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Antiulcereogenic effects of Ageratum conyzoides extract in male albino rats

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Abstract
Aims: Antiulcereogenic effect of methanol extract of *Ageratum conyzoides* was evaluated in indomethacin- induced gastric ulceration in male albino rats.
Methods: The ground plant leaves were macerated to prepare a methanol extract. Twenty male albino rats were divided into five groups of five rats each. The extract was administered orally at the doses of 100, 200 and 400 mg/kg body weight for the experimental groups. Distilled water (2 ml/kg) and cimetidine (32 mg/kg) were administered orally to the control and reference groups respectively. The extract treatments were carried out 60 minutes before oral administration of indomethacin (30 mg/kg) for ulcereogenic inducement. The rats were sacrificed after 8 hours.
Results: Rats pre- treated with extracts of *A. conyzoides* showed non-significant ($P > 0.05$) inhibition of gastric ulcer induced by indomethacin, however, pre- treatment with Cimetidine (reference drug) produced higher ulcer protection when compared with the extract. The rats pre-treated with 200 mg/kg. b.w of the extract showed higher ulcer inhibition than those pre-treated with 100 and 400 mg/kg b.w of the extract respectively, signifying non dose dependency.
Conclusion: *A. conyzoides*, a common weed, has some properties that can protect from ulcer induced by indomethacin. This suggests, it could be an easy source for antiulcer remedy.

Keywords: *Ageratum conyzoides*, antiulcereogenic, indomethacin and cimetidine.

1. Introduction
Ageratum conyzoides (A. conyzoides), Goatweed, is an erect, herbaceous annual, 30 to 80 cm tall [1]. It's traditional Igbo name is 'Ewu eri Okuko atu'. It has an erect stem and a strong, unpleasant smell. Its stems are covered with fine white hairs, leaves are opposite, pubescent with long petioles and include glandular trichomes [2]. The inflorescences contain 30 to 50 pink flowers arranged as a corymb and are self-incompatible [3, 4, 5]. In some countries the species is considered a weed, and control is often difficult [6, 7, 8, 9]. It is a common weed of the tropical zone. It grows sometimes as an ornamental plant [10] and is commonly used as a traditional medicine [11, 12]. *Ageratum* ranges from Southeastern North America to Central America, but the centre of origin is in Central America and the Caribbean. Most taxa are found in Mexico, Central America, the Caribbean, and Florida. *A. conyzoides* now is found in several countries in tropical and sub-tropical regions, including Brazil [13]. The plant is classified into two subspecies, *latifolium* and *conyzoides*. Subspecies *latifolium* is found in all the Americas and subsp. *conyzoides* has a pantropical distribution [14].
It succeeds in full sun and in a sheltered position in any reasonably fertile moisture-retentive soil that does not dry out in the summer [10]. Plants are reasonably tolerant of shade, though can be outcompeted by taller plants. Plant vigour and flowering periods are much reduced on dry soils [10]. The plant is a common weed in the tropicsb [10]. It can flower and produce fruit all year round; individual plants can produce 40,000 seeds and, in some areas, one-half of the seeds will germinate shortly after they are shed. Seeds are mainly spread by wind and water and will germinate under a wide range of conditions. The fresh plant is malodorous [10]. There is high variability in the secondary metabolities of *A. conzyoides* which include flavonoids, alkaloids, cumarins, essential oils, and tannins [1]. Precocene I and precocene II were identified in an *A. conyzoides* plant collected in India [16]. These compounds have been shown to affect insect development, as antijuvenile hormones, resulting in sterile adult [16]. About 51 terpenoid compounds, including precocene I and precocene I1 have been identified [17]. Up to11 cromenes in essential oils was found in the plant which includes a new cromene, 6.

