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Original Research Article

Gastro-Protective Effects of the Leaf Extract and Fractions of *Fleurya aestuans* L (Urticaceae)

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Abstract

Purpose: To investigate the gastroprotective properties of the methanol leaf extract and fractions of *Fleurya aestuans* L (Urticaceae) in rodents.

Methods: Anti-ulcer effect was evaluated in three ulcer models induced by ethanol, indomethacin and hypothermic-restraint stress in rats. Other anti-ulcer related activities of the extract such as the effects on gastrointestinal motility, and the activity on contractions evoked by standard agonists on isolated guinea pig ileum were also determined.

Results: Increasing concentrations of the extracts and fractions did not produce spasmogenic effect on the isolated guinea pig ileum, but produced a dose-related inhibition of contractile responses to histamine and acetylcholine with IC₅₀ range of 0.245-0.525 and 0.525-1.525 mg/ml, respectively. In the ethanol-induced ulcer models, administration of the extracts of *F. aestuans* at 400 mg/kg reduced the ulcer indices of all the treated groups, but significant ($p < 0.05$) ulcer protection was shown by the n-hexane and the methanol fractions. The rats were also significantly protected from the indomethacin-induced ulceration by the methanol extract ($p < 0.05$). The methanol extract and the n-hexane fraction conferred significant ($p < 0.05$) gastro-protection against ulcers induced by cold restraint (stress) in rats. Administration of the ethylacetate fraction (EF, 400 mg/kg) and the n-hexane fraction (HF, 400 mg/kg) produced significant ($p < 0.05$) anti-peristaltic activity reducing gastrointestinal motility in mice in a dose-related manner.

Conclusion: The leaf extracts of *F. aestuans* possess gastroprotective properties could justify folklore uses of the plant in peptic ulcer diseases.

Keywords: Anti-ulcer activity, *Fleurya aestuans*, gastro-protection, peptic ulcers

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Introduction

Peptic ulcer disease (PUD) is a spectrum of diseases consisting of gastritis, gastric ulcers, and duodenal ulcers¹. It is known to occur when the endogenous defense mechanisms of the protective mucosal barrier have failed to sufficiently counteract the aggressive factors (hydrochloric acid, pepsin, and *Helicobacter pylori*) and is characterized by gnawing or burning sensation in the abdomen². Duodenal ulcers occurs more frequently (about 80 % of PUDs) than gastric ulcers. The life time prevalence of PUDs is about 10 %³. PUDs are recurrent and most clinical studies have shown that approximately 50 % of all ulcer patients will have recurrence within one year of diagnosis.

Although advances have been made in the treatment of PUDs due to an increased understanding of the mechanisms associated with the development of ulceration in the gastrointestinal tract, the morbidity and mortality toll is still very high. In the United States, for example, a study estimated about 6500 deaths each year on ulcer-related complications⁴.

The available drugs for the management of PUDs are associated with high relapse rates and limiting side effects.^{5, 6, 7} Validation of the efficacy and harnessing of medicinal plants used in folk medicine for the treatment of peptic ulcer diseases is a very promising approach to overcome the limitations of orthodox medicines. Already, there is an avalanche of scientific evidences in support of the efficacy of medicinal plants in the management of ulcers of different aetiologies⁶⁻¹⁴

Fleurya aestuans is an erect, annual herb growing up to 1.5 m high with long stinging hairs. The leaves are greenish, alternate, more or less spirally arranged, and oval shaped of about 10-15 cm long and 8-12 cm wide, cordate at the base and narrowly

pointed at the apex, and coarsely toothed with hairs on both sides¹⁵. The decoctions of *F. aestuans* roots and leaves are used as antidote for poisoning especially from snake bites¹⁶. The leaves have been reported to be used for the treatments of rickets in children, constipation, wound dressing, and as a post-partum tonic¹⁷. In Western India, it is used to relieve rheumatic pain and as a diuretic.

