Afr. J. Biomed. Res. 13 (May 2010); 119 -123

Research article

Antiulcerogenic Activity of Ethanolic Leaf Extract of *Croton zambesicus* in Rats

[*1]Okokon Jude E., [2]Umoh Uwem F., [1]Udobang John A., [3]Etim Emmanuel I

Departments of [1]Pharmacology and Toxicology, [2]Pharmacognosy and Traditional Medicine, and [3]Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.

ABSTRACT: *Croton zambesicus* Muell. Arg. is often used in traditional medicine by Ibibios of Niger Delta region of Nigeria for the treatment of several diseases including gastrointestinal disorders especially ulcer. The antiulcer activity of the ethanolic extract of the crude leaf extract was investigated against indomethacin, ethanol and histamine – induced ulcer models in rats. The crude leaf extract of *Croton zambesicus* (200 – 600mg/kg) significantly ($p<0.001$) inhibited chemically – induced ulcers in rats. These effects may in part be due to phytochemical constituents of the extract. The results of this work suggest that the leaf extract of *Croton zambesicus* possesses antiulcer activity, supporting the ethnomedical use of the plant among the Ibibio tribe of Niger Delta.

Key Word: *Croton zambesicus,* antiulcer, gastroprotective, stomach, medicinal plant

INTRODUCTION

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 Guineo – congolese species widely spread in tropical Africa. Ethnobotanically, the leaf decoction is used in Benin as anti-hypertensive and anti-microbial (urinary infections) (Adjanohoun *et al,* 1989) and in parts of Nigeria as antidiabetic and malarial remedy (Okokon *et al*., 2005a, 2006). The roots are used as antimalarial, febrifuge and antidiabetic by the Ibibios of Niger Delta region of Nigeria (Okokon and Nwafor, 2009a). The root is also used in Sudan for menstrual pain (El-Hamidi, 1970) and as aperients (Ngadjui *et al*., 1999). Boyom *et al*. (2002) studied the composition of essential oils from the leaves, stem and roots of *Croton zambesicus* and found that the three types of oils are similar in composition, with those from the leaves and stem rich in monoterpenes, while that of the root bark contains sesquiterpenes. The root and stem bark oils were found to be rich in oxygen-containing compounds, with spathulenol and linalool as major components. Okokon and Nwafor (2009a) reported that the root extract whose LD_{50} is 273.86 mg/kg contains alkaloids, saponins and terpenes. Others were tannins, phlobatannins, anthraquinones and cardiac glycosides, while flavonoids absent. Block *et al. (*2002) isolated entrachyloban-3β-ol, an ent-trachylobane diterpene from dichloromethane extract of the leaves and reported that the diterpene has a cytotoxic activity on HeLa cells. Similarly**,** two new trachylobane – and one isopimarane type diterpenoids; ent-18-hydroxy-trachyloban-3-one, ent-trachyloban-3- one, isopimara-7,15-dien-3β-ol, together with transphytol, β-sitosterol, α-amyrin and stigmaterol have been isolated from the leaves (Block *et al*., 2004). Crotonadiol, a labdane diterpenoid, clerodane, crotocorylifuran and two trachylobanes; 7β-acetoxytrachyloban – 18 – oic acid,

*Address for correspondence: Tel: +234-802-3453678
E-mail address: judeefiom@yahoo.com

Received: December 2009; Revised version Accepted: May, 2010

trachyloban - 7β, 18 – diol, lupeol, β-sitosterol and its 3-β-glucopyranosyl derivative were isolated from the stem bark (Ngadjui *et al*, 1999). Ngadjui *et al*., (2002) further isolated three clerodane diterpenoids, crotozambefurans A, B and C from the stem bark.

Studies have reported on the antimicrobial properties of the leaf and stem (Abo *et al* **.,** 1999) as well as roots(Okokon and Nwafor, 2010). The ethanolic leaf extract has been reported to possess antiplasmodial (Okokon *et al.*, 2005a), antidiabetic (Okokon *et al*., 2006), anti-inflammatory, analgesic and antipyretic activities (Okokon *et al.,* 2005b), while the root extract has been reported to possess antimalarial (Okokon and Nwafor, 2009a) and anticonvulsant and antiulcer activities (Okokon and Nwafor, 2009b).

Information on biological activity of the leaf are scarce. We therefore investigated the gastroprotective activity of the leaf extract in rats in order to ascertain the antiulcer potentials of the plant (leaf) as claimed by the indigenous tribe of Ibibio of Niger Delta Region of Nigeria.

