

Original Research Article

Exploring the anti-ulcer potential of *Crassocephalum crepidioides* in *in vivo* and *in silico* experimental models

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Abstract

Purpose: To evaluate the anti-ulcer potential of *Crassocephalum crepidioides* by integrating both experimental and computational approaches.

Methods: Dried cold-macerated methanol extract of *C. crepidioides* was fractionated to obtain ethyl acetate, methanol and *n*-hexane fractions. The acute toxicity of the extract was determined by the Lorke method. The extract and ethyl acetate fraction were fingerprinted using the high-performance liquid chromatography-diode array detector (HPLC-DAD) technique. The *in vivo* anti-ulcer activity in Wistar rats was evaluated using ethanol- and indomethacin-induced models with oral Tween 80 and omeprazole as controls. The identified compounds were docked on human prostaglandin E receptor EP4, *Helicobacter pylori* urease and monoclonal porcine pepsin ulcerogenic factor targets for *in silico* studies.

Results: The extract was safe up to a 5000 mg/kg dose, with no lethality or delayed overt toxicity. The HPLC-DAD identified fingerprints such as flavonoids (quercetin, kaempferol, rutin and catechin), phenolic acids (chlorogenic, gallic, ellagic and caffeic acids), and many others. The ethyl acetate fraction (500 mg/kg) elicited the highest ulcer inhibition (72.92 %), significantly ($p < 0.05$) higher than 20 mg/kg omeprazole (56.25 %) in the indomethacin-induced model. The binding parameters of the ligands showed that among the flavonols and phenolic acids, rutin ($E = -12.12$ kcal/mol, $K_i = 0.001$ μ M) and chlorogenic acid ($E = -10.13$ kcal/mol, $K_i = 0.037$ μ M) respectively elicited significantly stronger binding to the EP4, a defensive ulcerogenic protein target.

Conclusion: *In vivo* and *in silico* evidence show that *C. crepidioides* possesses potent gastro-protective properties, which could be attributed to its polyphenolic constituents.

Keywords: Ethyl acetate fraction, Gastro-protective, Gastric ulcer, Molecular docking, Rutin

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INTRODUCTION

Phytotherapy is the utilisation of medicinal plants for the treatment of various ailments and has been practised since ancient times [1]. This

application of medicinal plants is derived from a huge reservoir of diverse secondary metabolites in various morphological parts such as seeds, bark, roots, leaves, and fruits of plants. Many

plants employ these phytochemicals as a defence mechanism against pathogens [2].

Peptic ulcer disease (PUD) is characterised by the presence of damage or ulcers (open wounds) in the mucosal lining of the gastrointestinal tract, which leads to the excessive secretion of gastric acid or pepsin. Typically, these symptoms appear within the gastric region and the proximal segment of the duodenum [3]. Several plants have been utilised in the management of PUD in ethnomedicine [4]. *Crassocephalum crepidioides* (Benth.) S. Moore (Asteraceae), commonly called fireweed in English, Obuinenawa in Igbo (Nigeria) and Gbolo in Benin Republic, is one such plant. It is an edible, non-timber forest herb that is consumed as a vegetable in many countries in Africa, including Nigeria, Benin Republic, Kenya, Tanzania and Uganda, due to the succulence of the leaves and stems [5]. Its ethnomedicinal value is found in the treatment of wounds, boils, burns, indigestion, and stomach ulcers [5]. The folkloric use of *C. crepidioides* in ethnomedicine has elicited a lot of interest in the management of ulcer diseases. Epidemiological data have established that the prevalence of PUD is a reflection of *Helicobacter pylori* infection prevalence, increasing with non-steroidal anti-inflammatory drugs (NSAIDs) and acetylsalicylic acid use, as well as with an ageing population [6]. A pooled prevalence of PUD in Africa at 15.2 % has been established, with duodenal ulcer as the most prevalent at 10.2 %, followed by gastric ulcer at 5.8 %, while 0.6 % of cases had both types [7]. Regional variations showed the prevalence rate at 19, 15, 12 and 8 %, in Western, Eastern, Northern and Southern Africa, respectively. Additionally, 57.1 % of patients tested positive for *H. pylori* infection, with its prevalence reaching 76.4% among those diagnosed with PUD [8]. These epidemiological data represent a high burden on the populace, considering their impacts on the quality of life.