Although the aqueous leaf extract of *F. aestuans* is popular in traditional medicine practice in south-eastern Nigeria as a palliative in a variety of stomach disturbances, there has not been any scientific report on the anti-ulcer properties of the plant. This motivated the present study on the anti-ulcer and other related gastro-protective properties of the *F. aestuans*.

Material and Methods

Animals

Adult albino rats (120-200 g), adult Swiss albino mice (20-27 g) of both sexes, adult guinea pigs (250-300 g) and rabbits (1.5-2.0 kg) obtained from the animal house of Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. The animals were acclimatized for 5 days and housed under standard conditions of temperature ($25 \pm 2^\circ\text{C}$) and 12-h light/dark cycle. The rats and mice were fed with standard livestock feed and the guinea-pig and rabbit fed with a green grasses, predominantly *Panicum maxima* Jacq (Poaceae). All animals were allowed unrestricted access to drinking water.

Collection and identification of plant material

The leaves of *F. aestuans* were collected from Nsukka, Enugu State, Nigeria in the month of October, 2004. The plant material was identified and authenticated by Mr JMC Ekekwe, a plant taxonomist, of the Department of Botany, University of Nigeria.

Extraction

The fresh leaves were washed in clean tap water, air-dried and milled into a coarse powder which was divided into two portions of 300 g each. The first portion of the leaf powder was extracted by maceration in 2.5 L of methanol for 48 h with intermittent agitations. The leaf powder was exhaustively extracted with repeated washings with fresh portions of methanol. The methanol extract, ME (19.5 g) was recovered after evaporation of the filtrate *in vacuo* at 40 °C using a rotary evaporator. The second portion of the leaf powder (300 g) was successively extracted with n-hexane, ethylacetate, and then methanol in that order of increasing solvent polarity to give HF (5.8 g), EF (12.5 g), and MF (7.6 g), respectively. About 2.5 L of each solvent was used in each step to macerate for 48 h with intermittent agitations. The marc was always removed and the solvents removed by spreading on a clean nylon mat before re-introducing into the container for each subsequent extraction.

The extract and fractions were subjected to phytochemical analysis according to the methods of Evans (2004)¹⁸.

Acute toxicity (LD₅₀) test

The acute toxicity test of the methanol extract (ME) of *F. aestuans* was estimated in mice using the method of Lorke (1983)¹⁹. Briefly, the tests involved two phases. The first phase is determination of the toxic range. The mice were placed in three groups (n = 3) and were given ME (10, 100 and 1000 mg/kg; p.o.) solubilised in a solution of 3 %, v/v Tween 85. The treated mice were observed for 24 h for number of deaths. The death pattern in the first phase determined the doses used for the second phase. Since there was no deaths recorded in the first phase, a fresh batch of four mice received 1000, 1600, 2900, and 5000 mg/kg of the extract. The treated animals were observed for lethality or signs of acute intoxication for 24 h. The LD₅₀ is the geometric mean of the

highest non-lethal dose and the least toxic dose¹⁹.

Anti-ulcer tests

The extracts (ME, HF, EF, and MF) were suspended in 3 % Tween 85 and tested orally for anti-ulcer activities using three models of experimental gastric ulcers. The extracts of *F. aestuans* were tested and compared for antiulcer properties at 400 mg/kg, determined as an effective dose in a prior dose-efficacy study using the whole methanol extract. All the rats employed were fasted 18 h prior to the tests but were allowed free access to water. Cimetidine (100 mg/kg) was given as a reference drug to the positive control group while the negative control group received 3 % Tween 85 (5 ml/kg).

Ethanol-induced ulcer model: The six groups of adult albino rats (n = 5) received either the vehicle (5 ml/kg), cimetidine (100 mg/kg), or 400 mg/kg of the extracts (ME, HF, EF, and MF) and 1 h later was administered with ethanol (1 ml of 96 %, v/v). The animals were sacrificed 1 h after the administration of the ethanol²⁰.

Indomethacin-induced ulcer model: In this study, indomethacin (100mg/kg) was administered orally to six groups of albino rats (n = 5) 1 h after the various treatments with the vehicle (5 ml/kg), cimetidine (100 mg/kg), or 400 mg/kg of the extracts (ME, HF, EF, and MF). The animals were sacrificed 8 h after the indomethacin treatment²¹.

