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Gastroprotective and Molecular Docking Evaluation of *Ageratum conyzoides* Juice Extract in Ethanol-Induced Gastric Ulceration

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Abstract

Peptic ulcer is a multifactorial and debilitating gastrointestinal disorder affecting about 10 million people worldwide. The effectiveness of many anti-ulcer drugs is often limited by adverse effects, thereby making the need for safer, natural alternatives necessary. In this study, experimental rats were orally gavaged for 14 days with 1.25, 2.5, or 5.5 mg/kg body weight of lyophilized goat weed (*Ageratum conyzoides*) juice extract (ACJE). Gastric ulceration was induced in fasted animals by oral administration of ethanol (96%, 5 mL/kg). Animals were subsequently euthanized to evaluate the ulcer index, stomach histology, and biochemical markers of toxicity and oxidative stress. Untreated, induced rats had significantly ($p < 0.001$) higher ulcer scores (9-fold) and gastric juice secretion (1.5-fold), as well as lower gastric juice pH (1.5-fold). These effects were markedly reversed by ACJE pretreatment. Rats administered 2.5 mg/kg ACJE demonstrated similar protective benefits to those of ranitidine, including significant reductions in ulcer severity, gastric juice volume, and acidity. Furthermore, ulcer-associated dyslipidemia and changes in LDL and HDL cholesterol levels were mitigated in ACJE-pretreated animals. The gastroprotective effect of ACJE was validated by histological findings and the restoration of oxidative stress in rats. In silico analysis revealed that ACJE phytochemicals bind strongly to urease and pepsin, with higher docking scores compared to standard drugs. Overall, ACJE shows promising gastroprotective potential, necessitating more mechanistic research.

Keywords: *Ageratum conyzoides*; gastric ulcer; oxidative stress; inflammation; molecular docking

Academic Editor: Shih-Yi Huang

Received: 10 April 2026
Revised: 16 June 2026
Accepted: 22 June 2026
Published: 30 June 2026

1. Introduction

Gastric ulcer is a common gastrointestinal disorder characterized by erosion of the gastric mucosa and associated abdominal pain and discomfort [1]. The debilitating disease also comes with a sizable social and economic burden as peptic ulcer, the umbrella term comprising gastric and duodenal ulcers, is estimated to have a lifetime prevalence of 5–10% and an annual incidence of 0.1–0.3% in the general population in Western countries [2]. An imbalance between gastric protective and aggressive factors leads to the development of gastric ulcer lesions, which have various etiologies. Physical stress, alcohol and tobacco use, non-steroidal anti-inflammatory medicines, genetic susceptibility, and *Helicobacter*

pylori infection are among the aggressive variables implicated in the formation of stomach ulcers [3,4].

According to Paulraj et al. [5], heavy drinking is strongly associated with damage to the gastric mucosa, which prompted the use of ethanol-induced gastric ulcer in the search for anti-ulcer drugs. Inflammation and oxidative stress may play major roles in gastric injury, as with ethanol-induced gastric mucosal injury [6]. The recruitment of neutrophils to the site of injury promotes the production of reactive oxygen species (ROS) and other pro-inflammatory mediators, resulting in oxidative stress [7]. Though there are several synthetic anti-ulcer medications available, their potency is sometimes coupled with side effects and other undesirable outcomes, including osteoporosis, hypergastrinemia, cardiovascular disease risk and impotence [8,9], prompting the search for potent natural alternatives with safer profiles.

Goat weed (*Ageratum conyzoides* L.; Figure 1) is a herbaceous tropical plant belonging to the family Asteraceae (Compositae) and is widely distributed across Central and South America, Asia, West Africa, and Australia [10]. Although the plant is a widely known agricultural weed, it has a rich but varied history of use in traditional medicine by many civilizations worldwide. The plant also contains bioactive phytochemicals with antioxidant and anti-inflammatory properties that may contribute to gastroprotection. In folkloric medicine, goat weed is used to treat skin infections and for wound healing, ulcers, diarrhea, and pain management [11]. Secondary metabolites from the plant possess insecticidal activity and have been tested with considerable efficacy against protozoan and fungal infections [12,13]. Traditionally, the fresh aerial parts of *Ageratum conyzoides* are squeezed without solvent addition to obtain the juice, which is orally consumed in folkloric medicine for the management of gastrointestinal discomfort and ulcer-related symptoms. Despite the fact that the fresh juice of *Ageratum conyzoides* has popular ethnomedicinal applications in the treatment of gastroduodenal ulcers, there is a dearth of scientific evidence to support these claims. The present study was therefore designed to evaluate the antiulcerogenic effect of *Ageratum conyzoides* juice extract in an ethanol-induced gastric ulcer model and to investigate the interactions of its phytochemicals with ulcerogenic targets using in silico molecular docking analysis.

Figure 1. *Ageratum conyzoides* L. (family Asteraceae), commonly known as goat weed, showing the aerial parts (leaves, stems, and inflorescences) used in this study.

2. Materials and Methods

2.1. Chemicals

Ethanol, malonaldehyde bis-(dimethyl acetal) (MDA), thiobarbituric acid (TBA), and 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) were procured from Sigma-Aldrich (St. Louis, MO, USA). Total cholesterol, triglyceride, high-density lipoprotein (HDL-c), and low-density lipoprotein (LDL-c/VLDL-c) assay kits were obtained from Fortress Diagnostics

Ltd. (Antrim, Northern Ireland, UK). Other chemicals were of analytical grade, and the water used was glass-distilled.

2.2. Preparation of Ageratum conyzoides Juice Extract

Fresh whole *Ageratum conyzoides* plants (500 g) were harvested from a private farm in Ado-Ekiti (7.6124° N, 5.2371° E), Nigeria, between 15 and 17 June 2020. Botanical identification and authentication were performed at the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria, where a voucher specimen (number FHI 114208) was deposited. The plants were properly cleansed with sterile water, and the aerial parts of the plant were extracted with a juicer without the use of water. The remaining juice was pressed out of the husk with a clean laboratory towel. The juice was frozen at −50 °C and subsequently lyophilized to obtain the powdered form of the extract (freeze-dried ACJE). A solvent-free extraction method was used to closely reproduce the traditional preparation of the fresh juice for ulcer treatment while allowing accurate dose standardization after lyophilization. The extraction yield following lyophilization was approximately 140–280 mg from 200 to 400 mL of fresh juice. This yield was used to estimate the equivalent dry extract corresponding to ethnomedicinal consumption. The freeze-dried extract was stored at −20 °C and then reconstituted in water before use.

2.3. High-Performance Liquid Chromatography (HPLC)

Phytochemical profiling and quantification of the juice extract were performed using a Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan). The extract (10 g) was extracted with 20 mL of acetonitrile (Sigma-Aldrich, St. Louis, MO, USA) under continuous agitation for 30 min, and the mixture was centrifuged (4000 rpm, 10 min) to enable phase separation. The resulting supernatant was then filtered through a 0.22 µm membrane filter (MilliporeSigma, Burlington, MA, USA) into a 25 mL volumetric flask and made up to volume with acetonitrile. Chromatographic separation was done using a reversed-phase C18 column (250 × 4.6 mm i.d., 5 µm particle size; Agilent Technologies, Santa Clara, CA, USA). The mobile phase, which was delivered in a gradient elution mode, consisted of solvent A (water containing 0.1% formic acid; Sigma-Aldrich, St. Louis, MO, USA) and solvent B (acetonitrile). The gradient program was first set at 10% B (0–2 min), then increased to 70% B (2–20 min), and eventually to 95% B (20–30 min), after which it was re-equilibrated to the initial conditions. The flow rate and column temperature were kept at 1.0 mL/min and 30 °C, respectively. Detection was done using a diode array detector (DAD) with multi-wavelength monitoring at 210, 254, 280, and 360 nm. The injection volume was 10 µL. Reference standards for representative compounds were prepared and injected to establish retention times, UV spectra, and calibration curves. In addition, calibration curves were constructed using at least five concentration levels, showing good linearity ($R^2 \geq 0.99$). Retention time and spectral matching with valid standards were used to identify the compounds, and external standard calibration was used for quantification.