angeloyloky-7- methoxy-2, 2-dimethylcromen [18].
Flavones were identified in India, including some considered new such as *ageconyfavones* A, B, and C [19]. Hexametoxyflavone has also been reported [20]. The species contains alkaloids, mainly the pirrolizidinic group, which suggest that it may be a good candidate for pharmacological studies. Several alkaloids were found including 1, 2-desifropirrolizidinic and licopsamine which can have hepatotoxic activity [21]. *A. conyzoides* is widely utilized in traditional medicine by various cultures worldwide, although applications vary by region. In Central Africa, it is used to treat pneumonia, but the most common use is to cure wounds and burns [22]. Traditional communities in India use this species as a bacteriocide, antidysenteric, and antilithic[16], and in Asia, South America, and Africa, aqueous extract of this plant is used as a bacteriocide [23,17]. In Cameroon and Congo, traditional use is to treat fever, rheumatism, headache, and colic [24, 25]. In Reunion, the whole plant is used as an antidysenteric [26]. Aqueous extracts of leaves or whole plants have been used to treat colic, colds and fevers, diarrhea, rheumatism, spasms, or as a tonic [27]. *A. conyzoides* has quick and effective action in burn wounds and is recommended by Brazilian Drugs Central as an antirheumatic [1]. Effective analgesic action was reported in rats using aqueous extract of *A. conyzoides* leaves (100 to 400 mg/kg) [25]. The action of cromenes (precocenes I and II) isolated from *Ageratum* plants was reported to accelerate larval metamorphosis,this resulted in juvenile forms or weak and small adults [19]. The juvenilizing hormonal action of precocene I and II in insects was demonstrated, the most common effect being precocious metamorphosis, producing sterile or dying adults [17]. Peptic ulcers are sores that develop in the lining of the stomach, lower esophagus or small intestine. It is one of the most frequent gastrointesinal diseases, which causes a high rate of morbidity particularly in the population of non-industrialised countries [29]. Gastric ulceration is a benign lesion on the mucosal epithelium upon contact of the stomach to excess acid and aggressive pepsin activity. It is the most common gastrointestinal disorder ever known, accounting for an estimated 15 mortality out of every 15,000 complications yearly in the world [30]. Pathophysiology of ulcer is due to an imbalance between aggressive factor such as acid, pepsin, *Helictobater pylori*, non steroidal anti-inflammatory drugs and local defense factors including mucus, bicarbonate, blood flow, prostangladins [31]. Gastro duodenal mucosa integrity is maintained through a homeostatic balance between aggressive and defensive factors [32]. Because prostaglandins have a protective effect on the lining of the stomach, long term inhibition of cycloxygenase (COX-1) with NSAIDs can promote gastrointestinal bleeding [33].

2. Materials and Methods

2.1. Chemicals and drugs

All chemicals and drugs used in this study were of analytical grade and products of Sigma Aldrich, Germany.

2.2 Plant Materials

The plant *A. conyzoides* were collected from Uli and Egbuoma of Oguta Local Government Area of Imo State, Nigeria. They were authenticated by Dr. C. J. Ukpaka, a botanist, in the Department Biological sciences, Chukwuemeka Odimegwu Ojukwu University, Uli campus. The leaves were plucked from the plants and then dried at room temperature (29-35^{O}C) for four weeks, after which they were blended into fine powder. The ground leaves were macerated for 92 hours in 80% methanol. This was filtered through muslin cloth on a plug of glass wool in a glass column. The resulting methanol extract was finely filtered using Whatman No 1 qualitative filter paper and concentrated using rotary evaporator (IKA, Germany) at an optimium temperature of 40 - 50^{O}C (to avoid denaturation of the active ingredients).

2.3 Animals used

Male albino rats of wistar strain weighing (139-215) g obtained from the animal unit of the department of Zoology and Environmental Biology, University of Nigeria, Nsukka were used for the study. They were housed in ventilated metal steel cages with sufficient space to ease their movement. The rats were fed with growers mash (Niger feeds, Nigeria) purchased from the Local market and water. They were allowed to acclimatize in the laboratory for seven days before the experiment and were given free access to water.
All the animals were carefully monitored and maintained in accordance with accepted principles for laboratory animal use and care by National Institute of Health Guide for care and use of laboratory animal (Pub No. 85-23, revised 1985).

