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ANTIULCEROGENIC ACTIVITY OF ETHANOLIC LEAF EXTRACT OF LASIANATHERA AFRICANA

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University of Uyo, Uyo, Nigeria.***E-mail:** judeefiom@yahoo.com**Abstract**

The effect of ethanolic leaf extract of *Lasianthera africana* on experimentally induced ulcer was studied in rats. The extract (1000 – 3000mg/kg) inhibited ethanol-induced, indomethacin – induced and reserpine –induced ulcer models in a dose dependent fashion. The various degrees of inhibitions were statistically significant (p<0.01). The effect of the extract was comparable to that of the standard drugs used. Thus, *Lasianthera africana* extract demonstrated a good antiulcer activity which supports the antiulcer effect of this plant in traditional medicine.

Key words: *Lasianthera africana*, antiulcer, vegetable**Introduction**

From time immemorial plants have served as food and medicine to man. Vegetables and leaves of some trees domesticated or wild are used by the Ibibios of Niger Delta region of southern Nigeria in the preparation of their soup daily. Some of these edible plants are equally medicinal and are used in the therapy of some diseases, majority of which have been reported to contain vital chemical compounds of medicinal importance.

Lasianthera africana (P.Beav.) is a perennial glabrous shrub of the family Icacinaceae whose height may reach from 61 to 136 cm and is widely distributed in the tropical rain forest (Hutchinson and Dalziel, 1973). There are four ethnovarieties distinguished by their taste, leaf colour and ecological distribution. The leaves are consumed as vegetable in southern Nigeria. Ethnobotanically, *L. africana* is used as antacid, analgesic, antispasmodic, laxative, antipyretic, antiulcerogenic, antidiabetic and antimalarial. *Lasianthera africana* has been reported to be bacteriostatic (Itah, 1997), fungicidal (Itah, 1996) antidiabetic (Ekanem, 2006) and antiplasmodial (Okokon *et al.*, 2007). The leaf whose LD₅₀ was 5000mg/kg contains alkaloids, terpenes, saponins, tannins, flavonoids, anthraquinones and cardiac glycosides (Okokon *et al.*, 2007). The aim of the present study was to evaluate the antiulcer potential of the ethanolic extract of the dark green variety on some experimentally induced –ulcer models in rodents.

Materials and Methods**Plant materials**

Fresh leaves of *Lasianthera africana* were collected in August, 2006 from a farmland in Uruan, Akwa Ibom State, Nigeria. The plant was identified and authenticated by Dr. Margaret Bassey, a taxonomist in the Department of Botany, University of Uyo, Uyo, Nigeria. Herbarium specimen (herbarium specimen no. FPH. 33C) was deposited at Faculty of Pharmacy Herbarium. The fresh leaves (2kg) of the plant were dried on laboratory table for 2

weeks and reduced to powder. 100g powder was macerated in 95% ethanol (300ml) for 72 hrs. The liquid filtrate obtained was concentrated in vacuo at 40°C. The yield was 3.98% w/w. The extract was stored in a refrigerator at 4°C until used for experiment reported in this study.

Animals

Male albino rats (170 -200g) were obtained from the University of Uyo animal house and used for the study. 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.