Method Validation

The analytical method was validated in line with standard guidelines for linearity, sensitivity, precision, and accuracy. Calibration curves for reference standards over relevant concentration ranges demonstrated good linearity, with R^2 values ≥ 0.99. The limits of detection (LOD) and quantification (LOQ) were determined based on signal-to-noise ratios of 3:1 and 10:1, respectively. They were found to range from 0.01 to 0.05 mg/g for LOD and 0.03 to 0.15 mg/g for LOQ based on the analyte. Precision was evaluated using intra-day and inter-day variability by repeated analysis of samples (n = 3). The relative standard deviation (RSD) values that were below 5% show there is good repeatability and intermediate precision.

2.4. Animals

Adult Wistar rats, weighing between 180 and 220 g, were obtained from an institution-approved preclinical animal house, kept in cages, and fed commercially available standard pelleted feed and water ad libitum. All animal experiments were conducted according to the guidelines of the National Institute of Health (NIH publication 85-23, 1985) for laboratory animal care and use. The animal study protocol was approved by the Departmental Animal Care and Use Ethical Committee of the Federal University, Oye-Ekiti (No: 2020A000012, 10 March 2020).

Animal Grouping and Treatment

Age-matched Wistar rats (200 ± 20 g) were randomly divided into six groups (I–VI; $n = 6$) and treated once daily for 14 consecutive days as follows: Group I (normal control) received distilled water (1 mL/kg, p.o.) without ethanol induction; Group II (negative control) received distilled water prior to ethanol-induced gastric ulceration; Group III received ranitidine (30 mg/kg, p.o.); and Groups IV, V, and VI received *Ageratum conyzoides* juice extract (ACJE) once daily for 14 consecutive days at doses of 1.25, 2.5, and 5.5 mg/kg (p.o.), respectively. The doses were based on estimated human consumption of the fresh juice. Ethnomedicinal use indicates that an average adult (70 kg) consumes approximately 200–400 mL of the juice, which corresponds to about 140–280 mg of lyophilized extract, equivalent to approximately 2–4 mg/kg body weight. Accordingly, doses of 1.25, 2.5, and 5.5 mg/kg were chosen to approximate and bracket this range. Following the treatment period, all animals were fasted for 24 h. Gastric ulcers were induced in Groups II–VI by oral administration of 96% ethanol (5 mL/kg). Following the treatment period, all animals were fasted for 24 h. Gastric ulcers were induced in Groups II–VI by oral administration of 96% ethanol (5 mL/kg). Ethanol (96%, 5 mL/kg) was selected based on its established use in experimental models for the rapid induction of gastric mucosal injury and ulceration [14]. All animals were anesthetized with ketamine (80 mg/kg, i.p.) and xylazine (13 mg/kg, i.p.) prior to decapitation 1 h after ethanol administration. The stomachs were immediately excised for the evaluation of gastric lesion index, gastric juice volume and pH, and biochemical analyses.

2.5. Stomach Isolation and Gastric Juice Collection

The abdomen of the animal was opened after decapitation, and the stomach was excised. The stomach was then opened along its greater curvature, and the gastric contents were drained for volume and pH determination. The cleaned stomachs were preserved in 0.1 M phosphate saline buffer (1:4 w/v, pH 7.4) prior to macroscopic examination and homogenization.

2.6. Collection of Blood Samples and Preparation of Serum

Blood samples were collected by cardiopuncture into plain serum tubes and allowed to stay for 1 h. The clotted blood was centrifuged at 4 °C for 10 min at 3500 g. The serum was transferred into clean tubes and stored at −20 °C until needed for use.

2.7. Evaluation of Serum Lipid Profile

Total cholesterol, triacyglycerols, low-density lipoprotein-cholesterol (LDL-c), and high-density lipoprotein-cholesterol (HDL-c) concentrations in rat serum were determined using commercially available kits (Fortress Diagnostics, Antrim BT41 1QS, UK) according to the manufacturer's instructions.

2.8. Gastric Lesion Index Evaluation

Ulcer scoring was done as reported by Sabiu et al. [15] and proceeded as follows:

0-Almost normal mucosa; 1-Vascular congestion; 2-One or two lesions; 3-Severe lesions; 4-Very severe lesions; 5-Mucosa full of lesions.

Briefly, cleaned stomachs were pinned on a corkboard, and ulcers were scored using a dissecting microscope with a square-grid eyepiece based on grading on a 0–5 scale as described above. The ulcer index was calculated for each group, and the percentage inhibition (or protection) of ulceration was determined using the following formula:

$\%\ inhibition = [(Ulcer\ index\ of\ ulcer\ control - Ulcer\ index\ of\ treated\ group)/Ulcer\ index\ of\ ulcer\ control] \times 100$. Similarly, percentage changes in gastric juice volume and pH were calculated relative to the ulcer control group using the same formula.

2.9. Assessment of Antioxidant Status and Membrane Lipid Peroxidation

The levels of the endogenous antioxidant molecule, reduced glutathione (GSH), and the amount of membrane lipid peroxidation were measured in stomach and hepatic tissues using standard laboratory methods and assay kits [16]. The excised tissues were rinsed in ice-cold 1.15% KCl, then chopped with scissors in four volumes of 50 mM Tris-HCl buffer (pH 7.4) and homogenized with a Teflon homogenizer. The homogenates were centrifuged at 12,000$\times$ *g* for 10 min at 4 °C. Lipid peroxidation was determined by measuring the formation of thiobarbituric acid–reactive substances (TBARS). TBARS are formed when malondialdehyde (MDA), an index of lipid peroxidation, reacts with thiobarbituric acid (TBA). The resulting pink chromogen absorbs maximally at 532 nm, allowing for the calculation of MDA levels using an MDA standard curve. The reaction of 5′,5′-dithiobis(2-nitrobenzoic acid) (DTNB) with GSH yielded a yellow chromophoric product, which was used to assess GSH concentration. The resultant 2-nitro-5-thiobenzoic acid was spectrophotometrically measured at 412 nm, and its absorbance was proportional to the amount of GSH.

2.10. Histopathological Examination

The histological assessment of the excised stomach tissues was carried out as earlier described by [17]. The stomach tissues were dehydrated using varying concentrations of ethanol after prior fixing in 10% formalin. Following xylene clearing, the tissues were embedded in paraffin and mounted on microscope slides. They were then sectioned at a thickness of approximately 5 µm before staining with hematoxylin and eosin (H&E). A Leica SCN 4000 slide scanner (Leica Biosystems, Nussloch, Germany) was used to view and analyze the slides.

