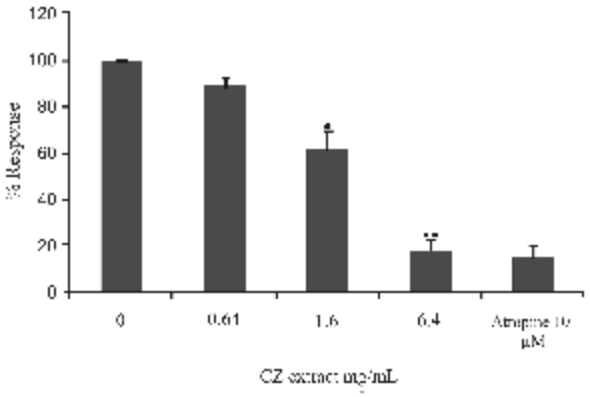

Figure 1a: Inhibitory effect of leaf extract of *Croton zambesicus* on ACh-induced contraction of rat ileum n=5, *$P<0.05$ and **$P<0.01$ vs control.

Effect of *Croton zambesicus* Extract on $CaCl_2$-Induced Contraction of Rat Ileum

The aqueous extract of *Croton zambesicus* (0.64 mg/mL) had no significant inhibitory effect on calcium chloride-induced contraction of the rat ileum. However,1 at concentrations of 1.6 and 6.40 mg/mL

the extract significantly (P<0.05 and P<0.001) inhibited calcium chloride-induced contraction respectively with an IC_{50} = 2.7±0.1 mg/mL (Fig. 1b).

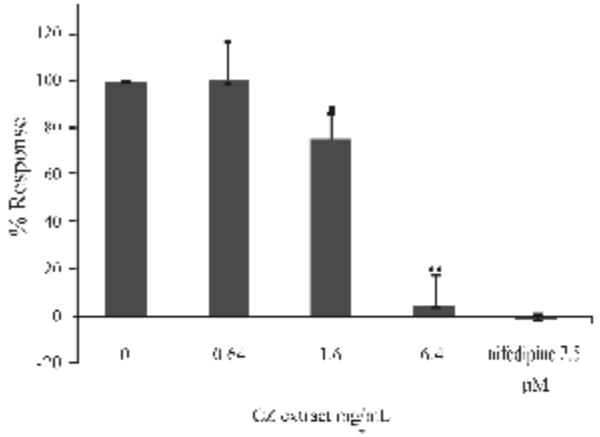

Figure 1b: Inhibitory effect of leaf extract of *Croton zambesicus* on calcium chloride-induced contraction of rat ileum n=5, *P<0.05, **P<0.001 vs control.

Effect of *Croton zambesicus* Extract Angiotensin II (Ang II)-Induced Contraction of rat Ileum

The extract (0.64 and 6.4 mg/ml) produced significant (*P*<0.01 and *P*<0.001) inhibition of Ang II-induced contraction of the rat ileum with an IC_{50} of 0.50±0.03 mg/ml (Fig. 2a). The inhibition was concentration-dependent and similar to that produced by valsartan an angiotensin type 1 (AT1) receptor antagonist (Fig 2b).

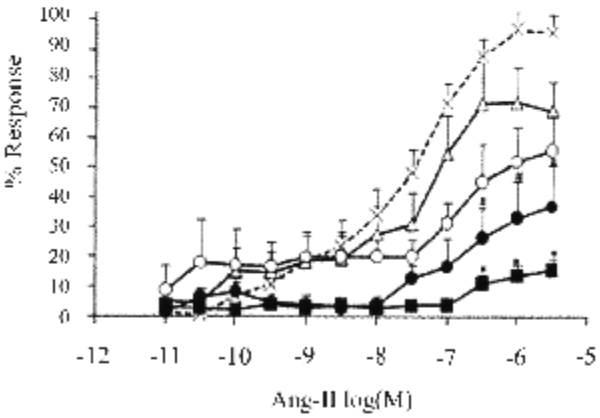

Figure 2a: Inhibitory effect of aqueous extract of *Croton zambesicus* on Ang II-induced contraction of rat ileum n=5*P<0.01 and **P<0.001 vs control. x Ang II, Ä CZ 0.08 mg/ml, Ï CZ 0.32 mg/ml, %CZ 0.64 mg/ml, % CZ 6.4 mg/ml CZ: Aqueous extract of *Croton zambesicus*