Indomethacin-induced ulcer

Male adult albino rats were used for the experiment. They were randomized into six groups of six rats each. Food was withdrawn 24 hrs and water 2hr before the commencement of experiment (Alphin and Ward, 1967). Group 1 (control) received only indomethacin (Sigma, 60mg/kg p.o. dissolved in 5% Na₂CO₃); Groups 2- 4 were pretreated with *Lasianthera africana* extract (1000,2000 and 3000 mg/kg p.o. respectively) as an aqueous solution; Group 5 received cimetidine (100mg/kg p.o. dissolved in 5% Tween 80), while Group 6 received cimetidine (100mg/kg.p.o), 10 mins later, extract (2000mg/kg. p.o) was given. One hr later, groups 2 - 6 were administered with indomethacin. Four hr after indomethacin administration, animals were killed by cervical dislocation. 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), preventive ratio (PR) and degree of ulceration (DU) 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 procedure was similar to that used in indomethacin-induced ulceration. The rats were randomly assigned into six groups of six rats each. Food was withdrawn 24 hrs and water 2hr before the commencement of experiment (Alphin and Ward,1967). Group 1(control) received only ethanol (2.5 ml/kg p.o), Groups 2- 4 were pretreated with *Lasianthera africana* extract (1000, 2000 and 3000 mg/kg p.o. respectively) as an aqueous solution; Group 5 received propranolol (40mg/kg p.o. dissolved in distilled water), while Group 6 received propranolol (40mg/kg. p.o dissolved in distilled water), 10 mins later, extract (2000mg/kg.p.o) was given. One hr later, groups 2 - 6 were administered with ethanol. Four hrs after ethanol administration, animals were killed by cervical dislocation. 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.,2000).

Reserpine-induced gastric ulceration in rats

Male adult albino rats weighing 120 – 170g were used for the experiment. They were randomized into six groups of six rats each. Food was withdrawn 24 hrs and water 2hr before the commencement of experiment (Alphin and Ward, 1967). Group 1 (control) received only reserpine (Sigma, 8mg/kg p.o. dissolved in Tween 80); Groups 2- 4 were pretreated with *Lasianthera africana* extract (1000, 2000 and 3000 mg/kg p.o. respectively) as an aqueous solution; Group 5 received cimetidine (100mg/kg p.o. dissolved in 50% Tween 80), 1 hr prior to reserpine administration, while Group 6 were pretreated with cimetidine (100mg/kg.p.o), 10 mins later, extract (2000mg/kg. p.o) was given. One hour later, groups 2 - 6 were administered with reserpine. Four hrs after reserpine administration, animals were killed by cervical dislocation. 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), preventive ratio (PR) and degree of ulceration (DU) of each of the groups pretreated with extract were calculated using standard methods (Zaidi and Mukerji 1985; Nwafor et al., 2000).

Statistical Analysis

Data are reported as mean \pm standard error of the mean (SEM) and were analyzed statistically using One way ANOVA followed by Tukey-kramer multiple comparison test and values of $P < 0.001$ and 0.05 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 ($P < 0.05$) compared to control (Table 1). The effect was comparable to that of the standard drug, cimetidine.

Ethanol-induced gastric Ulceration

The extract significantly protected rats from ethanol-induced ulcer (Table 2). There was a significant ($P < 0.05$) dose-dependent reduction in the ulcer indices relative to control.

Reserpine-induced ulceration

Administration of the extract significantly ($P < 0.05, 0.001$) reduced reserpine-induced gastric ulceration in a dose dependent fashion compared to control (Table 3)

Table 1: Effect of *L. africana* extract on indomethacin induced ulcer

TREATMENT	DOSE(mg/kg)	ULCER INDICES	PREVENTIVE RATIO
Control(indomethacin)	60	16.33 \pm 3.88	-
<i>L. africana</i> extract p.o.	1000	12.50 \pm 1.80**	23.45
	2000	1.16 \pm 0.23*	92.28
	3000	1.00 \pm 1.41*	93.38
Cimetidine	100	0.66 \pm 0.47*	95.95
Cimetidine + <i>L.africana</i>	100 + 2000	0.66 \pm 0.62*	95.95

Data were expressed as mean \pm SEM. significant at * $P < 0.001$, ** 0.05 when compared to control $n = 6$.

Table2: Effect of *Lasianthera africana* extract on ethanol induced ulcer

Treatment	Dose(mg/kg)	Ulcer indices	Preventive ratio
Control(ethanol)	-	4.33 \pm 0.47	-
<i>L. africana</i> extract p.o	1000	2.33 \pm 0.47*	46.19
	2000	2.00 \pm 0.00*	53.38
	3000	1.00 \pm 0.00*	76.90
Propranolol	40	1.00 \pm 0.81*	76.90
Propranolol + <i>L. africana</i>	40 + 2000	0.50 \pm 0.22*	88.84

Data were expressed as mean \pm SEM. significant at * $P < 0.001$ when compared to control $n = 6$.