2.11. Molecular Docking Analysis

Molecular docking studies were carried out to evaluate interactions between characterized phytochemicals/drugs and target proteins as previously described [18]. Docking was aimed at determining the most favorable binding conformations between ligands and their target receptors, as indicated by the lowest binding free energy [19]. Fifteen phytochemicals and three standard drugs were retrieved from PubChem in SMILES format, energy-minimized using ACD/ChemSketch version 12.0 (Advanced Chemistry Development Inc., Toronto, ON, Canada), converted to 3D structures with PyMOL version 2.5 (Schrödinger, LLC, New York, NY, USA), and prepared using AutoDock Tools (ADT) version 1.5.6 (The Scripps Research Institute, La Jolla, CA, USA) by adding polar hydrogens and charges. Prepared ligands were saved in PDBQT format. AlphaFold-predicted structures of human pepsin (UniProt ID: P0DJD9) and *Helicobacter pylori* urease (UniProt ID: 14916) were obtained from UniProt and prepared using ADT by removing water molecules and existing ligands. In addition, grid boxes defining the active sites were generated for docking while docking simulations were conducted using AutoDock Vina v1.2.3 [20]. To ensure the reliability of the docking protocol, three standard reference compounds were

included as controls, and blind docking was performed to assess the consistency of ligand binding across the target structures. Omeprazole and cimetidine were additionally included in the molecular docking analysis to provide broader comparative evaluation with antiulcer drugs of different mechanisms of action. Finally, the binding poses with the lowest binding energies were selected, and protein–ligand interactions were visualized using ezLigPlot (https://www.dxulab.org/software accessed on 21 June 2026). Molecular docking analyses were conducted using AutoDock Vina version 1.2.3, AutoDock Tools (ADT) version 1.5.6, PyMOL, and ezLigPlot. Human pepsin was used for the docking analysis because of the availability of curated structural datasets and its high functional conservation across mammalian species. Nevertheless, possible species-specific differences between human and rat pepsin should be considered when interpreting the docking results.

2.12. Statistical Analysis

All values were expressed as mean ± SEM ($n = 6$). Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using GraphPad Prism version 6.0a (GraphPad Software, San Diego, CA USA). A value of $p < 0.05$ was considered statistically significant. Graphing and curve fitting were also performed using the same software.

3. Results

3.1. Phytochemical Constituents of Ageratum conyzoides Juice Extract (ACJE)

HPLC-DAD analysis revealed the presence of many bioactive phytochemicals in ACJE at varying concentrations (Table 1; Figure S1). Identified compounds and their corresponding quantities (mg/g), based on retention time and spectral matching with reference standards, included terpenoids and related compounds (β-caryophyllene (1.04), α-humulene (0.07), prococene I (0.20), prococene II (0.06), campinas (0.08), and chromene (0.07)), phenolic compounds and derivatives (eugenol (2.31), coumarin (0.69), caffeic acid (0.30), and fumaric acid (0.58)), flavonoids (kaempferol (3.39), quercetin (8.46), and scutellarein (0.07)), phytosterols (β-sitosterol (3.19) and stigmasterol (0.13)), and an alkaloid (lycopsamine (0.07)).

Table 1. Chromatographic characteristics and concentrations of identified phytochemicals in *Ageratum conyzoides* juice.

Component	Retention	Area	Height	External	Concentration (mg/g)
β-Caryophylene	1.266	1205.910	33.877	0.0000	1.04
Eugenol	2.750	2691.102	26.083	323.8877	2.31
Coumarin	4.450	797.9090	13.365	87.2386	0.69
Caffeic acid	5.466	343.8010	8.516	0.0000	0.30
Prococene 1	6.483	227.5580	6.7444	0.0000	0.20
Prococene 2	7.333	73.3870	4.886	0.0000	0.06
α-humulene	7.950	83.6470	4.223	0.0000	0.07
Campinas	9.350	96.7250	4.445	0.0000	0.08
Quercetin	11.50	9857.227	163.450	0.0000	8.46
Kaempferol	12.166	3948.262	69.356	0.0000	3.39
β-Sitosterol	13.700	3714.881	46.453	0.0000	3.19
Stigmasterol	16.250	152.1400	7.627	0.0000	0.13
Fumaric acid	17.616	669.8370	5.768	0.0000	0.58
Scutelarein	18.900	87.1550	5.768	0.0000	0.07
Chromene	19.416	87.0920	5.572	0.0000	0.07
Lycopsamine	20.233	76.7560	5.119	0.0000	0.07

3.2. Gastric Ulcer Indices and Gross Appearances of Stomach

Ethanol instillation in fasted rats caused significant gastric mucosal ulceration, as demonstrated by the increase in ulcer scores from 0.33 ± 0.14 in the normal control group to 3.33 ± 0.21 (9-fold) in the ulcer-induced group ($p < 0.001$), increased gastric juice volume, and decreased gastric juice pH (Figure 2A–C). Prior treatment with ACJE (2.5 and 5.5 mg/kg) and ranitidine (30 mg/kg) markedly attenuated these alterations. Treatment with 2.5 mg/kg ACJE was comparable to ranitidine treatment in terms of the reversal of ulcer scores (68 vs. 37%), gastric juice volume, (65 vs. 62%), and pH (54 vs. 52%). The existence of ulceration hemorrhage in ulcerated rats, as opposed to normal control animals, demonstrated macroscopic damage in ethanol-induced, untreated animals (Figure 3B). Apart from scattered vascular congestion, the morphological integrity of the stomach mucosa was preserved in animals pre-treated with ranitidine (30 mg/kg) and ACJE at 2.5 and 5.5 mg/kg (Figure 3C,E,F) as compared to the untreated, ulcerated group. Pre-treatment with 1.25 mg/kg ACJE revealed only macroscopic evidence of mild inflammation but also no clear evidence of adverse mucosal injury (Figure 3D).