MATERIALS AND METHODS

Plant Materials

The fresh leaves of *Croton zambesicus* were collected from the premises of the University of Uyo and were identified and authenticated as *Croton zambesicus*. Muell. Arg (Euphorbiaceae) by Dr. (Mrs.) Margaret Bassey of the Department of Botany and Ecological Studies**,** University of Uyo and deposited at herbarium of Department of Pharmacognosy and Traditonal Medicine, University of Uyo (DPNM.31c).

Extraction

The leaves of the plant were air-dried, pulverized using a pestle and mortar and cold-macerated for 72 hours using ethanol. The liquid ethanolic extract that was obtained by filtration was concentrated in vacuo at 40^{o}C and all the ethanol was completely removed. The ethanolic extract was stored at -4^{O}C until used.

Animals

Albino rats (105 – 165g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water ad libitum. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

Evaluation of antiulcer activity
Indomethacin-induced ulcer

Male adult albino rats (150 – 170g) were randomly divided into five groups of six rats each. The animals were starved of food for 24 hours and water 2h before the commencement of experiment (Alphin and Ward, 1967). They were treated as follows; Group 1 (control) received only indomethacin (Sigma, 60 mg/kg p.o. dissolved in 5% Na_2Co_3); Groups 2 - 4 were pretreated with *Croton zambesicus* extract (200, 400 and 600 mg/kg p.o. respectively); Group 5 received cimetidine (100mg/kg p.o. dissolved in 50% Tween 80). One hour later, groups 2-5 were administered with indomethacin. Animals were killed by cervical dislocation, four hours after indomethacin administration,. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored (Nwafor *et al*., 1996). Ulcer index (UI) and preventive ratio (PR) of each of the groups pretreated with extract were calculated using standard methods (Zaidi and Mukerji, 1958 ; Nwafor *et al*., 2000).

Ethanol-induced gastric ulceration

The procedures adopted were similar to that used in indomethacin-induced ulceration except that the negative control group (group 1) received only ethanol (2.5 ml/kg p.o), Groups 2-4 were pretreated with *Croton zambesicus* extract (200, 400 and 600 mg/kg p.o. respectively);and positive control group (Group 5) received propranolol (40 mg/kg p.o. dissolved in distilled water). Time of administration of ulcerogen and sacrifice, stomach processing and examination as well as ulcer scoring were as in indomethacin-induced ulceration

Histamine-induced gastric ulceration in rats

The procedures were similar to that used in indomethacin-induced ulceration except that the negative control group (group 1) received only received only histamine acid phosphate (Sigma, 100mg/kg i.p. dissolved in distilled water) (Maity et al.,1995); and positive control group (Group 5) received cimetidine (100mg/kg p.o. dissolved in 50% Tween 80**).** Groups 2 - 4 were pretreated with *Croton zambesicus* extract (200, 400 and 600 mg/kg p.o. respectively); Group 5 received cimetidine (100 mg/kg p.o. dissolved in 50% Tween 80), Time of administration of ulcerogen (histamine acid phosphate, 100mg/kg**.,** i.p), stomach processing and examination as well as ulcer scoring were similar to that used in indomethacin-induced ulceration except that the animals were killed by cervical dislocation 18 hours after histamine administration.

Statistical Analysis

Data are reported as mean ± standard error of the mean(SEM) and were analyzed statistically using One way ANOVA followed by Tukey-kramer multiple comparison test and values of $p<0.01$ were considered significant.

RESULTS

Indomethacin-induced gastric ulceration

The extract (p.o.) pretreatment on indomethacin-induced gastric ulceration showed a dose-dependent reduction in ulcer indices in pretreated groups relative to control. The reduction was statistically significant ($P<0.001$) compared to control. (Table 1). The extract exhibited more protective effect when compared with the standard drug.

Table 1:
Effect of *C. zambesicus* extract on indomethacin- induced ulcer

Treatment	Dose (mg/kg)	Ulcer Indices	Preventive Ratio
Control (IND)	60	15.16± 1.24	-
C. zambesicus extract p.o.	200	6.66 ± 1.32*	56.06
	400	1.66 ± 0.76*	89.05
	600	1.50 ± 0.50*	90.10
Cimetidine	100	2.50 ± 0.27*	83.50

*Data were expressed as mean ± SEM. significant at *p< 0.001 when compared to control (n = 6.); IND = indomethacin*

Ethanol-induced gastric ulceration

The extract significantly protected rats from ethanol–induced ulcer (Table 2). There was a significant ($P<0.001$) dose-dependent reduction in the ulcer indices relative to control. The effect of the extract was more than that exhibited by the standard drug, propranolol.