A triple combination of an antibiotic (amoxicillin or clarithromycin), proton pump inhibitor (omeprazole) and histamine receptor antagonist (famotidine) has remained the most accepted treatment option [9]. In addition to their side effects, the increasing prevalence of antibiotic-resistant strains of *H. pylori*, a common cause of ulcers, poses a significant challenge to existing therapeutic strategies. In some cases, alternative therapeutic approaches have been explored without attaining the optimal outcome. While options such as phytotherapy have been common, a major setback is the poor understanding of the molecular mechanism of action, as well as limited targets in the human body [10]. However, the multi-target mechanism

of natural products in eliciting pharmacological actions has always provided guaranteed pathways in discovering antiulcer therapy [7]. This study, therefore, investigated the potential mechanism of anti-ulcer activity of *C. crepidioides* phytochemical constituents by (i) identifying the constituents using the high-performance liquid chromatography (HPLC) technique, (ii) *in vivo* bioassay of anti-ulcer activity and (iii) *in silico* docking of the constituents on targets representing the proteins of the defensive, aggressive and microbial ulcerative factors.

EXPERIMENTAL

Animals

Wistar rats (mean weight = 125 g) and mice (mean weight = 65 g) of either sex were housed in individual cages with softwood chips as bedding at 25 ± 2 °C on a 12/12-h diurnal cycle. They were given unlimited access to standard pellet feed and water *ad libitum* and adapted to the laboratory environment for seven days before initiating the actual experiment. The experiment was undertaken in compliance with local ethical approval granted by the animal care and use committee of the International Centre for Ethnomedicine and Drug Development, Nigeria (BDGP/25/InterCEDD/A.007) and OECD regulations for the use, care, and welfare of laboratory animals.

Crassocephalum crepidioides sample

The aerial parts of *Crassocephalum crepidioides* were collected from Gashaka-Gumti National Park (Longitude: 07°21'00"N, Latitude: 11°31'12"E), Gashaka, Taraba, Nigeria, in January 2025 and were identified and authenticated by Mr Isaac Ossai, a taxonomist at the Centre for Bioresources Development and Conservation Programme (BDGP), Nigeria. The voucher specimen InterCEDD/D53/016_ *Crassocephalum crepidioides* was prepared and deposited in the centre's herbarium.

Preparation of plant extract

The plant material was air-dried for 14 days, ground into coarse powder and 800 g of the sample was cold macerated in 3.75 L aqueous methanol (95 %v/v) for 48 h with frequent agitation [11]. The mixture was then strained and filtered using Whatman No. 3 filter paper. The extract was concentrated using a rotary evaporator. The extract (80 g) was subjected to silica gel (1:70) column chromatographic

fractionation using gradients of n-hexane (1.8 L), ethyl acetate (2.0 L) and methanol (1.5 L) successively. Each solvent eluate was pooled and concentrated *in vacuo* at 40 °C.

Qualitative phytochemical screening

Standard methods were adopted to conduct phytochemical screening for the presence of alkaloids, flavonoids, phenolic compounds, saponins, tannins, phytosterols, terpenoids and glycosides in the crude extract and solvent fractions [12].

High-performance liquid chromatography (HPLC) analysis

The HPLC system (Shimadzu Nexera Mx), μ Bondapak C18 column, 100 mm $\times$ 4.6 mm, 7 μ m particle size, mobile phase of acetonitrile: water or methanol: water, flow rate of 1.0 mL/min (standard); 0.08 mL/min (nano), injection volume of 5 μ L (standard); 10 μ L (alternate), detection was by UV at 254 nm / Diode Array Detector (DAD) and pump pressure of 15 MPa. Ten grams (10 g) of the sample was dispersed in a mixture of 20 mL acetonitrile and methanol (1:1 v/v) and vortexed for 30 min. Thereafter, the organic phase was transferred into a 25 mL volumetric flask and made up to volume with the same solvent mixture. The resulting solution was filtered for HPLC analysis. Before sample (extract and ethyl acetate fraction) injection, the HPLC system was calibrated to define the detection window for the test samples. The prepared sample was then injected into the HPLC system to obtain the chromatograms, and the peak areas corresponding to the analytes of interest were recorded. Quantification was achieved by comparing the peak area of the sample with that of the standard [13].

Acute toxicity test of the extract

Acute oral toxicity testing was conducted in healthy Wistar mice following the Lorke method. The first phase required 9 mice, which were divided into three (3) groups of three (3) animals each. The extract was administered orally to each group at the doses of 10, 100, and 1000 mg/kg, respectively [14]. The mice were observed for lethality, and signs of toxicity, such as behavioural (alertness, restlessness, irritability, and fearfulness), autonomic profiles (salivation, lacrimation, perspiration (diaphoresis), piloerection, incontinence, and defecation), neurologic (CNS) profile (spontaneous activity (drowsiness), reactivity, touch response, pain response, convulsions, tremors, and gait), physical states like loss of

appetite, for morbidity or mortality and other signs of toxicity. At the expiration of time, and while no animal died in the first phase, another set of animals was starved for 24 h with free access to water and divided into three (3) groups of three (3) animals each. The extract was administered orally to each group at the doses of 1500, 1900, and 2900 mg/kg, respectively. The animals were monitored for 24 h for both death and behavioural profile. At the end of the monitoring period, and while no mortality was recorded in the second phase, two mice were kept in separate cages, one mouse each. The extract was administered orally to each mouse at the doses of 4000 and 5000 mg/kg, respectively. The animals were also monitored for 24 hours for both death and behaviour profile.