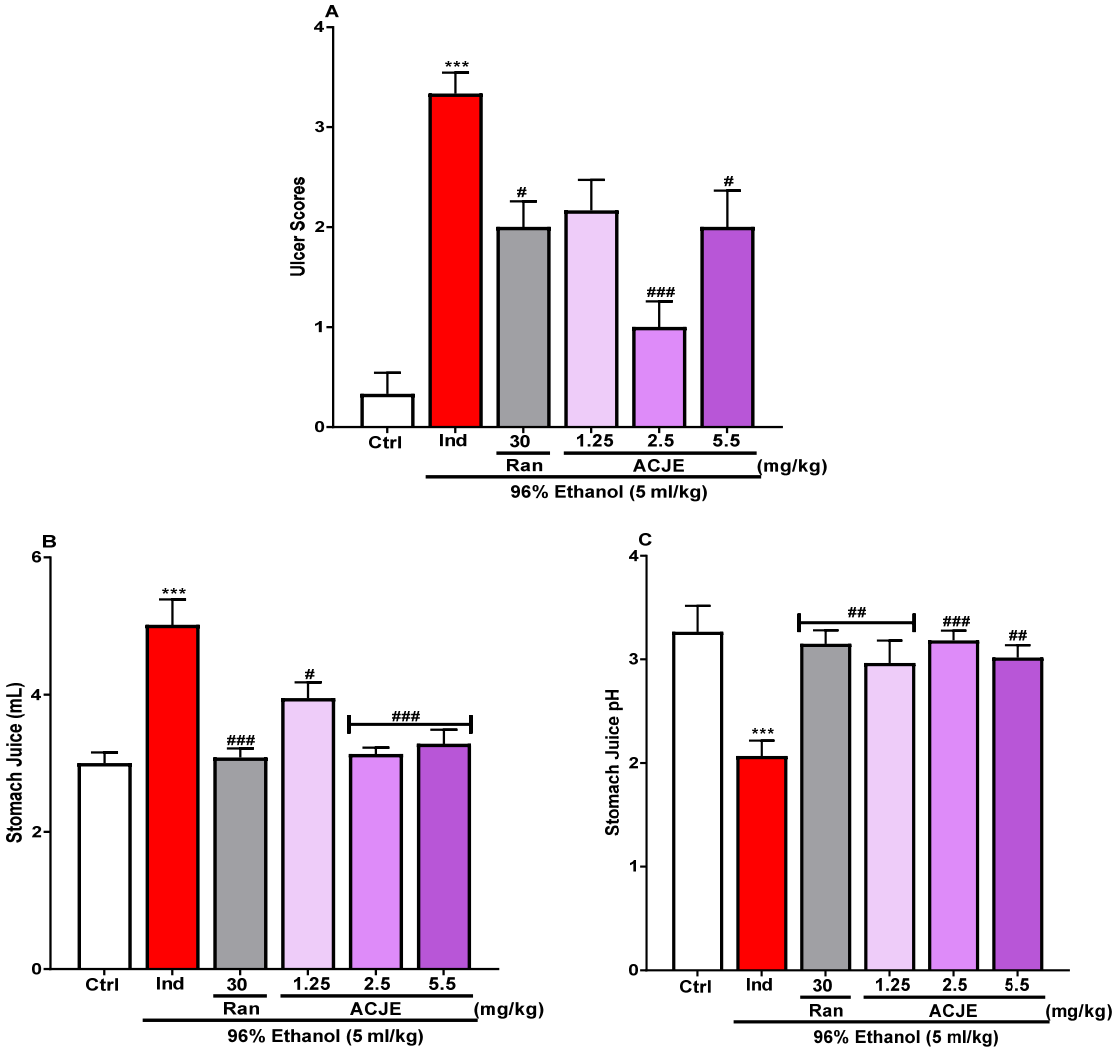

Figure 2. Influence of *Ageratum conyzoides* juice extract, ACJE on (**A**) ulcer scores, (**B**) stomach juice volume and (**C**) stomach juice pH following ethanol-induced gastric ulceration in rats. ACJE = *Ageratum conyzoides* juice extract; Ind = ethanol-induced ulcer control; Ran = ranitidine. Values are expressed as mean ± SEM ($n = 6$). *** $p < 0.001$ vs. normal control group; $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$ vs. induced, untreated group.

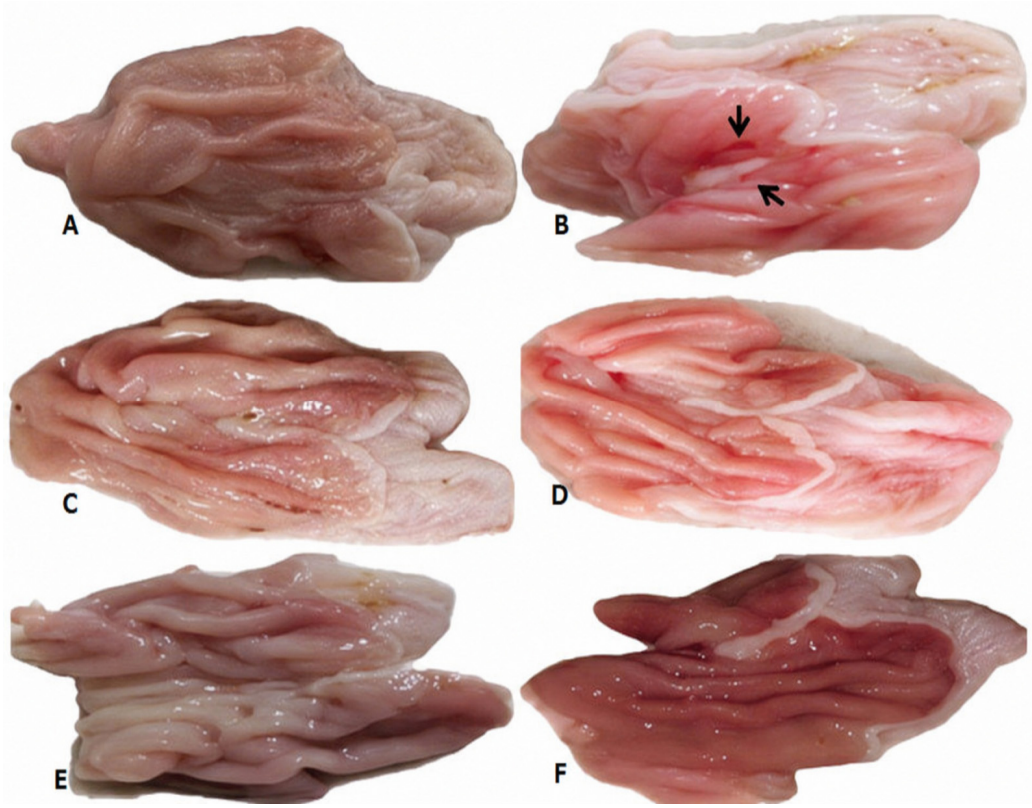

Figure 3. Effect of *Ageratum conyzoides* juice extract, ACJE, on the macroscopic appearance of the gastric mucosa of rats following ethanol-induced gastric damage. (**A**) Normal control group with preserved gastric mucosa and no injury. (**B**) Untreated ulcer group with severe hemorrhagic bands in the gastric mucosa. (**C**) The ranitidine group showed only scattered vascular congestion but no substantive injuries to the gastric mucosa. (**D**) Rats pre-treated with 1.25 mg/kg ACJE showed only a "mild redness" of the mucosa but without any adverse mucosal injury. (**E**) Rats pre-treated with 2.5 mg/kg ACJE and (**F**) 5.5 mg/kg ACJE revealed no mucosal injuries.

3.3. Endogenous Antioxidant Status and Index of Oxidative Damage

The ethanol group demonstrated considerable ($p < 0.01$; $p < 0.001$) oxidative damage in the stomach tissues as seen by enhanced membrane lipid peroxidation and a low level of reduced glutathione compared to the normal control group (Figure 4A,C). However, the animals that received ACJE at 1.25 and 2.5 mg/kg dosages were protected against ethanol-induced damage to the gastric mucosa, as the increase in membrane peroxidation and reduction in the endogenous antioxidant molecule was prevented. The effect of ethanol induction on the antioxidant profile was not obvious in the hepatic tissues since both membrane peroxidation and GSH levels were unaffected (Figure 4B,D).

3.4. Serum Lipid Profile

The increase in serum total cholesterol (TC) of the ethanol-induced animals was not significant ($p > 0.05$) although treated animals showed lowered TC compared with the ulcerated, untreated control. However, ethanol instillation increased LDL cholesterol while decreasing HDL cholesterol (Table 2). ACJE (2.5 and 5.5 mg/kg) treatment prior to induction reversed these effects. Ethanol induction or ACJE pretreatment had no effect on serum triglyceride levels.

