

Assessment of gastroprotective activity of leaf extracts of *Ageratum conyzoides* Linn. using pyloric-ligation method in animal model

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Abstract

Ageratum conyzoides Linn (Asteraceae) is an annual herbaceous, tropical plant with numerous ethno-medicinal uses, pharmacological applications and biological activities. This study was done to assess the gastroprotective activity of aqueous and methanolic leaf extracts of *A. conyzoides* L. in rats using pyloric-ligation method. Phytochemical analysis of extracts was done using Gas Chromatography–Mass Spectrometer (GCMS) technique. 40 male Wistar rats (180-200g) used for this study were divided equally into eight groups (A-H), groups A and B were used normal and test controls while groups C-E and F-H were respectively treated with aqueous and methanolic extracts of *A. conyzoides* L. at dose rate of 100mg/kg, 300mg/kg and 500 mg/kg (body weight). Gastric mucosal injury was induced via pyloric ligation method for groups B-H. Gross and histological examination of gastric tissues was done to assess the degree of gastric mucosal protection or erosion. The phytochemical analysis showed that both extracts of *A. conyzoides* L. contain bioactive compounds with known anti-oxidant activity. The macroscopic and microscopic examination of gastric tissues of experimental animals revealed mild mucosal surface erosion in treated groups (C-H) but intense erosion in test control group B following the prolonged exposure to acidic gastric secretions. Based on the findings of this study, both aqueous and methanolic extracts of *A. conyzoides* L. showed potent gastroprotective activity which may be closely associated with the anti-oxidant properties of their constituents phytochemical compounds.

Keywords: *Ageratum conyzoides* L., Gastroprotection, Pyloric-ligation.

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1. Introduction

Ageratum conyzoides Linn (Asteraceae) is an annual herbaceous, tropical plant commonly referred to as goat weed or Appa grass and as a tropical plant, it is very prominent in West Africa and different parts of Asia and South America. It is soft hairy, erect and may grow to about 1m in height with curved stems and leaves coated with fine white hairs [1,2]. Its ethno-medicinal uses, pharmacological application and biological activities have been well documented. In Brazil, *A. conyzoides* L. was widely used in traditional medicine for treatment of colds, fever, rheumatism, diarrhea and spasm [3-5]. Further studies done

in Nepal, reported the application of *A. conyzoides* L. leaf extracts on wounds and cuts, to serve as antiseptic and anti-hemorrhagic agent [6]. Moreover, the alcoholic extracts of leaves, roots and the whole plant of *A. conyzoides* L. have been reported to possess anti-inflammatory, neuromuscular-blocking and wound-healing activities in rats [7-11]. In addition to its several therapeutic uses, the allelopathic potential of acetone extracts of *A. conyzoides* L. plant shoots has been reported relating it to allelochemical constituents of the extracts [12]. Still, there remains the need for more information about pharmacological uses of ethnomedicinal plants and their phytochemistry vis-à-vis

these pharmacological applications. Therefore, this study was aimed at phytochemically profiling the aqueous and methanolic leaf extracts of *A. conyzoides* L. using gas chromatography-mass spectrometer technique; assessing the gastric mucosal protective activity of each extract against prolonged exposure to acidic gastric secretion during pyloric-ligation method and relating the activity with properties of their constituent phytochemicals.

2. Materials and Methods

2.1 Plant material

Fresh whole *A. conyzoides* L. plant was authenticated at the Herbarium of Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria and sample deposited with voucher number UBH_A344. The plant material for the study was obtained from suburb of Okada community, Ovia North-East Local government, Edo State, Nigeria.

2.1.1 Plant extraction

The leaves of the plant *A. conyzoides* L. were detached, air-dried and grinded into powdered form using mechanical grinder. The powdered leaves were each dissolved in distilled water and methanol for 72hours, filtered and evaporated to dryness using rotary evaporator. Residues obtained were cooled, weighed and respectively used as aqueous and methanolic extracts used for the study.

2.2 Phytochemical analysis using Gas Chromatography-Mass Spectrometer (GC-MS)

0.1g of aqueous and methanolic extracts was dissolved in 1ml of methanol and filtered. 1 μ l of each filtrate was used for the GC-MS phytochemical analysis of aqueous and methanolic extracts.