Anti-ulcer screening of the extract and active fraction

Animal grouping

In each of the two experimental models, 70 albino rats were randomly allocated into six groups (A–F). Group A (negative control) received only the vehicle (Tween-80 in normal saline, 0.50 mL). Groups B–E were treatment groups, each administered three graded doses (125, 250, and 500 mg/kg) of either the crude extract (Group B), hexane fraction (Group C), ethyl acetate fraction (Group D) or methanol fraction (Group E). Group F (positive control) received omeprazole (20 mg/kg). All the treatments were administered orally after the induction of ulceration.

Ethanol-induced anti-ulcer activity model

The rats were first fasted for 24 h, with free access to water, which was removed 1 h before induction of gastric ulcers. After the fasting period, a single dose (5 mL/kg) of absolute ethanol was administered orally to all groups. After a 1 h interval, the rats in groups A–F received their respective treatments orally once daily for three days. The rats were anaesthetised using chloroform and then humanely sacrificed on the fourth day, and their stomachs were removed and opened along the greater curvature, delicately washed with normal saline to remove gastric contents and blood clots, and then examined by a 10 $\times$ magnifying lens to assess the degree of ulceration [15].

Indomethacin-induced anti-ulcer activity model

The rats were first fasted for 24 h, with free access to water, which was removed 1 h before

induction of gastric ulcers. After the fasting period, a single dose (30 mg/kg body weight) of indomethacin was administered orally to all groups. After a 6 h interval, the rats received their respective treatments orally daily for three days, as previously described. The rats were anaesthetised using chloroform and then humanely sacrificed on the fourth day, and their stomachs were removed and opened along the greater curvature, delicately washed with normal saline to remove gastric contents and blood clots, and then examined by a 10× magnifying lens to assess the formation of ulcers. The number of ulcers was counted and scored as follows: no lesion (0), red colouration (0.5), haemorrhagic streak (1), small ulcer (2), multiple ulcers (3) and deep ulcers (4). Ulcer index (UI) was determined by computing the mean ulcer score for each rat using the formula [3].

$$UI = \frac{\text{Total ulcer score for group}}{\text{Number of animals in the group}}$$

The ulcer inhibition (% UI) is calculated using;

$$UI (\%) = \frac{UI_{\text{control}} - UI_{\text{treated}}}{UI_{\text{control}}} \times 100$$

Where UI_{control} = ulcer index of negative control and UI_{treated} = ulcer index of test group

In silico studies

Protein targets

The 3D structures of three target proteins were retrieved from the Protein Data Bank. *Helicobacter pylori* urease with its inhibitor (PDB ID: 6ZJA; resolution 2.00 Å) served as the

microbial factor. Monoclinic Porcine Pepsin (PDB ID: 4PEP; resolution 1.80 Å) represented the aggressive ulcer factor, while the human prostaglandin E receptor EP4 in complex with Fab and ONO-AE3-208 (PDB ID: 5YWY; resolution 3.20 Å) served as the defensive ulcer factor [16-18].

Ligands

The six most abundant fingerprints identified from the HPLC analysis served as the ligands. The compounds (Figure 1) included quercetin (1), kaempferol (2), rutin (3), gallic acid (4), chlorogenic acid (5) and 4-hydroxybenzoic acid (6).

Preparation of protein targets

All crystallographic waters and non-standard heteroatoms were removed to clean the structures. The cleaned models were exported to the University of California, San Francisco, USA (UCSF) Chimera for energy minimisation, applying Steepest descent minimisation for 100 steps (step size 0.02 Å), and conjugate gradient minimisation for 100 steps (step size 0.02 Å) [19].

Molecular docking study

Docking was carried out in AutoDock Tools 1.5.7 using a rigid-receptor/flexible-ligand technique. Preparation steps: The protein and ligand files were converted to PDBQT, followed by the addition of polar hydrogens to the receptor, merged with non-polar hydrogens.