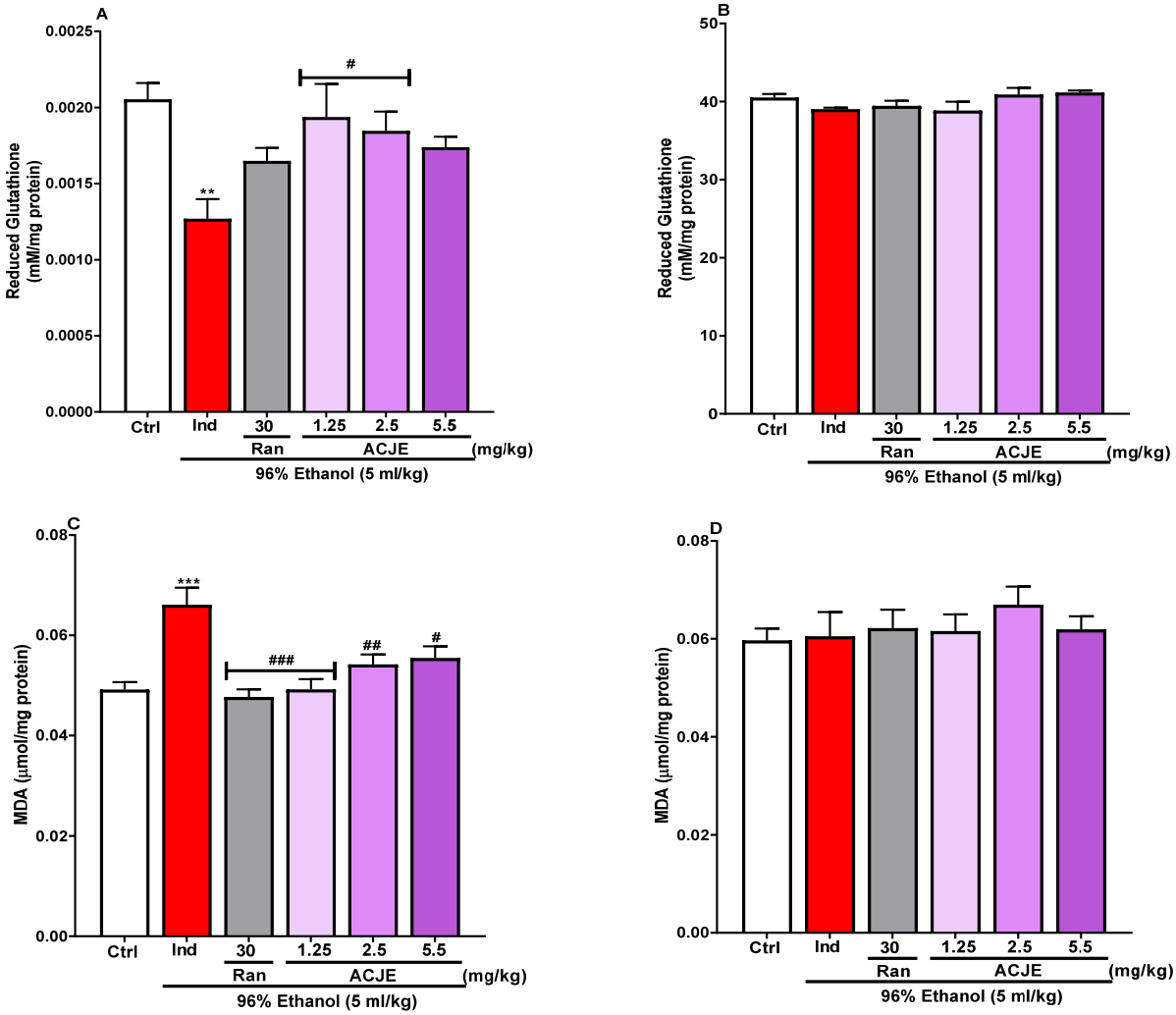

Figure 4. Oxidative stress indices as typified by the concentration of reduced glutathione in the stomach (**A**) and liver (**B**) as well as the extent of membrane peroxidation in the stomach (**C**) and liver (**D**) of rats subjected to ethanol-induced gastric mucosa injury. ACJE = *Ageratum conyzoides* juice extract; Ind = ethanol-induced ulcer control; Ran = ranitidine. Values are expressed as mean ± SEM (n = 6). $^{**}p < 0.01$; *** $p < 0.001$ vs. normal control group; $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$ vs. induced, untreated group.

Table 2. Serum lipid profile of ethanol-induced ulcerated rats treated with *Ageratum conyzoides* juice extract.

Group	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	LDL-Cholesterol (mg/dL)	HDL-Cholesterol (mg/dL)
Control	111.9 ± 8.02	264.8 ± 15.4	48.84 ± 5.60	42.81 ± 5.59
Ethanol (Eth)	133.7 ± 11.2	238.3 ± 7.22	97.49 ± 3.68 ***	17.36 ± 1.53 ***
Eth + Ran	102.2 ± 3.87 #	248.5 ± 5.92	55.38 ± 9.03 ###	31.87 ± 3.16
Eth + ACJE 1.25	108.9 ± 4.23	234.1 ± 4.73	87.84 ± 9.03	31.82 ± 3.03
Eth + ACJE 2.5	92.22 ± 4.39 ##	232.2 ± 7.94	48.43 ± 6.53 ###	35.88 ± 3.25 ##
Eth + ACJE 5.5	85.93 ± 4.06 ###	244.3 ± 4.97	39.03 ± 5.02 ###	34.44. ± 2.88 #

Values are expressed as mean ± SEM (n = 6). *** $p < 0.001$ vs. normal control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs. induced, untreated group.

3.5. Histopathology

Consistent damage to the histological architecture of the stomach typified by necrotic areas and cellular degeneration was visible in rats administered 96% ethanol when com-

pared to control animals. However, the gastric mucosa of animals that received prior ACJE at 1.25, 2.5, and 5.5 mg/kg and ranitidine (30 mg/kg) treatment was protected against the corrosive effect of ethanol, preserving vital histological aspects when compared to the induced, untreated animals (Figure 5).