Table 3: Effect of *Lasianthera africana* extract on reserpine induced ulceration in rats

Treatment	Dose(mg/kg)	Ulcer index	Preventive ratio
Control(reserpine)	8	17.42 \pm 0.34	-
<i>L. africana</i> extract	1000	12.30 \pm 0.51*	29.39
	2000	5.15 \pm 0.28*	70.43
	3000	2.25 \pm 0.42*	87.08
cimetidine	100	2.63 \pm 0.81*	84.90
Cimetidine+ <i>L.africana</i>	100 +2000	1.28 \pm 0.67*	92.65

Data were expressed as mean \pm SEM. significant at * $P < 0.001$ when compared to control $n = 6$.

Discussion

Lasianthera africana leaves though used as a vegetable has been reported by Okokon et al., (2007) to be used traditionally in the treatment of ulcer. For this reason, the antiulcer activity of the leaves extract was evaluated using indomethacin, reserpine and ethanol – induced ulcer models. Indomethacin, a known ulcerogen especially in an empty stomach (Bhargava et al.,1973) causes ulcer mostly on the glandular (mucosal) part of the stomach (Evbuonwa and Bolarinwa,1990; Nwafor et al.,1996) by inhibiting prostaglandin synthetase through the cyclooxygenase 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 results in increased susceptibility of 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 reduced significantly 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 C4 (LTC4) (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). Although the mechanism of reserpine induced gastric damage is poorly understood, it has been suggested by Salim (1990) to be similar to that of ethanol as discussed above. As such, the reduction of reserpine-induced ulcer by the extract in this study may be linked to its cytoprotective effect through antioxidant activity. Okokon et al., (2007) reported that the leaf extract contains flavonoids, terpenes, saponins, alkaloids and cardiac glycosides among others. Flavonoids such as quercetin has been reported to prevent gastric mucosal lesions in various experimental models (Di carlo et al., 1999; Zayachkivska, 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 inhibiting histamine secretion from mast cells by inhibition of histidine decarboxylase. Free radical scavenging ability of flavonoids has been reported to protect the gastrointestinal tract from ulcerative and erosion lesion (Borrelli and Izzo, 2000). Saponins, especially triterpenes type have been implicated in antiulcer activity mediated by formation of protective mucus on the gastric mucosa and also protect the mucosa from acid effects by selectively inhibiting prostaglandin F 2a.(PGF2 α) (Agwu and Okunji, 1986; Lewis and Hanson, 1991).

In conclusion, the results of the present study show that *Lasianthera africana* leaf extract displays gastroprotective activity as demonstrated by significant inhibition of the formation of ulcers induced through three different ulcer models studied. The antiulcer activity of the extract maybe due to the action of its phytochemical compounds present in the extract. The observation justifies the ethnomedical uses of the plants as antiulcer and antacid in addition to its nutritional values.

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References

1. Agwu,C.N. and Okunji, C.O. (1986). Gastrointestinal studies of *Pyrenacantha staudii* leaf extracts. *J. Ethnopharmacol.* **15**:45 – 55.
2. Alphin,R.S. and Ward, J. W. (1967). Action of hexopyrroonium bromide on gastric secretion in dogs and on gastric secretion and ulceration in rats. *Archives Internationales de Pharmacodynamie et de Therapie.* **270**:128 -140.
3. Bhargava,K.P.,Gupta M.B. and Tangri,K.K.(1973).Mechanism of ulcerogenic activity of indomethacin and oxyphenbutazone .*European J. Pharmacol.*, **22**:191 – 195.