2.4 Phytochemical Analysis

The phytochemical analysis of the methanol extract of *A. conyzoides* was carried out to identify the secondary metabolites according to the methods of Harbourne[34] and Trease and Evans [35].

2.5 Lethal Toxicity Studies (LD_{50})

Lethal toxicity study was determined following the method of Lorke[36]. The animals were divided into two phases with each phase subdivided into three groups made up of three mice each. The mice were monitored closely for 24 hours for signs of toxicity and lethality.

2.6 Gastric ulceration

An experimental indomethacin model of inducing experimental gastric ulcers was used to assess the antiulcer activity of *A. conyzoides* extract [37].
This was carried out as described by Ukwe and Nwafor[38]. Food was withdrawn 24 hours and water, 1 hour before drug treatment. Twenty male albino rats were used for study. The rats were randomly divided into five groups of five rats each. Animals in groups 1 and 2 received distilled water and cimetidine (32 mg/kg b.w.) respectively. Those in groups 3, 4 and 5 were pre-treated with 200, 400 and 800 mg/kg b.w. of the extract respectively. The animals were treated per orally with normal saline and varying doses of *A. conyzoides* extract as mentioned above. The drug (5 ml/kg) dose and extract were freshly prepared as suspension in normal saline and administered per oral to the animals. After one hour, indomethacin 30 mg/kg (dissolved in 5% sodium bicarbonate solution) was administered orally to all the rats. Seven hours later, the rats were sacrificed by cervical dislocation. The rats' stomachs were removed, washed gently and each opened along the greater curvature, pinned flat on a board, examined with a hand lens(x10) and scored for ulcer. The ulcer score was calculated for each animal according to arbitrary scale used by Singh *et al.* [39]. Where 0 = no lesion, 1= hyperemia, 2 = one or two slight lesions, 3 = very severe and 4 = mucosal full of lesion.
Ulcer index was calculated as mean ulcer scores [40].
Percentage inhibition to ulcer formation in rats by the extract was calculated as follows

$$\% \text{ inhibition of ulceration} = \frac{(\text{ulcer index}_{\text{control}} - \text{ulcer index}_{\text{test}}) \times 100\%}{\text{Ulcer index}_{\text{control}}} \quad [31]$$

2.7 Statistical Analysis
Results obtained were subjected to statistical tests using Statistical Package for Social Sciences (SPSS). All values were expressed as mean ± SEM. Data were analysed by one way ANOVA and difference between means will be assessed by two- tailed students' t-test. $P < 0.05$ was considered statistically significant.

3. Results
3.1 Lethal toxicity study
Result of acute toxicity showed that the oral administration of the methanol leaf extract of *A. conyzoides* to albino mice up to the dose of 5000 mg/kg b.w. did not record any mortality. However, a mild clinical sign of writhing and tremors in mice treated with the dosage of 5000 mg/kg b.w. was observed. This indicated that the extract could be safe at 5000 mg/kg b.w.

3.2 Phytochemical Screening
Phytochemical analysis showed that methanol extract of *A. conyzoides* contains flavonoids, glycosides, saponins, phenol and tannins.

3.3 Gross evaluation of gastric lesions
Indomethacin-induced ulcers in 100 % of the animals in the negative control (distilled water; 2 ml/kg group (Table 1). The ulcer index was 3.67 ± 1.00, which was characterized with severe disruption of surface epithelium of gastric mucosa. Pre – treatment with cimetidine significantly ($p < 0.05$) reduced the severity of indomethacin – induced ulcers compared to rats pre- treated with distilled water (ulcer control). The *A. conyzoides* leaf extract were also shown to exert some level of cyto protective effect but it does not have as much effect as cimetidine.