Hypothermic restraint-stress ulcer model: The albino rats were placed into groups (n = 5) and administered the vehicle (5 ml/kg), cimetidine (100 mg/kg), or 400 mg/kg of the extracts (ME, HF, EF, and MF). One hour later, the animals were immobilized individually in a restraining cage and put in a refrigerator maintained at 4 ± 1° C. The rats were sacrificed after 1 h of cold-immobilisation²².

In each of these study models, the stomach of the sacrificed rats was removed, opened along the greater curvature, and then rinsed under a stream of tap water. The stomach was pinned flat on a corkboard and was observed using a hand lens ($\times 10$ magnification). Erosions formed on the glandular portions of the stomach were counted and each given a severity rating on a 0-3 scale based on the diameter of ulcer (0, no ulceration; 1, ulcers ≤ 1 mm; 2, ulcers > 1 mm ≤ 2 mm; 3, ulcers > 3 mm)^{6,7,14,23}. The total ulcer score for each stomach divided by a factor of 10 was calculated for each animal and expressed as ulcer index (U.I.). The degree of ulcer protection for each treatment group was calculated as a percentage with respect to the mean ulcer index of the negative control group.

Effect of extract on gastrointestinal transit

Forty adult albino mice (of either sex) were fasted for 24 h prior to the experiment, but were allowed unrestricted access to drinking water. They were randomized into ten groups ($n = 5$). One group received Tween 85 (5 ml/kg, p. o.) and a second group received atropine (10 mg/kg, p. o.). The extracts (ME, HF, EF, and MF) were each administered at 200, 400 mg/kg, p.o. to different groups of the mice. After 1 h of treatment, each mouse received 0.5 ml of charcoal meal (5 % charcoal in 10 % tragacanth mucilage) orally. The mice were sacrificed, 30 min later, with excess chloroform anaesthesia and the intestine carefully removed and displayed. The intestinal distance moved by the charcoal plug from the pylorus was measured for each mouse and expressed as a percentage of the total distance from the pylorus to the ileocaecal junction for the respective animal.

In vitro pharmacological studies

The effects of the extracts on isolated guinea pig ileum and isolated rabbit jejunum preparations were studied. Segments of the tissues, 2-3 cm long, were suspended in 20

ml organ bath filled with Tyrode solution of composition (mM/L): NaCl-136.7, KCl-2.7, CaCl₂-1.8, NaHCO₃-11.9, MgCl₂-1.0, Na₂HPO₄-0.4, and glucose-5.5 maintained at 37 ± 1 °C and aerated with air. The preparations were set up under a resting tension of 0.5 g and allowed to equilibrate for 60 min during which the bathing fluid was changed every 10 min. At the end of equilibration period, the extracts were tested for any spasmogenic or spasmolytic activity by adding increasing concentrations of each of these extracts (100–3200 μ g/ml) on guinea pig ileum and rabbit jejunum preparations. The effects of the extracts on submaximal responses of standard drugs (ACh and Histamine) were also determined and the IC₅₀ calculated for each treatment. The contact time for the activity of each extract was 120 seconds while the standard spasmogen acted for 30 seconds in a 3 minute time cycle. Responses were determined in triplicate and recorded on Ugo Basile Unirecorder (7050) through an isometric transducer (7004).

Antimicrobial screening

Helicobacter pylori, the enteric organism implicated in peptic ulcer disease was not used in this screening test because of the difficulty in culturing the organism. However, other enteropathogenic and related microorganisms were employed. The organisms used were: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Salmonella typhi*, *Candida albican* and *Aspergillus niger*. They were clinical strains obtained from the Pharmaceutical Microbiology Laboratory, University of Nigeria, Nsukka. The organisms were maintained by weekly subculturing and incubating at 37 °C (for bacteria) and 25 °C (for fungi and yeast). 24 h old cultures of the microorganisms were used in the screening. The agar disc diffusion method was employed²⁴. Wells of 8 mm diameter were bored on seeded gelled agar dish containing 1.0×10^6 cfu/ml of the respective organism and varying concentrations (5-50 mg/ml) of the extracts applied to the appropriately

labeled wells. The plates were incubated at 37 °C for 24 h for bacteria and 48 h for fungi and yeast. The effects of the extract on the growth of the microorganisms were studied by observing the zones of inhibitions. The experiments were carried out in triplicates and the mean inhibition zone diameter obtained in each case.