2.2.1 GC-MS phytochemical analysis

The GCMS analysis of the plant extract was performed using gas chromatograph (7890A series) coupled to TSQ quantum XLS mass spectrometer with an injector device (7683B series). The temperature and pressure of column heater were set at 250^oC and 10.15psi respectively, the average velocity was 66.45cm/sec and carrier gas was Helium with 1mL/min flow rate. The electron impact (EI) of the MS was set at 70eV, injection volume was 1 μ l, scan interval set at 0.5seconds, scan mass range from 50 – 650amu and polarity was positive. The phytochemical constituents of extracts were separated on a column TG-5ms (with dimensions – 30mL X 0.25mmID X 0.25 μ m film thickness) and flame ionization detector (at 250^oC) used to determine percentage composition of the phytochemicals. The phytochemical compounds present in each extracts were identified by comparing their mass spectra with reference spectra in the database of National Institute of Standard and Technology (NIST) library.

2.3 Experimental Animals and groupings

This study involved 40 male Wistar rats weighing between 180g-200g and equally grouped into eight groups

A–H (n=5). Group A animals given distilled water (5mls/kg body weight) represented normal control. Group B animals given distilled water (5mls/kg body weight) represented test control that induced by pyloric ligation. Group C – E animals were given 100mg/kg, 300mg/kg and 500mg/kg aqueous extracts of *A. conyzoides* L. Group F – H animals were given 100mg/kg, 300mg/kg and 500mg/kg methanolic extracts of *A. conyzoides* L. The dosages of extracts used for this study were considered safe without toxic effects [13]. The period of treatment was 28 days and the mode of treatment was oral using flexible oro-gastric gavage. After the treatment period, experimental animals in groups B-H were subjected to pyloric ligation of inducing gastric mucosal injury.

2.4 Induction of gastric mucosal injury using pyloric ligation method

Prior to the pyloric-ligation method, experimental animals were fasted for 24hours in separate cages but allowed free access to water. The experimental animals were anaesthetized by intraperitoneal injection of Ketamine/Xylazine (50mg/kg at 1:1). Then, a small midline incision was made on the abdominal wall of each experimental animal to access pyloric part of the stomach. The gastric pylorus was ligated, stomach gently returned into abdominal cavity and the abdomen closed. Experimental animals were allowed for an observatory period of five hours, afterwards sacrificed, stomach tissue harvested and prepared for tissue processing [14].

2.5 Ethical approval

This study was duly approved by the Research and Ethics committee of Igbinedion University, Okada, Edo State, Nigeria and all the study protocols were in compliance with National Institute of Health guidelines for care and handling of laboratory animals [15].

2.5 Macroscopic examination

The internal aspects of stomach tissues were photographed to document all observable gross gastric mucosal erosion following the prolonged exposure to acidic gastric juice during pyloric-ligation method.

2.6 Tissue Processing

The gastric tissues were fixed in 10% neutral buffered formalin, dehydrated using ascending grades of alcohol (two changes each of 70%, 90% and absolute alcohol for 30 minutes each), cleared in xylene for 30minutes and embedded in molten paraffin and allowed to cool to form tissue blocks.

2.7 Sectioning

Blocks of tissue were cut into sections 5 μ thickness by using rotary microtome, mounted on microscope slides and used for histological study using Haematoxylin and Eosin histological staining technique.

2.8 Haematoxylin and Eosin staining technique

Tissue sections were dewaxed in xylene for 15 minutes, hydrated with 100%, 90% and 70% alcohol and

distilled water for 3 minutes each, Stained in Haematoxylin for 10 minutes, washed in running water for 2 minutes, differentiated in 1% acid alcohol (1% HCl in 70% alcohol) for 1 minute, blue in Scoh's tap water for 10 minutes, rinsed in water, stained in Eosin for 3 minutes, rinsed in water, dehydrated with 70%, 90% and 100% alcohol for 2 minutes each, cleared in xylene for 2 minutes and mounted with DPX [16].

2.9 Photomicrography

Photomicrographs were generated from stained microscope slides using 10MP digital camera for microscope.