Table 1: Effects of *A. conyzoides* on gastric ulceration by indomethacin

Groups	Pre treatment	Dosage	mean ulcer ± SEM	Percentage protection
1.	distilled water	2 ml/kg	3.67± 1.00	0.00
2.	cimetidine	32 mg/kg	1.30 ± 0.47	64.58
3.	extract	100 mg/kg	3.00 ±1.41	18.26
4.	extract	200 mg/kg	1.67 ± 0.47	54.5
5.	extract	400 mg/kg	3.00 ± 1.41	18.26

Discussion
The present study was designed to investigate the anti-ulcer activity of methanolic leaf extract of *A. conyzoides* against indomethacin-induced gastric ulceration in albino rats. The results of this study demonstrated that methanol extract of *A. conyzoides* leaves significantly protected against mucosal damage induced by indomethacin and curative ratios of plant extract 100, 200 and 400 mg/kg body weight were 18.26 %, 54.50 % and 18.26 % respectively. The effect of the extract at 200 mg/kg b.w. compared favourably to cimetidine 32 mg/kg (positive control). As shown in Table 1, cimetidine produced a stronger anti-ulcer than any of the doses of the extract. Indomethacin at doses higher than the toxic dose (20 mg/kg) produced visible ulceration in rats [37]. Research has shown that, indomethacin when administered on an empty stomach shows ulcereogenic properties [41]. The ulcereogenic activity of indomethacin and other non – steroidal anti-inflammatory agents as postulated might be due to their ability to inhibit prostaglandin synthesis [42], an action essential for making pain and fever better [43]. However, prostaglandins have protective actions on the mucosa and have the ability to stimulate mucus and bicarbonate output [44] and increase mucosal blood flow [45].

Prostaglandins serve a cytoprotective role in the stomach. PGE2 is synthesized by epithelial and smooth muscle cells in the stomach, where it reduces gastric acid secretion while stimulating the production of protective mucus. For this reason, synthetic prostaglandins are helpful in promoting the healing of gastric ulcers [33]. Evidence had shown that NSAIDS and aspirin –like drugs can also chemically reduce the hydrophobicity of the mucus gel layer that protects the surrounding tissue from the acidic content of the gut [46, 47]. *A. conyzoides* protected the stomach from ulcers induced by indomethacin at comparable rates as cimetidine, a standard drug. We cannot give specific mechanism for reasons why lower and highest dose produced the same level of ulcer reduction which is lower than the intermediate dose given. The cytoprotection observed may be as a result of an increase in prostaglandins release [37]. Phytochemical study of this plant showed flavonoids, glycosides, saponins, phenols and tannins as major components. Flavonoids, which A. conyzoides has in abundance, are among the important constituents of plant for which anti ulcer activity have been exclusively confirmed [48, 49, 50].

Conclusion
The findings of this study show that methanol extract of leaves of *A. conyzoides* have some anti-ulcer protective activity. This gives a scientific basis for its use by traditional herbal givers as stomach cooling agent.

Declaration of interest
Authors declare that we have no competing interest.

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References
1. Ming LC. *Ageratum conyzoides*: A tropical source of medicinal and agricultural products. In: J Janick (ed.), Perspectives on New Crops and New Uses. ASHS Press, Alexandria, VA, 1999, 469-473.
2. Jhansi P, Ramanujam CGK. Pollen analysis of extracted and squeezed honey of Hyderabad, India. Geophyte. 1987; 17:237-240.
3. Kaul MLH, Neelangini S. Male sterility in diploid Ageratum conyzoides L. Cytologia. 1989; 54:445-448.
4. Ramanujam CGK, Kalpana TP. *Tamarindus indica* L. an important forage plant for *Apis florea* F. in south central India. Apidol. 1992; 23:403-413.
5. Kleinschmidt G. Colony nutrition on the Atherton tableland. Austrl Beekeep. 1993; 94:453-464.
6. Kalia BD, Singh CM. Studies on weed management in maize. In: Proc. Ind. Soc. Weed Sci. Int. Symp. 1993;