Statistical analysis

The results were analysed using one-way ANOVA, subjected to least squared difference (LSD) post Hoc tests and expressed as mean \pm standard error of mean. Significant differences between paired mean observations were taken at $p \leq 0.05$.

Results

The phytochemical study showed that the whole methanol extract of *F. aestuans* contains saponins, tannins, flavonoids, terpenoids, steroids and glycosides. After 24 h of oral administration, the methanol extract of *F. aestuans* did not produce lethality or any sign of acute intoxication in the mice at a dose up to 5000 mg/kg.

The extracts have no inherent spasmogenic activity on the isolated guinea pig ileum. Increasing concentrations of the extracts and fractions did not produce contractile

response of the isolated guinea pig ileum, but produced a dose-related inhibition of contractile response to histamine and acetylcholine. The concentration of each extract which produced 50 % inhibition of the maximal response to histamine IC_{50} and ACh are shown in Table 1.

Table 1: The IC_{50} of the extracts and fractions on histamine and acetylcholine-induced contraction of isolated guinea pig ileum

Extract	Median inhibitory concentration IC_{50} (mg/ml)	
	Histamine	ACh
Methanol extract	0.525	1.120
Hexane fraction	0.395	0.525
Ethyl acetate	0.245	0.880
Methanol fraction	0.395	1.525

In the ethanol-induced ulcer models, administration of the extract and the fractions of *F. aestuans* at 400 mg/kg reduced the ulcer indices of the treated groups. However, a higher and significant ($p < 0.05$) ulcer protective effect was recorded for the n-hexane and the methanol fractions (Table 2). The extract and fractions (400 mg/kg) also

Table 2: Effect of *F. aestuans* extracts on ulcers induced by different ulcerogens

Treatment	Ulcer indices for the different ulcerogens		
	Ethanol-ulcer (1 ml of 96 %, p. o)	Indomethacin-ulcer (30 mg/kg, p. o)	Stress-ulcer (hypothermic-restraint)
Methanol extract (400 mg/kg)	1.64 \pm 0.36 (52.05 %)*	1.56 \pm 0.56 (53.85 %)*	2.82 \pm 0.39 (46.29 %)*
Hexane fraction (400 mg/kg)	0.9 \pm 0.32 (97.37%)*	2.12 \pm 0.81 (37.28 %)	0.82 \pm 0.40 (84.25 %)*
Ethyl acetate (400 mg/kg)	1.28 \pm 0.45* (62.57 %)	1.98 \pm 0.79 (41.42 %)	4.3 \pm 0.53 (18.41 %)
Methanol fraction (400 mg/kg)	0.68 \pm 0.16 (80.12 %)*	3.34 \pm 0.45 (10.18 %)	0.33 \pm 0.86 (37.38 %)
Cimetidine (100 mg/kg)	0.94 \pm 0.27 (72.51 %)*	0.66 \pm 0.23 (80.47 %)*	1.50 \pm 0.99 (71.53 %)*
Negative control (Tween 85, 1 ml/kg)	3.42 \pm 0.48	3.38 \pm 0.55	5.27 \pm 1.04