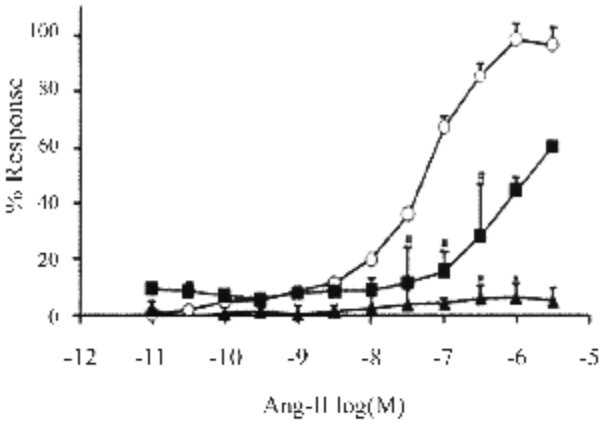

Figure 2b: Effect of valsartan on Ang II-induced contraction of rat ileum n=5, #P<0.05 *P<0.001 vs control. Ï Ang II, % valsartan 1 nM, % valsartan 10 nM.

Effect of *Croton zambesicus* Extract on Castor oil-induced Diarrhoea

The aqueous leaf extract of *Croton zambesicus* (400 and 800 mg/kg) produced significant (*P*<0.05 and *P*<0.001) reduction in the frequency of loose and dry stools which was dose-dependent. Similarly, atropine (6 mg/kg) produced a significant (P<0.01) reduction in the frequency of loose and dry stools (Fig 3a).

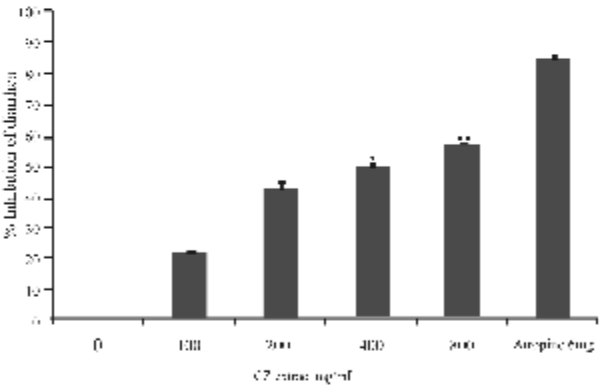

Figure 3a: Inhibitory effect of *Croton zambesicus* (CZ) extract on castor oil-induced diarrhoea n=5 *P<0.05 and **P<0.001 vs control. CZ: Aqueous extract of *Croton zambesicus*

Effect of *Croton zambesicus* Extract on Gastrointestinal Motility

The aqueous leaf extract of *Croton zambesicus* (400 and 800 mg/kg) also produced significant (*P*<0.05 and *P*<0.001) inhibition of gastrointestinal motility. The inhibition produced by atropine (6 mg/kg) was significant (P<0.05) and similar to that produced by extract 400 mg/kg (Fig 3b).

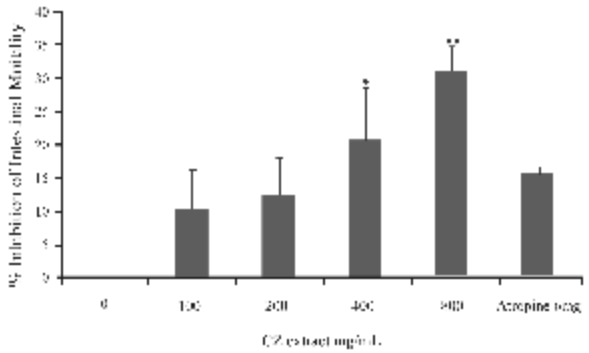

Figure 3b: Inhibitory effect of *Croton zambesicus* (CZ) extract on intestinal motility in rats n=5 *P<0.05 and **P<0.001 vs control. CZ: Aqueous extract of *Croton zambesicus*

Discussion

Phytomedicines of proven safety and efficacy contribute to the goal of ensuring majority of people have access to health care (WHO, 2013). *Croton zambesicus* was selected for this study based on its ethnomedicinal use in the management of gastrointestinal disorders such as diarrhoea and dysentery (Adjanohaun, 1989). The data obtained from this study provide evidence that the leaves of *Croton zambesicus* contain antispasmodic principles with potential benefits in the management of gastrointestinal disorders.

The extract significantly inhibited ACh-induced contraction of the rat ileum indicating a smooth muscle relaxant effect (Pintérova *et al*., 2011). Acetylcholine (ACh) acts on muscarinic M 3 receptors coupled to G-proteins which results in activation of IP_3, efflux of extracellular calcium and release from intracellular stores (Guibert *et al*., 2008; Pintérova *et al*., 2011). The role of calcium channels in antispasmodic activity of CZ was investigated and $CaCl_2$-induced contraction was significantly inhibited by CZ extract in a pattern also similar to nifedipine. This finding may suggest the extract inhibits contractile mechanisms involving extracellular calcium influx (Morel *et al*., 1997).