3:89-90.
7. Lam CH, Lim JK, Jantan B. Comparative studies of a paraquat mixture and glyphosate and/or its mixtures on weed succession in plantation crops. Planter. 1993; 69:525-535.
8. Waterhouse DF. Prospects for biological control of paddy weeds in southeast Asia and some recent success in the biological control of aquatic weed. Camber. Ext Bul Australia, 1993, 366.
9. Kshattryya S, Sharma GD, Mishra RR. Fungal succession and microbes on leaf litters in two degraded tropical forests of worth-east India. Pedobiologia. 1994; 38(2):125-137.
10. Huxley A. The new RHS dictionary of Gardening. Macmillan press, 1992, 57-89.
11. Manandhar NP. Plants and people of Nepal, Timber press. Oregon, 2002, 57-69.
12. Nguyen VD, Doan TN. Medicinal plants in Vietnam. World heaith organization, 1989.
13. Baker HG. Characteristics and modes of origin of weeds Academic Press, New York, 1965.
14. Johnson MF. A monograph of the genus *Ageratum* L. (Compositae, Eupatorieae). Ann. Missouri Botan Gar. 1971; 58:6-88.
15. Borthakur N, Baruah AKS. Search for precocenes in *Ageratum conyzoides* Linn. of North-East India. J Ind. Chem. Soc. 1987; 64:580-585.
16. Amaefule KI, Ifemeje JC, Mere, CA. Effect of crude oil pollution on phytochemical and anti-diabetic potentials of *Oxytenanthera abyssinica* (rhizomes) in normal and alloxan induced diabetic rats. IJTSRD. 2019: 3:1072-1075.
17. Ekundayo O, Sharma S, Rao EV. Essential oil of Ageratum conyzoides. Plant and Medicine. 1988; 54:55-57
18. Gonzales AG, Thomas G, Ram P. Chromenes form Ageratum conyzoides. Phytochemy. 1991; 30:1137-1139.
19. Vyas AV, Mulchandani NB. Structure reinvestigation of conyzorigun, a new chromone from *Ageratum conyzoides*. J Chem. Soc. Perkin. Trans. 1984; 1:2945-2947.
20. Horie T, Tominaga H, Kawamura Y. Revised structure of a natural flavone from *Ageratum conyzoides*. Phyto. 1993; 32:1076-1077.
21. Trigo JR, Campos S, Pereira AM. Presença de alcalóides pirrolizidinicos em *Ageratum conyzoides* L. In: Simposio de Plantas Medicinais do Brasil, Sao Paulo. (Resumos), 1988, 13.
22. Durodola JJ. Antibacterial property of crude extracts from herbal wound healing remedy—*Ageratum conyzoides*. Plant Med. 1977; 32:388-390.
23. Almagboul AZ, Farroq AA, Tyagi BR. Antimicrobial activity of certain Sudanese plants used in folkloric medicine: Screening for antibacterial activity, part II. Fitoterap. 1985; 56:103-109.
24. Menut C, Sharma S, Luthra C. Aromatic plants of tropical central Africa, Part X-Chemical composition of essential oils of *Ageratum houstonianum* Mill. And *Ageratum conyzoides* L. from Cameroon. Flav Frag J. 1993; 8(1):1-4.
25. Bioka D, Banyikwa FF, Choudhuri MA. Analgesic effects of a crude extract of *Ageratum conyzoides* in the rat. Acta Horticul. 1993; 332:171-176.
6. Vera R, Chemical composition of the essential oil of *Ageratum conyzoides* L. (Asteraceae) from Reunion. Flav Fragr J. 1993; 8:256-260.
26. Oliveira F, Akisue MK, Garcia LO. Caracterização farmacognóstica da droga e do extrato fluído de mentrasto, *Ageratum conyzoides* L. Lecta. 1993; 11(1):63-100.
27. Raja SS, Singh A, Rao S. Effect of *Ageratum conyzoides* on *Chilo partelus* Swinhoe (Lepidoptera: Pyralidae). J Anim Morph Physiol. 1987; 34(1-2):35-37.
28. Folk GW. Cecil Essentials of Medicine. 5th ed. WB Saunders company, Edinburgh, 2001, 334-343