3. Results

3.1 Plant extraction

The weight of aqueous extract of *A. conyzoides* L. obtained was 22g which represent 4.4% yield while the weight of methanolic extract was 35g which represent 7.0% yield.

3.2 Phytochemical analysis

The gas chromatogram of aqueous and methanolic extracts of *A. conyzoides* L. (Figure 1 and 2) showed the retention time (RT) which represents the relative concentration of compounds being eluted while the peak height represents the relative concentrations of phytochemicals present in the extracts. The chromatogram of aqueous extracts of *A. conyzoides* L. showed 7 peaks indicating that the aqueous extracts contain 7 phytochemical compounds which include: 1,2,4-Trizol 4-amine N-(2-thienylmethyl)-ester (2.54%), 2-Pentadecenone 6,10,14-trimethyl ester (3.05%), n-Hexadecanoic acid (17.77%), 9,12,15-Octadecatrienoic acid (Z,Z,Z)- (9.12%), 9,12- Octadecadienoic acid (Z,Z)- (48.01%), Phytol (12.63%) and Methyl stearate (6.90%). The retention time (RT) and peak area (%) of phytochemical compounds identified in the aqueous extracts of *A. conyzoides* L. are given in Table 1.

Figure 1: Gas chromatogram of aqueous extracts of *A. conyzoides* L. using GC-MS

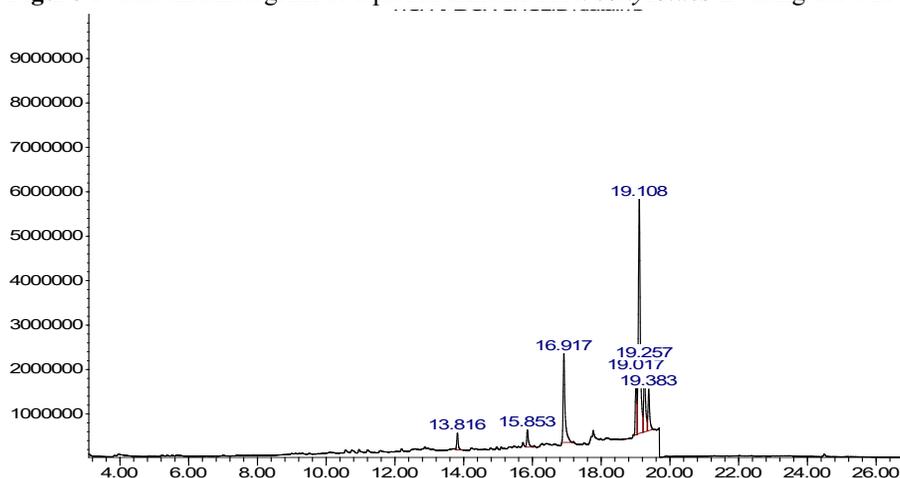
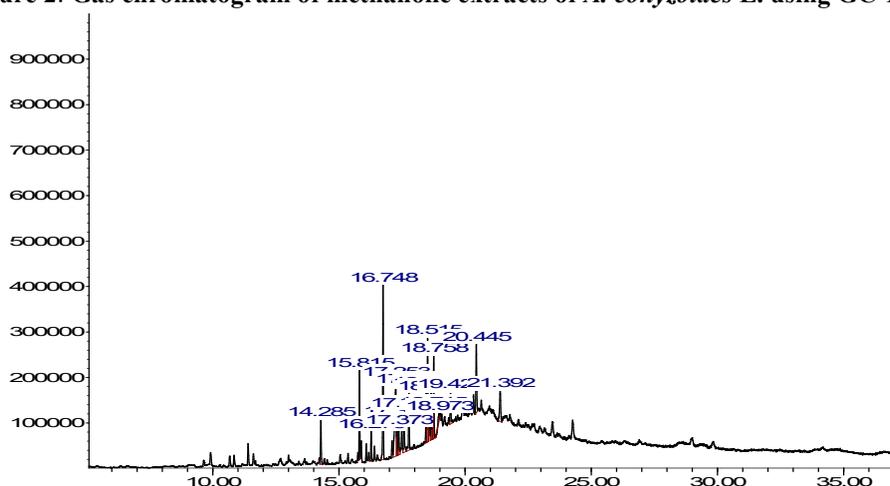