Table 2:
Effect of *C. zambesicus* extract on ethanol- induced ulcer

Treatment	Dose (mg/kg)	Ulcer indices	Preventive ratio
Control (ethanol)	-	3.66 ± 0.57	-
C. zambesicus extract p.o.	200	1.66 ± 0.47*	54.64
	400	0.33 ± 0.37*	90.98
	600	0.00 ± 0.00*	100
Propranolol	40	1.33 ± 0.47*	63.66

*Data were expressed as mean ± SEM. significant at *p< 0.001 when compared to control (n = 6.)*

Histamine–induced ulceration

Administration of the extract significantly ($P<0.001$) reduced histamine-induced gastric ulceration in a dose-dependent fashion compared to control (Table 3). The effect of the extract was more than that exhibited by the standard drug, cimetidine.

Table 3:
Effect of *C. zambesicus* extract on histamine- induced ulceration in rats

Treatment	Dose (mg/kg)	Ulcer index	Preventive ratio
Control (Histamine)	100	11.33 ± 0.68	-
C. zambesicus extract	200	6.33 ± 2.11*	44.13
	400	2.00 ± 0.73*	82.39
	600	1.00 ± 0.36*	91.17
Cimetidine	100	2.00 ± 0.77*	82.34

*Data were expressed as mean ± SEM. Significant at *p< 0.001 when compared to control (n = 6.)*

DISCUSSION

Croton zambesicus leaves though used in the treatment of various diseases have been reported to be used traditionally in the treatment of gastrointestinal disorders. For this reason, the antiulcer activity of the leaf extract was evaluated using indomethacin, ethanol and histamine–induced ulcer models. Indomethacin, is known to cause ulcer especially in an empty stomach (Bhargava *et al*., 1973) and mostly on the glandular (mucosal) part of the stomach (Evbuonwa and Bolarinwa, 1990; Nwafor *et al*., 1996) by inhibiting prostaglandin synthetase through the cycloxygenase pathway (Rainsford, 1987). Prostaglandins function to protect the stomach from injury by stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal turn over and repair (Hayllar and Bjarnason, 1995; Hiruma-Lima *et al*., 2006). Suppression of prostaglandins synthesis by indomethacin result in increased susceptibility of the stomach to mucosal injury and gastroduodenal ulceration. The extract was observed to significantly reduce mucosal damage in the indomethacin–induced ulcer model, suggesting the possible extract mobilization and involvement of prostaglandin in the anti- ulcer effect of the extract. Administration of ethanol has been reported to cause disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production (Salim, 1990). This is attributed to the release of superoxide anion and hydroperoxy free radicals during metabolism of ethanol as oxygen derived free radicals has been found to be involved in the mechanism of acute and chronic ulceration in the

gastric mucosa (Pihan *et al.,*1987). It was observed in this study that the extract significantly reduced ethanol-induced ulcer. This may be due to cytoprotective effect of the extract via antioxidant effects. Ethanol is also reported to cause gastric mucosal damage by stimulating the formation of leukotriene C_4 (LTC_4) (Whittle *et al*.,1985). The gastroprotective effect of the extract may in part be due to the suppression, by the extract of lipoxygenase activity (Nwafor *et al*.,1996). Histamine-induced ulceration is known to be mediated by enhanced gastric acid secretion as well as by vasospastic action of histamine (Cho and Pfeiffer,1981). The inhibition of ulcer due to histamine by the extract may be due to its suppression of histamine-induced vasospastic effect and gastric secretion. The leaf extract has been found to contain flavonoids, terpenes, saponins, alkaloids and cardiac glycosides among others. Flavonoids such as quercetin have been reported to prevent gastric mucosal lesions in various experimental models (Di Carlo *et al*., 1999; Zayachkivska *et al*, 2005) by increasing the amount of neutral glycoproteins (Di Carlo *et al*., 1999). Flavonoids have been reported to protect the gastric mucosa from damage by increasing the mucosal prostaglandin content and by inhibition of histidine decarboxylase in the mast cells. Free radical scavenging ability of flavonoids has been reported to protect the gastrointestinal tract from ulcerative and erosion lesion**s** (Borrelli and Izzo, 2000). Saponins, especially triterpenes type have been implicated in antiulcer activity mediated though the formation of protective mucus on the gastric mucosa and also protect the mucosa from acid effects by selectively inhibiting $PGF_{2\alpha}$ (Agwu and Okunji, 1986; Lewis and Hanson, 1991). The antiulcer activity of the root extract of *C. zambesicus* has been reported by Okokon and Nwafor (2009a). Similarly, essential oil from the bark of *Croton cajucara* has been reported to produce gastroprotective effect in rodent (Hiruma-Lima *et al.,* 2000). This further indicates that the antiulcerogenic effect of the extract may be inherent in the plant.