* $p < 0.05$; percentage ulcer protection are in parenthesis

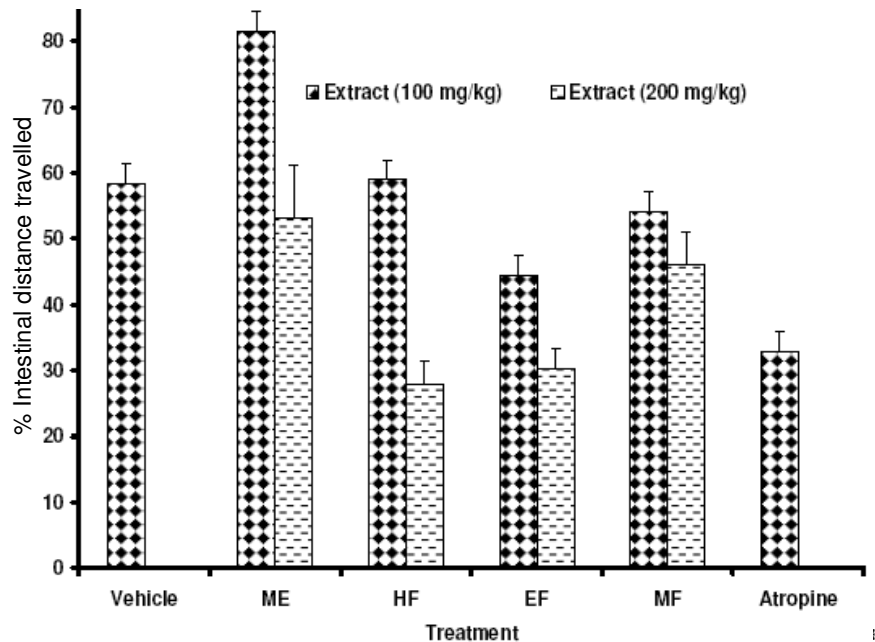


Figure 1: The effect of *F. aestuans* extracts on gastrointestinal motility
 ME = methanol extract, HF = Hexane fraction, EF = Ethyl acetate fraction, MF = Methanol fraction

conferred ulcer protective effects on the indomethacin-induced ulceration which is shown by the reduced ulcer indices of the treated groups, but only the whole methanol extract showed significant ($p < 0.05$) protection when compared to the negative control treatment (Table 1). The extract and fractions conferred some degree of protection against ulcers induced by cold restraint (stress) in rats though significant ($p < 0.05$) gastro-protective effect was shown by the whole methanol extract and the n-hexane fraction when compared to the negative control treatment. Administration of the extracts reduced gastrointestinal motility in mice significantly ($p < 0.05$) in a dose-related manner. In the control treatment group, the charcoal meal plug traversed 58.42 % of the length of the intestine while the EF (400 mg/kg) and the HF (400 mg/kg), which showed higher and significant levels of anti-peristaltic activity, reduced the distances traversed to 30.20 and 27.86 %, respectively (Figure1).

The extracts and fractions of *F. aestuans* did not inhibit the growth of bacteria and fungi used in the antimicrobial screening of the extracts at the concentrations tested.

Discussion

The different therapeutic options available for the treatment of ulcers are either employed to inhibit gastric secretions or to enhance mucosal defense^{2,25}. These anti-ulcer therapeutic strategies are aimed at balancing the mucosal aggressive factors against mucosal protective factors. These therapies are intended to relieve the patient from the ulcer pain, to facilitate the healing of the lesions, to prevent reoccurrence and the development of associated complications²⁵. Traditional folklore medicine practice has claimed a lot of success in the use of medicinal plants in the management of peptic ulcer diseases and these has encouraged our recent screening of some of these herbs for gastro-protective properties with a view to isolating potent and safe antiulcer drugs from medicinal

plants^{6,7,11,13,26,27}. The leaf of *F. aestuans* is popular among the herbalist in south-eastern Nigeria as a palliative in stomachache. In this study, we investigated the gastro-protective properties of the leaf extracts of the plant using three ulcer models induced by different ulcerogens (ethanol, indomethacin, and cold-restraint). These three models represent some of the most common causes of gastric ulcers in humans.