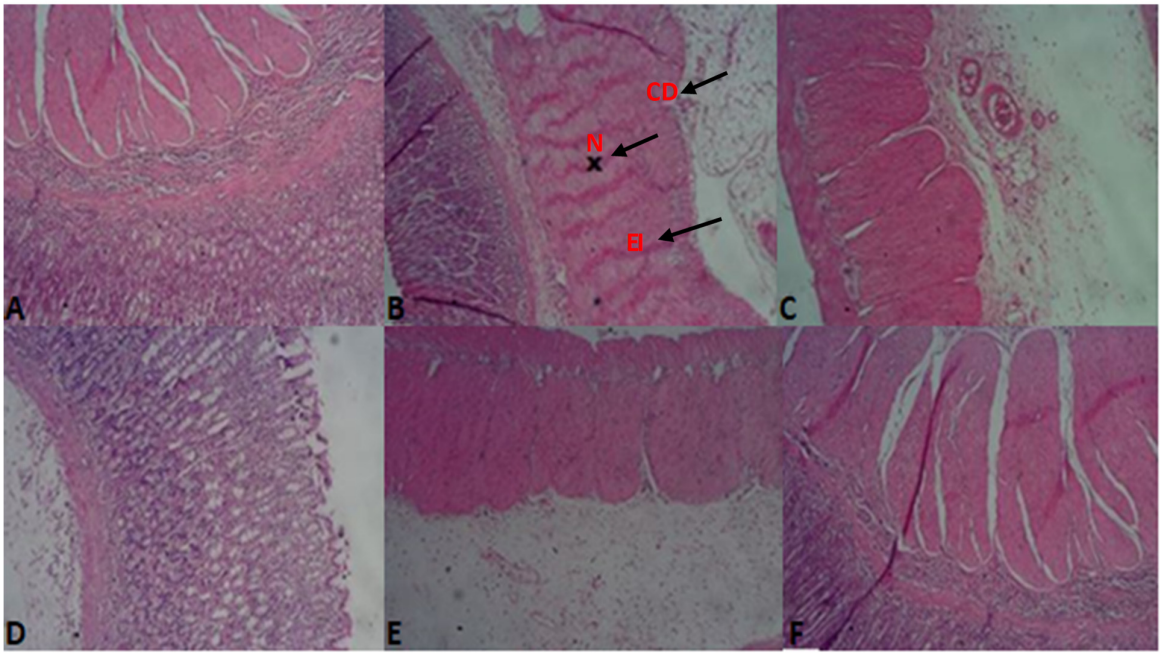

Figure 5. Effect of *Ageratum conyzoides* juice extract on the histological architecture of rat stomach following ethanol-induced gastric mucosal injury. Representative H&E-stained sections are shown. CD = cellular degeneration; N = cellular necrosis; EI = edema and inflammatory cell infiltration. Images were captured at ×200 magnification. Scale bar = 100 µm. (**A**) The normal control group showed well-outlined arrays of normal gastrointestinal tissues: the mucosa, submucosa, muscularis externa, and serosa. (**B**) The ulcer control group showed a marked disruption to the gastric mucosa and was characterized by necrotic areas and cellular degeneration. (**C**). The ranitidine group showed no observable cytoarchitectural distortion. (**D**) The 1.25 mg/kg ACJE, (**E**) 2.5 mg/kg ACJE, and (**F**) 5.5 mg/kg ACJE groups showed normal gastric mucosa and no cytoarchitectural distortion.

3.6. Molecular Docking of ACJE Phytochemicals with Ulcerogenic Targets

As shown in Table 3 and Figures 6 and 7, phytochemicals from ACJE exhibited significant binding interaction with key ulcerogenic proteins (pepsin and urease). The binding affinities of the studied ligands varied from −4.179 kcal/mol to −7.656 kcal/mol for human pepsin and −3.930 kcal/mol to −7.080 kcal/mol for *Helicobacter pylori* urease, respectively. Among the conventional medicines, omeprazole had the highest binding affinity, with values of −6.470 kcal/mol for human pepsin and −5.277 kcal/mol for *H. pylori* urease. Also, molecular docking studies revealed important interactions between selected phytochemicals and the residues of pepsin and urease. For pepsin, scutellarein formed hydrogen bonds with Arg 29, Thr 287, and Met 307 while also maintaining Van der Waals contacts with other residues. Quercetin showed nucleophilic interactions with Thr 136, Gly 96, and Ile 190; kaempferol created bonded interactions with Leu 47 and Phe 348, and stigmasterol had a single bonded interaction with Phe 348. Regarding urease, kaempferol established hydrogen bonds with Ala 115A, Lys 10A, His 14A, and Glu 45; scutellarein formed hydrogen bonds with Ser 143A and His 144A and a pi-pi stacking interaction with Phe 198A; quercetin bonded with Leu 38A and Leu 64 and interacted with several other residues.

Table 3. Docking parameters and binding affinities of *Ageratum conyzoides* phytochemicals and standard drugs against ulcerogenic targets.

Sn	Compound (Ligands)	PubChem Cid	Binding Affinities (kcal.mol^{-1})	
			Human Pepsin (af-p0djd9-f1-Model_v4)	*H. pylori* Urease (af-p14916-f1-Model_v4)
1	Quercetin	5280343	−7.350	−7.080
2	Eugenol	3314	−5.021	−5.044
3	Coumarin	323	−5.097	−4.944
4	Caffeic acid	689043	−5.482	−5.626
5	Prococene I	28619	−5.446	−5.472
6	Prococene II	12565	−4.992	−4.851
7	α-humulene	6508206	−5.828	−6.007
8	Kaempferol	5280863	−7.322	−6.558
9	β-sitosterol	222284	−6.516	−6.261
10	Stigmasterol	5280794	−6.938	−6.932
11	Fumaric acid	444972	−4.179	−3.930
12	Scutellarein	5281697	−7.656	−6.180
13	Chromene	9211	−5.095	−4.858
14	Lycopsamine	107938	−5.715	−4.294
15	β-caryophyllene	5281515	−5.242	−6.193
St1	Ranitidine	3001055	−4.842	−4.028
St2	Omeprazole	4594	−6.470	−5.277
St3	Cimetidine	2756	−4.206	−4.206

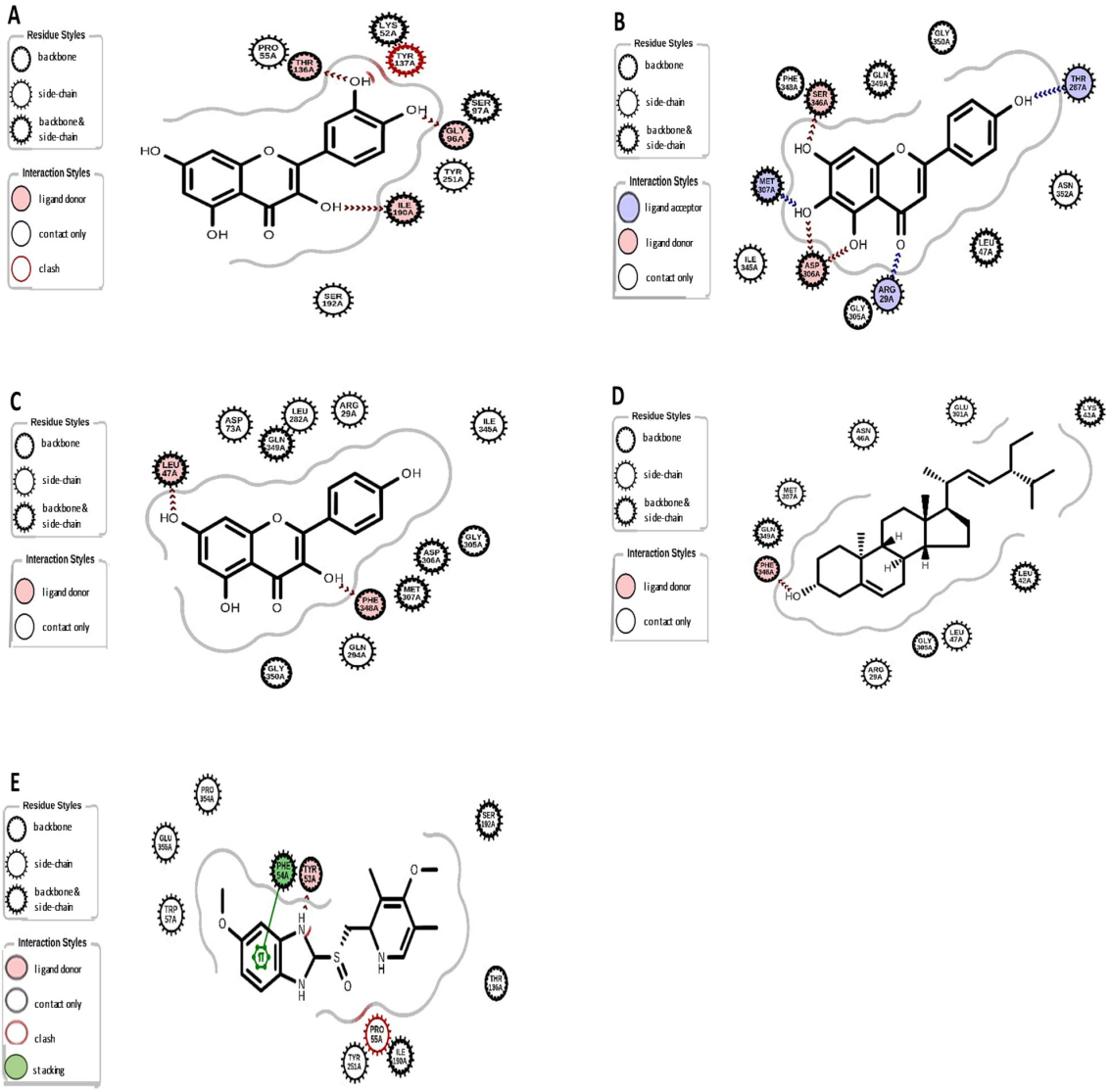

Figure 6. Binding interactions of the ulcerogenic enzyme pepsin with quercetin (**A**), scutellarein (**B**), kaempferol (**C**), stigmasterol (**D**), and omeprazole (**E**).