29. Del Valle J, Chey W, Scheiman J. Acid Peptic Disorders. In: Yamada T, Aplers D, Kaplowit ZN. (Eds). Textbook of Gastroenterology. 4th ed. Philadelphia,
30. Balogun ME, Jeje SO, Salami SA, Onwe PE, Folawiyo MA. Anti-ulcereogenic and gastric anti-secretory effects of *Nauclea latifolia* extract in male albino rats. E J Exp Biol. Lippincotn Williams and Wilkins 2003; 1321-1376. 2015; 5(2):74-80.
31. Hardman JG, Limbird LE. (ed). Goodman's and Gilman"s the pharmacological Basis of Therapeutics, 10th edition, Tata LL Mc Graw Hill New York, 2001, 1005-1019.
32. Rosenthal MD, Glew RH. Eicosanoids. Medical bio chemistry, Human Metabolism in Health and Disease. John Wiley and Sons Incorporated. River Street. Hoboken. 2009; 111:218-228.
33. Harborne JB. Phytochemical methods: A guide to modern techniques to plant analysis. 2nd ed. Chapman and Hall Ltd, London, 2000.
34. Trease G, Evans WC. Trease and Evans Pharmacognosy, Physicans guide to herbal medicine: 13th edition, Balliere, Tindall, London, 2002, 343-383.
35. Lorke D. A new approach to practical acute toxicity testing. Archives of Toxicology. 1983; 54:275-287.
36. Anosike C, Abonyi O, Ubaka C. Does the African garden egg offer protection against experimentally induced ulcers? As Pac J Trop Med, 2011, 163-6.
37. Ukwe CV, Nwafor SV, Anti-ulcer activity of leaf extract of Persea americana Nig. J Pharm Res. 2004; 3(1):91-95.
38. Singh S, Bani S, Singh GB. Anti-inflammatory activity of Lupeol. Fitotererap. 1997; 68:9-16.
39. Tan PV, Nditafon NG, Yewah MP, Dimo T, Ayafor FI. *Eremomoatax speciosa*: effect of leaf aqueous extract on ulcer formation and gastric secretion in rats. J Ethnopharmacol. 1996; 54:139-142.
40. Blaargwa KP, Gupta MB, Tangri KK. Mechanism of ulcereogenic activity of indomethacin and oxyphenbutazone. Eur J Pharm. 1993; 22:191-195.
41. Vane JR. Inhibition of prostaglandin synthesis as a mechanisun of action of Aspirin like drugs. Nat New Biol, 1971, 231-232.
42. Reisner L, Koo JSP. Pains and its management. In: Koda Kimble MA, Young LY, Kradjan WA, Gugliemo BJ. Applied therapeutics; the clinical use of drugs. 8th ed. Philadelphia: Lippincott Williams and Wilkins, 2005.
43. Gyires K. Gastric mucosal protection from prostaglandins to gene therapy. Curr Med Chem. 2005; 12:203-215.
44. Kotani T, Kobata A, Nakamura E, Amagase K, Takeuchi K. Roles of cyclooxygenase-2 and prostacyclin/IP receptors in mucosal defense against ischemia/reperfusion injury in mouse stomach. J Pharmacol Exp Ther. 2006; 316:547-55.
45. Darling RL, Romero JJ, Dial EJ, Akunda JK, Langenbach R, Lichtenberger LM. The effects of aspirin

on gastric mucosal intergrity, surface hydrophobicity, prostaglandin metabolism in cyclooxygenase knockout mice. Gastrentr. 2004; 127:94-104.
46. Lichtenberger LM, Zhou Y, Dial EJ, Raphael RM. NSAID injury to the gastrointestinal tract: evidence that NSAIDs interact with phospholipids to weaken the hydrophobic surface barrier and induce the formation of unstable pores in membranes. J Pharm Pharmacol. 2006; 58:1421-8.
47. Di Carlo G, Mascolo N, Izzo AA, Capasso F. Flavonoid: Old and new aspects of a class of natural therapeutic drugs. Life Sci. 1999; 64:337-357.
48. Borrelli F, Izzo AA. The plant kingdom as a source of anti-ulcer remedies. Phytother Res. 2001; 53:82-88.
49. Galati EM, Monoforte MT, Tripodo MM. Anti-ulcer activity of Opuntia ficus indica L. (Cactaceae): Ultra structural study. J Ethnopharco. 2001; 76:19.