Table 1: Phytochemical compounds identified in the aqueous extract of *A. conyzoides* by GC-MS

| S/N | Retention Time (Min) | Name of Compound | Peak Area (%) |
|-----|----------------------|---|---------------|
| 1 | 13.82 | 1,2,4-Trizol 4-amineN-(2-thienylmethyl)-ester | 2.54 |
| 2 | 15.85 | 2-Pentadecenone 6,10,14-trimethyl ester | 3.04 |
| 3 | 16.92 | n-Hexadecanoic acid | 17.77 |
| 4 | 19.02 | 9,12,15- Octadecatrienoic acid (Z,Z,Z)- | 9.12 |
| 5 | 19.11 | 9,12- Octadecadienoic acid (Z,Z)- | 48.00 |
| 6 | 19.26 | Phytol | 12.63 |
| 7 | 19.38 | Methyl stearate | 6.90 |

The chromatogram of methanolic extracts of *A. conyzoides* L. showed 17 peaks indicating that the methanolic extracts of *A. conyzoides* L. contain 17 phytochemical compounds which include: 1,7-dibromoheptane (3.73%), 2,6,6-trimethyl bicyclo-3,1,1-heptane (6.87%), 3,7,11,15- tetramethyl-2-hexadecen-1-ol (2.65), Hexadecanoic acid (14.00%), n-Hexadecanoic acid (12.73%), Thiophene-2-acetic acid dodec-9-ynyl ester (3.39%), cyclohexylmethyl pentadecyl ester (2.60%), 2-Methoxybenzoic acid (4.57%), 2- Furanmethanol (5.96%),

9,12-Octadecadienoic acid (4.50%), 9-Octadecenoic acid (9.72%), 5-Eicosene (4.41%), Dioctyl disulphide (2.22%), Octadecanoic acid, methyl ester (7.40%), 9,17-Octadecadienal (0.91%), Docosane (3.31%), Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-ester (6.73%) and Octacosane (4.30%). The retention time (RT) and peak area (%) of phytochemical compounds identified in the methanolic extracts of *A. conyzoides* L. are given in Table 2.

Figure 2: Gas chromatogram of methanolic extracts of *A. conyzoides* L. using GC-MS**Table 2: Phytochemical compounds identified in the methanolic extract of *A. conyzoides* by GC-MS**

| S/N | Retention Time (Min) | Name of Compound | Peak Area (%) |
|-----|----------------------|---|---------------|
| 1 | 14.29 | 1,7-Dibromoheptane | 3.73 |
| 2 | 15.81 | 2,6,6-Trimethyl bicyclo-3,1,1-heptane | 6.87 |
| 3 | 16.28 | 3,7,11,15- Tetramethyl-2-hexadecen-1-ol | 2.65 |
| 4 | 16.75 | Hexadecanoic acid methyl ester | 14.00 |
| 5 | 17.25 | n-Hexadecanoic acid | 12.73 |
| 6 | 17.30 | Thiophene-2-acetic acid dodec-9-ynyl ester | 3.39 |
| 7 | 17.37 | cyclohexylmethyl pentadecyl ester | 2.60 |
| 8 | 17.58 | 2-Methoxybenzoic acid | 4.57 |
| 9 | 17.78 | 2- Furanmethanol | 5.96 |
| 10 | 18.45 | 9,12- Octadecadienoic acid (Z,Z)- | 4.50 |
| 11 | 18.52 | 9-Octadecenoic acid | 9.72 |
| 12 | 18.65 | 5-Eicosene | 4.41 |
| 13 | 18.72 | Dioctyl disulphide | 2.22 |
| 14 | 18.76 | Octadecanoic acid, methyl ester | 7.40 |
| 15 | 18.97 | 9,17-Octadecadienal | 0.91 |
| 16 | 19.43 | Docosane | 3.31 |
| 17 | 20.45 | Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-ester | 6.73 |
| 18 | 21.39 | Octacosane | 4.30 |

3.2 Macroscopic Examination

The gross appearance of the internal aspect of gastric tissues of experimental animals showed varying degrees of gastric mucosal erosion which ranges from

intense (Group B) to mild (Groups C – F) and null (Groups G & H) compared to normal gastric mucosa of the non-treated and non-induced animals (Group A) (Figure 3).