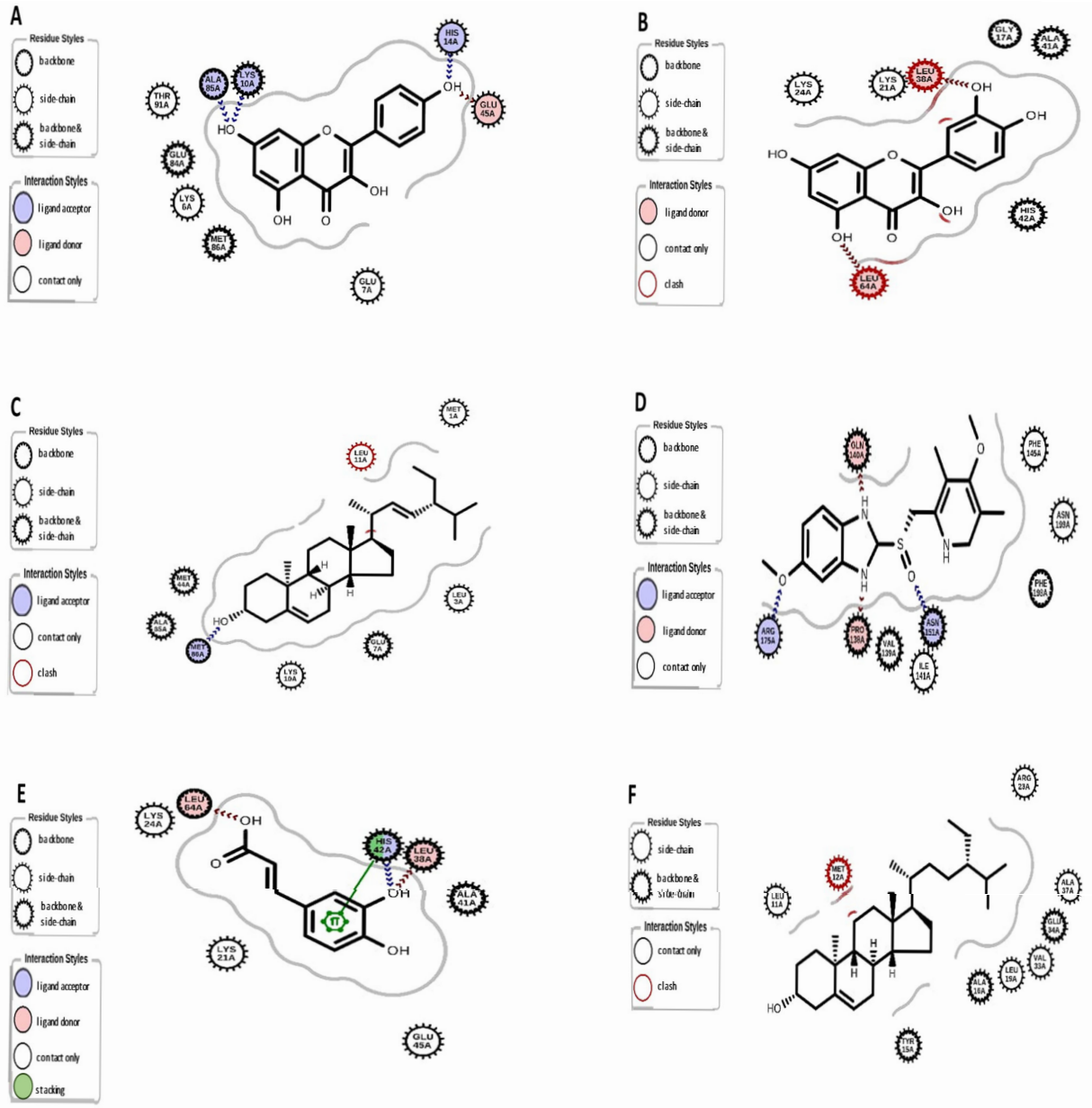

Figure 7. Binding interactions of the ulcerogenic enzyme urease and kaempferol (**A**), quercetin (**B**), stigmasterol (**C**), omeprazole (**D**), caffeic acid (**E**), and beta-sitosterol (**F**).

4. Discussion

Gastric ulcer remains a significant gastrointestinal disorder, and the search for safe and effective gastroprotective agents, particularly from natural sources, continues to be an important area of investigation. The present study was, therefore, conducted to evaluate the gastroprotective potential of *Ageratum conyzoides* juice in an ethanol-induced gastric ulcer model. Even though the fundamental mechanism of ethanol-induced stomach ulcers is not entirely understood, there is evidence that three events are involved: oxidative stress, apoptosis, and inflammatory responses [21]. Because heavy alcohol intake is arguably the most important cause of stomach ulcers in people [22–24], the ethanol model is viable and commonly used to screen for anti-ulcer potentials of medicines, and it may be superior to other models such as stress and pylorus ligation [23,24]. High alcohol infusion may directly erode the stomach mucosa, causing mucous membrane solubilisation and exposure to the degradative actions of hydrochloric acid, eventually resulting in mucosal lesions and upper gastrointestinal hemorrhage [25]. The gastroprotective and antioxidant effects observed

in this study are consistent with previous reports describing the anti-inflammatory and cytoprotective activities of flavonoids such as quercetin and kaempferol [26]. Similarly, earlier studies on *Ageratum conyzoides* have reported antioxidant and tissue-protective properties that may contribute to gastric mucosal protection [12].

In the present study, ethanol administration in fasting rats produced marked gastric mucosal injury, as evidenced by significant increases in ulcer scores (approximately 9-fold), gastric juice volume (1.5-fold), and a reduction in gastric pH, alongside visible macroscopic damage to the gastric mucosa. Pre-treatment with *Ageratum conyzoides* juice extract (ACJE; 2.5 and 5.5 mg/kg) and ranitidine (30 mg/kg) significantly attenuated these alterations, restoring gastric parameters and preserving mucosal integrity. Gastric ulcer induction in rats was validated in the current investigation by gastric mucosal injury as evidenced by high ulcer scores, macroscopic indication of gastric tissue abnormalities, low pH, and a high volume of gastric juice, consistent with prior findings [4,27,28]. One viable strategy to promote ulcer healing is to control gastric acid secretion since this crucially delays gastric ulcer healing [29]. *Ageratum conyzoides* juice extract was able to effectively reduce stomach acid output and improve other indices of gastric ulceration in rats in a comparable manner to ranitidine in the present study. Since ethanol induction of gastric ulcers has an oxidative stress underpinning, improving the antioxidant profile may reduce damage to the mucosal epithelium. In the current work, induced rats' stomachs experienced severe oxidative stress, as evidenced by decreased gastric GSH levels and increased membrane lipid peroxidation. The antioxidant properties of *Ageratum conyzoides* juice were demonstrated by the reversal of oxidative stress potentiation in gastric tissue of treated animals. In other words, antioxidant phytoconstituents in *Ageratum conyzoides* juice mopped up the increased reactive species caused by ethanol-induced mucosal damage [30] .