4. Borrelli, F. and Izzo A.A.(2000).The plant kingdom as source of anti ulcer remedies.Phytother. Res.**14**:581 – 591.
5. Di Carlo G.,Mascolo,N.,Izzo, A.A. and Capasso,F.,(1999).Flavonoids:old and new aspects of a class of a natural therapeutic drug. Life sciences **64**:337 – 353.
6. Ekanem, A.(2006).Antidiabetic activity of ethanolic leaf extract and fractions of *Lasianthera africana* on alloxan diabetic rats.M.Sc. Thesis.University of Uyo.Nigeria.
7. Evbuonwa,M.T. and Bolarinwa, A. F.(1990). Effect of diet on indomethacin-induced peptic ulceration in pregnant rats. Nig. J. Physiol. Sciences. **6**:189 – 191.
8. Hayllar J. and Bjarnason, I.,(1995). .NSAIDS, COX-2 inhibitor and the gut .Lancet 346 - 522.
9. Hiruma-Lima , C. A., Calvo, T.R., Rodriguez,C.M., Andrade,F. D. P., Vilegas, W. and Brito, A.R. M. (2006). Antiulcerogenic activity of *Alchornea castaneaefolia* effects on somatostatin,gastrin and prostaglandin.J. Ethnopharmacol.**104**:215 – 224.
10. Hutchinson J. and Dalziel, J. M. (1973). Flora of West tropical Africa.2nd edition.Crown Agents for Overseas Government. And Administration.Vol.1.part.2.p.638.
11. Itah, A. Y. (1996). Screening of plant's parts for fungicidal properties. Trans. Nig. Soc. Bio. Conserv. **4(1)**:26 – 40.
12. Itah, A.Y. (1997). Bactericidal and bacteriostatic effect of edible leafy vegetable extract on growth of canned food borne bacteria. Trans.Nig.Soc.Bio.Conserv.**6**:103 – 111.
13. Lewis, D.A. and Hanson, D. (1991).Anti-ulcer drugs of plants origin. Progress in Medicinal Chemistry. **28**:208 – 210.
14. Nwafor,P.A.,Effraim,K.D. and Jacks,T. W. (1996). Gastroprotective effects of acqueous extracts of *Khaya senegalensis* bark on indomethacin – induced ulceration in rats .West Afr. J. Pharmacol. Drug Res. **12**:46-50.
15. Nwafor,P.A.,Okwuasaba F.K. and Binda L. G. (2000). Antidiarrhoeal and antiulcerogenic effects of methanolic extracts of *Asparagus pubescens* root in rats. J. Ethnopharmacol.**72**:421 – 427.
16. Okokon, J. E., Antia, B. S. and Essiet, G. A. (2007). Evaluation of in vivo antiplasmodial activity of ethanolic leaf extract of *Lasianthera africana*. Res. J. Pharmacol.**1(2)**:30 – 33.
17. Pihan G.,Regillo, C. and Szabo S. (1987). Free radicals and lipid peroxidation in ethanol or aspirin – induced gastric mucosa injury. Digestive Diseases and Sciences.**32**:1395 – 1401.
18. Rainsford, K. D.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.
19. Salim, A.S. (1990). Removing oxygen derived free radicals stimulates healing of ethanol induced erosive gastritis in the rats. Digestion **47**:24 – 28.
20. Whittle, B. J. R., Oren-Wolman, N. and Guth, P. H. (1985). Gastric vasoconstrictor actions of leukotriene C₄ and PGF_{2 α} and thromboxane mimetic (U-4669) on rats submucosal microcirculation in vivo..Am. J. Physiol.**248**: G580 – G586.
21. Zaidi,S.H. and Mukerji, B. (1958). Experimental peptic ulceration. Part 1. The significance of mucus barrier. Indian J.Med. Res. **46**:27 – 37.
22. Zayachkivska, O. S., Konturek, S. J., Drozdowicz, D., Konturek P.C., Brzozowski T. and Ghegotsky, M.R. (2005). Gastroprotective effects of flavonoids in plants extracts. J. Physiol. Pharmacol. **56**: 216 231.