In conclusion, the results of the present study show that *Croton zambesicus* leaf extract displayed gastroprotective activity as demonstrated by significant inhibition of the formation of ulcers induced by chemical models studied. The antiulcer activity of the extract may be due to the action of the chemical compounds present in the extract. These observation**s** justify the ethnomedical uses of the plants as antiulcer agent and as an antacid.

Acknowledgements

The authors are grateful to Ms. Sifon Akpan of Department of Pharmacology and Toxicology for her technical assistance.

REFERENCES

Abo KA, Ogunleye VO and Ashidi JS (1999). Antimicrobial potential of *Spondias mombin, Croton zambesicus,* and *Zygotritonia crocea. Phytotherapy Research*, **13**(6): 494-497.

Adjanohoun EJ, Adjakide V and deSouza S (1989). Contribution to ethnobotanical and floristic studies in Republic of Benin.Vol.1. Agency for Cultural and Technical Cooperation, p.245.

Agwu, C. N., Okunji, C. O. (1986). Gastrointestinal studies of *Pyrenacantha staudii* leaf extracts. *Journal of Ethnopharmacology* 15: 45 – 55.

Alphin, R.S., Ward, J.W. (1967). Action of hexopyrronium bromide on gastric secretion in dogs and on gastric secretion and ulceration in rats. *Archieves Internationales de Pharmacodynamie et de Therapie* 270: 128 -140.

Bhargava, K.P., Gupta, M. B., Tangri, K. K. (1973). Mechanism of ulcerogenic activity of indomethacin and oxyphenbutazone. *European Journal of Pharmacology* 22:191 – 195.

Block S, Baccelli C, Tinat B, Meervelt L, Rozenberg R, Jiwan J, Llabres G, DePauw-Gillet M and Quetin-Leclercq J (2004). Diterpenes from the leaves of *Croton zambesicus*. *Phytochemistry*, 65: 1165-1171.

Block S, Stevigny C, Llabres G, deHoffman E, Adjakide V, DePauw-Gillet M and Quetin-Leclercq J (2002). Ent-Trachyloban-3β-ol, a new cytotoxic diterpene from *Croton zambesicus. Planta Medica*, **68**: 647-648.

Borrelli, F., Izzo, A. A. (2000). The plant kingdom as source of antiulcer remedies. *Phytotherapy Research* 14: 581 – 591.

Boyom FF, Keumdjio F, Dongmo PM, Ngadjui BT, Amvam-Zollo PH, Menut C and Bessiere JM (2002). Essential oil from *Croton zambesicus* Muell. Arg. growing in Cameroun. *Flavour and Fragrance Journal,* **17**: 215-217.

Cho, C. H., Pfeiffer, C. J., (1981). Gastrointestinal ulceration in the guinea pigs in response to dimaprit, histamine and H_1 and H_2 blocking agents. *Digestive Disease Science* 26: 306 – 311.

Di Carlo, G., Mascolo, N., Izzo, A. A., Capasso, F. (1999). Flavonoids: old and new aspects of a class of a natural therapeutic drug. *Life sciences* 64: 337 – 353.

El-Hamidi A (1970). Drug Plants of the Sudan Republic in native medicine. *Plantae Medica.* **18**: 278-280.

Evbuonwan, M. T., Bolarinwa, A. F. (1990). Effect of diet on indomethacin-induced peptic ulceration in pregnant rats. *Nigerian Journal of Physiological Sciences* 6: 189 – 191.

Hayllar, J., Bjarnason, I. (1995). NSAIDS, COX-2 inhibitor and the gut. Lancet 346 - 522.

Hiruma-Lima, C. A., Calvo, T. R., Rodriguez, C. M., Andrade, F. D. P., Vilegas, W., Brito, ARM. (2006). Antiulcerogenic activity of *Alchornea castaneaefolia* effects on somatostatin, gastrin and prostaglandin. *Journal of Ethnopharmacology* 104: 215 – 224.

Hiruma-Lima, C. A., Gracioso, J. S., Rodriguez, J. A., Haun, M., Nunes, D. S.,Souza Brito, ARM. (2000). Gastroprotective effect of essential oil from *Croton cajucara* Benth.(Euphorbiaceae). *Journal of Ethnopharmacology* 69: 229 – 234.