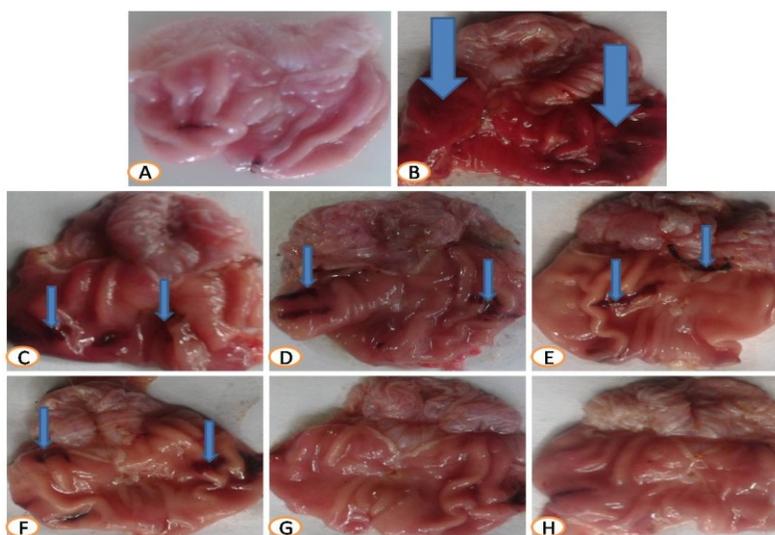


Figure 3: Macroscopic/Gross appearances of the internal aspect of gastric tissues of experimental animals (Groups A-H) Arrows point to varying degrees of gastric mucosal erosion due to exposure to the acidic gastric secretions during pyloric-ligation method: Group A represents normal gastric mucosa; Group B showing intense mucosal erosion; Groups C-F showed mild/focal mucosal erosion; Groups G & H showed very mild/null mucosal erosion.

3.3 Histological results

The histological examination of the gastric tissues of experimental animals showed relatively normal histological architecture of the gastric mucosa of treated animals (Groups C – F) with mildly eroded mucosal surface

and densely packed gastric glands which compared to the non-treated and non-induced animals (Group A) while the non-treated and induced animals (Group B) showed intense mucosal surface erosion (figure 4).

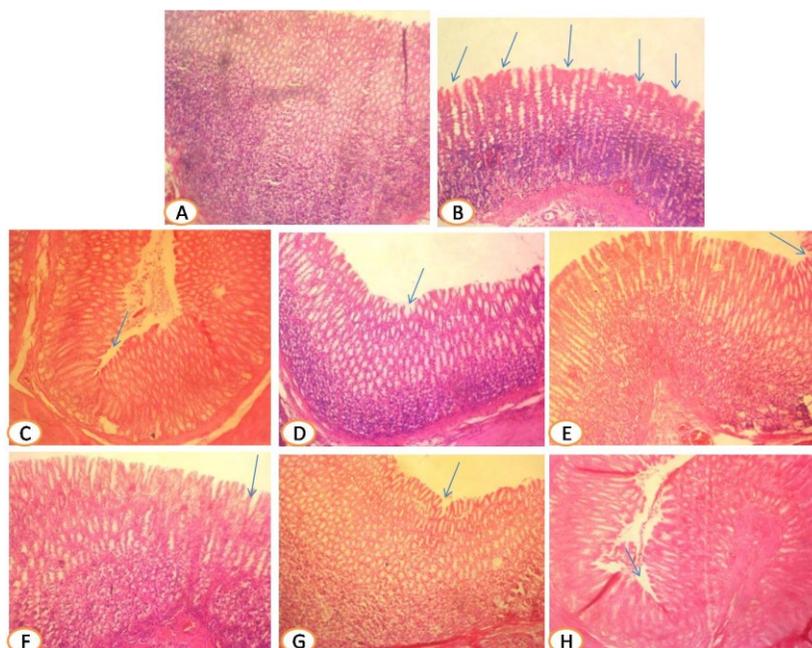


Figure 4: Photomicrograph of gastric tissue sections of experimental animals (Groups A–H) showing histo-architecture of gastric mucosa and different degrees of mucosal surface erosion (H & E X100). Arrows point to eroded gastric mucosal surface: Group A represents normal gastric mucosa; Group B showing prominent mucosal erosion; Groups C–H showed mild/focal mucosal erosion.