The antiulcer properties of some medicinal plants are due to multiple factors, including antioxidant boost, stimulation of mucus production, and decreased gastric acid secretion [27,31]. These events can accelerate ulcer reepithelization and healing by increasing cellular proliferation and migration, angiogenesis, and granulation tissue maturation [31]. Ethanol-induced gastric injury has been associated with disturbances in lipid metabolism secondary to oxidative stress and membrane damage [32,33]. Therefore, serum lipid parameters were evaluated as supportive indicators of ethanol-induced systemic alterations. The observed lipid profile abnormalities in ethanol-induced ulcerated rats are consistent with prior findings, which attributed this to disturbance of fatty acid metabolism and, indirectly, membrane lipid peroxidation and free radical production that often result from ethanol exposure [32,33]. As a result, stabilization of blood lipid profiles indicates that the juice extract can prevent or protect against ethanol-induced effects in experimental animals [32]. Histological evidence supported the healing response caused by *Ageratum conyzoides* juice, indicating that rats fed juice extracts had their stomach mucosa integrity restored [34].

In the present study, HPLC analysis identified many biologically active phytochemicals in the juice extract of goat weed. These include β-caryophyllene, eugenol, coumarin, β-sitosterol, chromene, kaempferol, quercetin, campinas, caffeic acid, lycopsamine, and scutellarein, among others. Quercetin, kaempferol, and β-sitosterol, which are the most abundant constituents in the juice, were earlier reported to possess inhibitory potential against ulcerogenic targets [35].

As a complement to experimental research, molecular simulations have often been used to provide mechanistic insights into biomolecular recognition governed by well-defined physicochemical forces [36]. The binding affinities and interaction profiles of the characterized phytochemicals and standard anti-ulcer drugs (omeprazole, ranitidine, and cimetidine) against human pepsin and *Helicobacter pylori* urease were evaluated in the present study using molecular docking approaches. Although ulceration was induced

by ethanol, molecular docking was included to explore possible interactions of ACJE phytochemicals with ulcer-related targets. Human pepsin and *Helicobacter pylori* urease were selected for their known role in gastric mucosal injury and peptic ulcer pathogenesis, respectively. Human pepsin was used for the docking analysis because of the availability of curated structural datasets and its high functional conservation across mammalian species. Nevertheless, possible species-specific differences between human and rat pepsin should be considered when interpreting the docking results. Docking scores reflect the thermodynamic favorability of ligand binding, and higher negative scores imply stronger and more stable interactions [37]. The binding affinities of the investigated ligands ranged from −4.179 to −7.656 kcal/mol for human pepsin and −3.930 to −7.80 kcal/mol for *Helicobacter pylori* urease. Omeprazole was found to exhibit the highest binding affinity toward both targets among all the reference drugs. This is consistent with previous reports demonstrating greater acid-suppressive and ulcer-healing efficacy of omeprazole compared with ranitidine and cimetidine [38,39]. Some phytochemicals, such as quercetin, kaempferol, scutellarein, stigmasterol, and β-sitosterol, in the juice extract showed superior binding affinities against the ulcerogenic enzymes compared to omeprazole. Other compounds in the juice extract, including caffeic acid and β-caryophyllene, also demonstrated enhanced binding to urease in line with prior docking studies [40,41]. However, it is important to state here that molecular docking provides theoretical predictions of ligand–protein interactions and does not establish direct biological activity or inhibitory potency. In this wise, the observed binding affinities should be interpreted as indicative of potential interactions rather than definitive evidence that multiple phytochemicals exert simultaneous activity on the studied targets. Pepsin is a highly active aspartic endoprotease that works under acidic conditions to damage the gastric mucosa [42]. *H. pylori* urease, conversely, enhances bacterial viability by neutralizing stomach acidity [43]. Inhibition of these enzymes has been associated with attenuation of ulcer formation. Quercetin and kaempferol, which are two of the compounds identified in the extract, have been previously reported to modulate pepsin activity and *H. pylori* proliferation [44,45]. As stated earlier, the docking results in this study primarily serve to highlight possible molecular interactions that may contribute to the observed gastroprotective effects of the extract, rather than confirming the activity of individual constituents. The presence of lycopsamine in ACJE is of concern since pyrrolizidine alkaloids have been implicated in hepatotoxicity upon chronic exposure [46]. In the present study, no significant changes were observed in hepatic oxidative stress indices, but a detailed assessment of liver histopathology was not performed. Hence, further toxicological evaluation is required to establish the long-term safety profile of ACJE. One of the study's limitations is the lack of isolation of individual phytochemicals in the juice extract to determine their precise roles in gastroprotection. Furthermore, in vitro enzyme inhibition experiments were not available to validate the molecular docking results. Pharmacokinetic and toxicity prediction analyses were not performed in this study; hence, future studies should include the ADMET/toxicity profiling of the identified phytochemicals to further evaluate their safety and drug-likeness. The acute ethanol-induced ulcer model may also not adequately represent other clinically relevant ulcer conditions, especially chronic *H. pylori*-associated ones. Additional research involving molecule isolation, mechanistic validation, and other experimental models will improve translational relevance.

5. Conclusions

Ageratum conyzoides juice extract showed significant gastroprotective activity against ethanol-induced gastric ulceration in rats as shown by reduced ulcer severity, improved gastric parameters, restoration of antioxidant status, and preservation of gastric histoarchi-

tecture. Molecular docking analysis also confirmed the antiulcer potential of the identified phytochemicals. However, additional studies investigating its long-term safety profile, particularly with respect to pyrrolizidine alkaloid-associated toxicity, are required before therapeutic development can be considered.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nutraceuticals6030044/s1, Figure S1: Representative HPLC-DAD chromatogram showing the identification of phytochemicals in *Ageratum conyzoides* juice extract.

Author Contributions: Investigation, resources, writing—original draft preparation, A.J.M.; conceptualization, investigation, data curation, writing—original draft preparation, T.R.K.; resources, investigation, W.O.A., P.D.O., S.E.D. and W.I.E.; methodology, supervision, formal analysis, writing—review and editing J.A.; conceptualization, supervision, methodology, formal analysis, writing—review and editing, K.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The animal study protocol was approved by the departmental Animal Care and Use Ethical Committee of the Federal University, Oye-Ekiti (No: 2020A000012, 10 March 2020).

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ACJE	*Ageratum conyzoides* juice extract
ADT	AutoDock Tools
b.w.	Body weight
DTNB	5,5′-Dithiobis(2-nitrobenzoic acid)
GSH	Reduced glutathione
H&E	Hematoxylin and eosin
HDL-c	High-density lipoprotein cholesterol
HPLC	High-performance liquid chromatography
LDL-c	Low-density lipoprotein cholesterol
MDA	Malondialdehyde
NIH	National Institutes of Health
p.o.	Per os (oral administration)
ROS	Reactive oxygen species
TBARS	Thiobarbituric acid–reactive substances
TBA	Thiobarbituric acid
VLDL-c	Very-low-density lipoprotein cholesterol

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