In the ethanol-induced ulcer models, administration of the extract and the fractions of *F. aestuans* protected the treated animals from ulceration, especially in the groups treated with n-hexane and the methanol fractions. It is possible that these extract may be acting by promoting the mucosal defensive mechanisms since ethanol-induced gastric mucosal lesions are known to be caused by direct toxic action of ethanol, reduction in bicarbonate secretion and depletion of gastric wall mucous²⁸. Ethanol is known to reduce endogenous glutathione and prostaglandin (PG) levels while the release of histamine, influx of calcium ions and generation of free radicals are increased²⁹. Research has also demonstrated that ethanol-induced gastric mucosal lesions are not inhibited by anti-secretory agents but by agents that enhance mucosal defensive factors²⁰.

The whole methanol extract of *F. aestuans* also showed a significant ($p < 0.05$) protection of the rats against indomethacin-induced ulcers. Non-steroidal anti-inflammatory agents such as indomethacin cause ulceration mostly at the glandular part of the stomach which is related to inhibition of endogenous prostaglandin synthesis³⁰. Prostaglandins have been demonstrated to serve useful gastro-protective functions which involve maintaining gastric microcirculation, stimulation of mucous and bicarbonate secretions and inhibition of gastric acid secretions³¹. This inhibition of prostaglandins is associated with over production of leukotrienes which induces mucosal vasoconstriction thereby reducing local blood flow³². This activity of the extract

also suggests cytoprotective mechanisms of action.

The extract and fractions of *F. aestuans* conferred some degree of protection against ulcers induced by cold restraint (stress) in rats though significant ($p < 0.05$) gastro-protective effect was shown by the whole methanol extract and the n-hexane fraction when compared to the negative control treatment. Hypothermic restraint ulcer model is associated with increase in gastric acid secretion and a decrease in pH. Histamine is believed to have an essential role in the pathogenesis of stress-induced ulcer since it is a potent stimulator of gastric acid secretion³⁴.

Reduction in intestinal motility ameliorates ulcer pain and hastens the healing of ulcer wounds³⁵. The extracts reduced gastrointestinal motility in mice significantly ($p < 0.05$) in a dose-related manner. The extract also showed a significant ($p < 0.05$) and dose-related reduction in gastrointestinal motility. The reduction in gastrointestinal motility may be related to the inhibition of contractile responses evoked by acetylcholine and histamine on isolated guinea pig ileum.

Microbial colonization of the gastrointestinal system has been associated with a variety of peptic ulcer diseases³⁶. *Helicobacter pylori* has been implicated in the pathogenesis of ulcer and has made antibiotics an essential component in the management of the PUDs. This stimulated our interest in screening the extracts of the extracts of *F. aestuans* for antimicrobial activity. Although we could not culture *H. pylori* for use in the antimicrobial studies, the effects of the extracts against other Gram negative enteric pathogens were tested, but the extracts did not inhibit microbial growth.

The extracts of *F. aestuans* were shown to possess non-specific gastro-protective activities against ulcers induced by the different ulcerogens. Phytochemically, the extracts of *F. aestuans* contain saponins,

tannins, flavonoids, terpenoids, steroids and glycosides. Some these bioactive constituents have been associated with gastro-protection and anti-ulcer effects in previous studies. The non-specific gastro-protective activities of the extracts may be the result of a combined effect of the different phyto-constituents present.

Flavonoidal compounds have shown anti-secretory and cytoprotective properties³⁷ and is also believed to increase capillary resistance and cause an improvement in microcirculation. This activity could render the cells less injurious to ulcer aggressive factors³⁸. The extracts of *F. aestuans* is also rich in saponins which have been shown to exhibit anti-ulcer properties through the formation of protective mucous on the gastric mucosa and by selectively inhibiting PGF₂ α ^{39,40}. The extracts also contain tannins which act as an astringent. Tannins generally have vasoconstrictive and protein precipitating effects. Precipitation of protein at ulcer sites forms impervious protective pellicle which renders the lesion less permeable to toxic substances and more resistant to attack of proteolytic enzymes⁴¹.

Conclusion

The extract of the leaves of *F. aestuans* possesses gastroprotective properties justifying the use of the plant leaves in peptic ulcer diseases by traditional healers.

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