4. Discussion

According to the results of this study (Figure 3), the gross appearance of the internal aspect of gastric tissues of experimental animals treated with both aqueous and methanolic leaf extract of *A. conyzoides* L. (Groups C–H) is relatively normal in morphology, indicating the protective effects of the leaf extracts against erosive effect of acidic gastric secretions. Similarly, the histological appearance of gastric tissues of experimental animals treated with both aqueous and methanolic extracts of *A. conyzoides* L. (Groups C–H) revealed relatively normal gastric mucosal histo-architecture with densely packed gastric glands when compared to non-treated and non-induced animals (Group A) (Figure 4). This may also be directly associated with protective effects of leaf extracts of *A. conyzoides* L. on gastric mucosa from the corrosive action of gastric juice since the non-treated animals (Group B) showed prominent mucosal surface erosion (Figures 3 & 4). The results of phytochemical profiling of *A. conyzoides* L. leaf extracts using GC-MS revealed the presence of phytochemicals with diverse biological activities and pharmacological relevance. Usually, therapeutic properties of herbal or medicinal plants may be accessed through determination of the biological activity of their phytochemical constituents [17,18]. Some biological activities associated with phytochemical constituents of *A. conyzoides* L. leaf extracts identified from the result of phytochemical analysis in this study include

anti-oxidant, anti-inflammatory anti-hyperlipidemia, allelopathic and analgesic activities [19,20]. The anti-oxidant property of *A. conyzoides* L. leaf extracts is of great relevance in understanding its gastroprotective mechanisms. For instance, one mechanism by which aggressive factors cause gastric mucosal injury is through production of free-radicals which cause peroxidation of polyunsaturated fatty acids in lipid bilayer of plasma membrane and leads to cycles of lipid peroxidation that result into damage of cell membrane and intracellular structures including the DNA [21]. However, in a counter-active response, the protective factors stimulate release of anti-oxidants (or free-radical scavengers) to ameliorate or inhibit deleterious effects of free radicals on gastric mucosa. According to findings by Sharwaikar et al (2003) [22], ethanol extract of *A. conyzoides* was reported to exhibit gastroprotective activity against ibuprofen-, ethanol- and stress-induced gastric ulceration. Similarly, Mahmood et al (2005) [13] reported gastroprotective activity of aqueous extract of *A. conyzoides* against ethanol-induced gastric ulcers. Both studies reported gastroprotective activity of *A. conyzoides* to be closely associated with anti-oxidant properties of the plant extract. These findings are in tandem with the results of this study in which gastroprotective effects of both aqueous and methanolic extracts of *A. conyzoides* were distinctly and comparatively assessed against prolonged exposure to acidic gastric secretion as the gastric mucosal aggressive

factor. Similar results obtained from another plant reported that anti-ulcer activity of herbal or medicinal plants is largely due to flavonoids components of their phytochemicals which act to inhibit formation of mucosal lesions in gastric tissues of experimental animals [23]. Another studies also posited that flavonoids derived from herbal or medicinal plants exhibit significant anti-oxidant effect and protect against toxic effects of reactive oxygen species (ROS) due to hydroxyl groups [24,25]. From the findings of this study, the phytochemical constituents (Tables 1 & 2) identified in aqueous and methanolic extracts of *A. conyzoides* L. have been documented to possess anti-oxidant activity. This invariably account for the potent gastroprotective activity exhibited by the extracts against the gastric mucosal aggressive factor.

5. Conclusion

The findings of this study showed that both aqueous and methanolic leaf extracts of *A. conyzoides* L. exhibited potent gastroprotective activity against prolonged exposure to acidic gastric secretion and this can be primarily associated with the anti-oxidant properties of their constituent phytochemical compounds.

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