Inhibition of extracellular Ca^{++} influx by medicinal herbs in both intestinal and vascular smooth muscle has been reported (Ajagbonna *et al*., 2001 and Giliani *et al*., 2010). Baccilli *et al* (2007); Martinsen *et al* (2010) reported inhibition of KC1-induced contraction of the rat aorta by diterpenes isolated from *Croton zambesicus*.

In the isolated intestinal muscle preparation of the rat, cummulative increase in concentration of angiotensin II produce maximal response between 10^{-7} and 10^{-6} M (Ewert *et al*., 2006 and Spark *et al*., 2008). The aqueous extract of CZ produced a significant, dose-dependent inhibition of angiotensin II-induced contraction, characterized by a decrease in maximal response with a shift of the dose-response curve to the right. Low concentrations of CZ produced maximal inhibition of angiotensin II-induced contractions of the rat ileum, while a 10-fold increase in concentration was required for ACh and calcium chloride. Ang II produces contraction of smooth muscle via distinct post-receptor mechanism (Guibert *et al*., 2008). It acts on angiotensin type 1 (AT1) receptors and causes contraction of intestinal smooth muscle, which is mediated by G protein-dependent signaling pathways (Akazawa *et al*., 2013). Inhibition of Ang II-induced contraction by CZ may be linked to its binding on AT1 receptors in the rat ileum. The role of AT1 receptors to small and large intestinal muscle contractility provides an opportunity to discover medicinal plants beneficial in motility disorders through AT1 receptor stimulation or inhibition (Garg *et al*., 2012).

Aqueous leaf extract of *Croton zambesicus* caused a significant decrease in the frequency of loose stools and this may be attributed to its relaxant effect on smooth muscles in the gastrointestinal tract. Castor oil increases gastrointestinal motility and volume of intestinal contents by preventing its resorption from the intraluminal space. The irritation and inflammation produced by rinoleic acid prevents the reabsorption of salt and water (Chitme *et al*. 2004).

In addition, the aqueous leaf extract produced a significant, dose-dependent inhibition of gastrointestinal motility with a resultant decrease in intestinal transit of charcoal meal (Omoboyowa *et al*., 2013). The effect produced by the extract was more potent compared with atropine. Inhibitory effect of atropine on muscarinic receptors produces relaxation of smooth muscles and consequently a decrease in intestinal transit time (Brown and Taylor 2001). The antispasmodic and antidiarrhoeal activities of CZ extract may be attributed to its inhibitory action on calcium, muscarinic and angiotensin type 1 (AT1) receptors in smooth muscles of the gastrointestinal tract. (Pintérova *et al*., 2011 and Garg *et al*., 2012).

Phytochemical screening of the aqueous extract of *Croton zambesicus* and column subfractions revealed the presence of flavonoids, alkaloids, terpenoids, saponins and tannins (Salatino *et al*., 2007 and Ayanniyi *et al*., 2014). The smooth muscle relaxant activity of medicinal plants has been reported to be due to the presence of alkaloids, flavonoids, steroids, tannins and/or terpenoids.

The results from this study show that aqueous leaf extract of *Croton zambesicus* produces relaxation of smooth muscles in the gastrointestinal tract. The extract has antidiarrhoeal activity mediated via inhibition on calcium channels, muscarinic and angiotensin 1 receptors. Further bioassay-guided

phytochemical studies will be carried out to isolate and characterize the active constituents.

Conflict of Interest

The authors declare there is no conflict of interest.

References

Adjanohaun EJ, Ajakidje V de Souza S. Contributiont o Ethnobothanical and Floristic Studies in Benin Republic Vol. 1 Agency for Cultural and Technical Cooperation. 1989: 245.

Akazawa H, Yano M, Yabumoto C, Kudo-Sakamoto Y, Komuro I.. Angiotensin II type 1 and type 2 receptor-induced cell signaling. Curr Pharm Des. 2013: 19(17):2988-2995.

Ayanniyi RO, Maiha BB, Salawu OA, Jimoh Akanbi H. Hypotensive activity of aqueous leaf extract of *Croton zambesicus* Linn. (Euphorbiaceae). Centrepoint Journal (Science edition). 2014: 24(1):1-9.

Ayanniyi RO, Wannang NN. Anticonvulsant activity of the aqueous leaf extract of *Croton zambesicus* in mice and rats. Iranian J Pharmacol Ther. 2008 a: 7(1):79-82.

Ayanniyi RO, Wannang NN. Neuropharmacological profile of the aqueous leaf extract of *Croton zambesicus* (euphorbiaceae) in some laboratory animals. Iranian J Pharmacol Ther. 2008 b. 7(2):161-164.