Lewis, D.A., Hanson, D. (1991). Anti-ulcer drugs of plants origin. *Progress in Medicinal Chemistry* 28: 208 – 210.

Maity, S.,Vedasiromoni, J. R.,Ganguly, D. K. (1995). Anti-ulcer effect of the hot water extract of black tea (*Camellia sinensis*). *Journal of Ethnopharmacology*. 46: 167 – 174.

Ngadjui BT, Abeghaz BM, Keumedjio F, Folefoe GN and Kapche GW (2002). Diterpenoids from the stem bark of *Croton zambesicus*. *Phytochemistry*, **60**: 345-349.

Ngadjui BT, Folefoc GG, Keumedjio F, Dango E, Sondegam BL and Conolly JD (1999). Crotondiol, a lablane diterpenoid from the stem bark of *Croton zambesicus*. *Phytochemistry*, **51**: 171-174.

Nwafor, P. A., Effraim, K. D., Jacks, T. W. (1996). Gastroprotective effects of aqueous extracts of *Khaya senegalensis* bark on indomethacin – induced ulceration in rats. *West African Journal of Pharmacology and Drug Research* 12: 46 – 50.

Nwafor, P. A., Okwuasaba, F. K., Binda, l. G. (2000). Antidiarrhoeal and antiulcerogenic effects of methanolic extracts of *Asparagus pubescens* root in rats. *Journal of Ethnopharmacology* 72: 421 – 427.

Okokon J. E., Nwajuakwu N., Udokpoh A. (2005b). Preliminary study of analgesic, anti inflammatory and anti pyretic activities of ethanolic leaf extract of *Croton zambesicus*. *Journal of Pharmacy and Bioresources* **2** (2): 75-79.

Okokon JE and Nwafor PA (2009b). Antiulcer and anticonvulsant activities of *Croton zambesicus* root extract. *Pakistan Journal of Pharmaceutical Sciences* 22(4):384 - 390.

Okokon JE, Ofodum KC, Ajibesin KK, Danladi B and Gamaniel KS. (2005a). Pharmacological screening and antiplasmodial activity of *Croton zambesicus* against *Plasmodium berghei berghei* infection in mice. *Indian Journal of Pharmacology* **37**(4): 243-246.

Okokon JE., Nwafor PA. (2010). Antimicrobial activity of root extract of *Croton zambesicus*. *Pakistan Journal of Pharmaceutical Sciences*. 23(1): 114 – 118.

Okokon, J. E. and Nwafor, P. A. (2009b). Antiulcer and anticonvulsant activities of *Croton zambesicus* root extract. *Pakistan Journal of Pharmaceutical Sciences* 22(4): 384 - 390.

Okokon, J. E., Bassey, A. L. and Obot, J. (2006). Antidiabetic activity of ethanolic leaf extract of *Croton zambesicus* on alloxan diabetic rats. *African Journal of Traditional,Complementary and Alternative Medicine* **3** (2): 21-26.

Okokon, J.E. and Nwafor, P. A. (2009a). Antiplasmodial activity of root extract and fractions of *Croton zambesicus*. *Journal of Ethnopharmacology* **121**: 74 -78.

Pihan G., Regillo, C., Szabo S. (1987). Free radicals and lipid peroxidation in ethanol or aspirin – induced gastric mucosa injury. *Digestive Diseases and Sciences* 32: 1395 – 1401.

Rainsford, K. D. (1987). The effects of 5- lipoxygenase inhibitors and leukotriene antagonists on the development of gastric lesions induced by nonsteroidal anti-inflammatory drugs in mice. *Agents and Action* 21, 316 – 319.

Salim, A. S. (1990). Removing oxygen derived free radicals stimulates healing of ethanol-induced erosive gastritis in the rats. *Digestion* 47: 24 – 28.

Whittle, B. J. R., Oren-Wolman, N., Guth, P. H. (1985). Gastric vasoconstrictor actions of leukotriene C_4 and $PGF_{2\alpha}$ and thromboxane mimetic (U-4669) on rats submucosal microcirculation *in vivo*. *American Journal of Physiology* 248: G580 – G586.

Zaidi, S. H., Mukerji, B., (1958). Experimental peptic ulceration. Part 1. The significance of mucus barrier. *Indian Journal of Medical Research* 46: 27 – 37.

Zayachkivska, O. S., Konturek, S. J., Drozdowicz, D., Konturek, P. C., Brzozowski, T., Ghegotsky, M. R. (2005). Gastroprotective effects of flavonoids in plants extracts. *Journal of Physiology and Pharmacology* 56: 216 - 231.