Baccelli C, Martinsen A, Morel Quetin-Leclercq, J. Vasorelaxant activity of essential oils from *Croton zambesicus* and some of their constituents. Planta Med. 2010: 76(14):1506–1511.

Brown JH, Taylor P. Muscarinic receptor agonist and antagonist. In: Hardman GL, Limbird LE and Gilman AG, eds. Goodman and Gilman's The Pharmacological Basis of Therapeutics 10th ed. New York: McGraw Hill, 2001: 162-163.

Capasso R, Borrelli F, Cascio MG, Aviello G, Huben K, Zjawiony JK, Izzo AA. Inhibitory effect of salvinorin A, from Salvia divinorum, on ileitis-induced hypermotility: cross-talk between kappa-opioid and cannabinoid CB(1) receptors. Br J Pharmacol. 2008. 155(5): 681-689.

Chitme HR, Chandra M, Kaushik S. Studies on anti-diarrhoeal activity of Calotropis gigantea R.Br. in experimental animals. J Pharm Pharm Sci. 2004. 7(1):70–75.

Dosso K, N'guessan B, Bidie A, Gnangoran B, Méite S, N'guessan D. Ehilé, E. Antidiarrhoeal Activity of an Ethanol Extract of the Stem Bark of *Piliostigma reticulatum* (Caesalpiniaceae) in Rats. Afr J Trad CAM. 2012. 9(2):242-249.

Ewert S, Spak E, Olbers T, Johnsson E, Edebo A, Fandriks L. Angiotensin II indued contraction of rat and human small intestinal wall musculature in vitro. Acta Physiol (Oxf). 2006. 188(1):33-40.

Garg M, Angus PW, Burrell LM, Herath C, Gibson PR, Lubel JS. The pathophysiological roles of the renin-angiotensin system in the gastrointestinal tract. Aliment Pharmacol Ther. 2012. 35(4):414-428.

Giliani AH, Mandukol S, Iqbal J, Yasinzai M, Aziz N, Khan A, Rehman N. Antispasmodic and vasodilator activities of *Morinda citrifolia* root extract are mediated through blockade of voltage dependent calcium channels. BMC Complement Altern Med. 2010. 1186/1472-6882-10-2.

Guibert C, Ducret, T and Savineau, JP. Voltage-independent calcium influx in smooth muscle. Prog. Biophys. and Mol. Biol. 2008. 98(1):10-23.

Martinsen A, Baccelli C, Navarro I, Abad A, Quetin-Leclercq J, Morel N. Vascular activity of a natural diterpene isolated from Croton zambesicus and of a structurally similar synthetic trachylobane. Vascul. Pharmacol. 2010. 52(1-2):63-69.

Morel JL, Macrez N, Mironneau J. Specific Gq Protein Involvement In Muscarinic M3 Receptor-Induced Phosphatidylinositol Hydrolysis And Ca2+ Release In Mouse Duodenal Myocytes. Br. J. Pharmacol. 1997. 121: 451-458.

Okokon JE, Nwafor PA. Antiinflammatory, analgesic and antipyretic activities of ethanolic root extract of *Croton zambesicus*. Pak . J. Pharm. Sci. 2010. 23(4):385-392.

Omoboyowa DA, Nwodo OFC, Joshua PE. Antidiarrhoeal activity of chloroform ethanol extracts of *Anacardium accidentale* kernal. Journal of Natural Products. 2013. 6:109-117

Patwardhan B, Warunde D, Pushpangadan P, Bhatt N. Evid based Complement Alternat Med. 2005. 2:465-473.

Pintérová M, Kuneš J, Zicha J. Review: Altered Neural and Vascular Mechanisms in Hypertension. Physiol. Res. 2011. 60:381-402.

Salatino A, Salatino MLF, Negri G. Traditional uses, chemistry and pharmacology of *Croton* species (Euphorbiaceae). J Braz Chem Soc. 2007. 18:11-33.

Spark E, Casselbrant A, Olbers T, Lonroth H, Fandricks L. Angiotensin II-induced contractions in human jejuna wall musculature in vitro. *Acta Physiol* (Oxf). 2008. 193:181-90.

Tijani AY, Okhale SE, Salawu TA, Onigbanjo HO, Obianodo LA, Akingbasote JA, Emeje M. Antidiarrhoeal and Antibacterial properties of crude aqueous stem bark extract and fractions of Parkia biglobosa (Jacq.) R. Br. Ex G. Don. Afri J Pharm Pharmacol. 2009. 3(7): 347-353. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-77953672811&partnerID=tZOtx3y1.

World Health Organization, 2013. *WHO traditional medicine strategy 2014-2023*, Available at: http://apps.who.int/iris/handle/10665/92455.