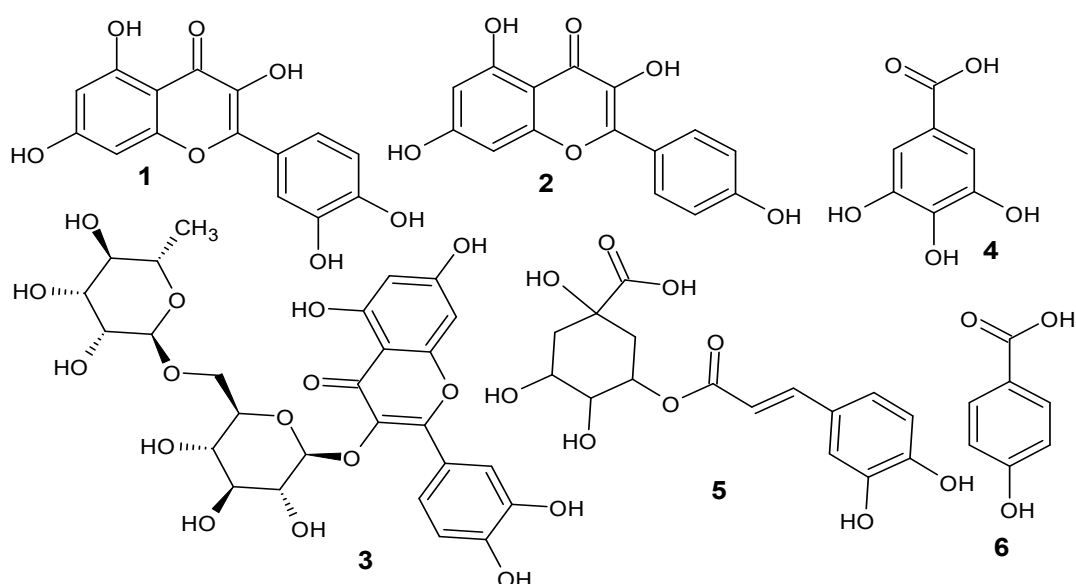


Figure 1: Chemical structures of test compounds

Then, Gasteiger charges and AutoDock4 atom types were assigned. The ligand torsions were automatically defined via ADT's Python scripts. For each protein, the grid box was centred on the native inhibitor's binding site with 0.375 Å spacing. Docking used the Lamarckian Genetic Algorithm with these settings: GA runs: 50, Population size: 300, Maximum energy evaluations: 2.5 million, Maximum generations: 270 000. All other parameters were kept at default settings during the docking simulations [20]. The native ligands were first re-docked into the *Helicobacter pylori* urease, monoclinal porcine pepsin and human prostaglandin E receptors to reproduce the original poses, after which the compounds from the HPLC analysis were docked into the receptors, replicating the parameters used in the native ligand re-docking. The best-scoring docked poses were imported into the protein-ligand interaction profiler for inspection of hydrogen bonds, hydrophobic contacts, and coordination interactions with active-site residues [21]. Interactive diagrams and 3D snapshots were generated to illustrate binding modes and support the interpretation of anti-ulcer activity.

Data analysis

The data were expressed as mean $\pm$ SEM and were analysed using Windows SPSS version 20.0. Comparisons were made between negative control, positive control, and treatment groups of varied doses using ANOVA followed by Post hoc Tukey's HSD multiple comparison tests. At a 95 % confidence interval, $p < 0.05$ was considered statistically significant.

RESULTS

Extraction of *C. crepidioides* and fractionation of the extract

The extraction of 800 g of powdered *C. crepidioides* with 3.75 L methanol (95 %v/v) yielded 83.5 g (10.4 %w/w). Bulk separation of the extract yielded 8.87, 6.85 and 3.75 %w/w of methanol, ethyl acetate and n-hexane fractions, respectively.

Qualitative phytochemical constituents of *C. crepidioides*

The qualitative screening of *C. crepidioides* extract and its fractions for the presence of saponins, phenolics, phytosterols, alkaloids, tannins, flavonoids, terpenoids and glycosides showed the absence of phytosterols in both the extract and fractions. There was no trace of terpenoids, saponins and glycosides in the ethyl acetate fraction, while the n-hexane fraction was the least diverse, containing only terpenoids.

HPLC profiles of *C. crepidioides* extract

The HPLC chromatograms of the most active fraction (ethyl acetate) of *C. crepidioides* (Figure 2) show the presence of flavonoids (quercetin, kaempferol, rutin and catechin) and phenolic acids (4-hydroxybenzoic acid, chlorogenic acid, gallic acid, ellagic acid and caffeic acid), simple phenols (orcinol, catechol and eugenol), terpenoids (α -guaiene, aromadendrene and citronellol), sterols (β -sitosterol), sugar alcohols (erythritol), aromatic heterocycles (benzofuran).

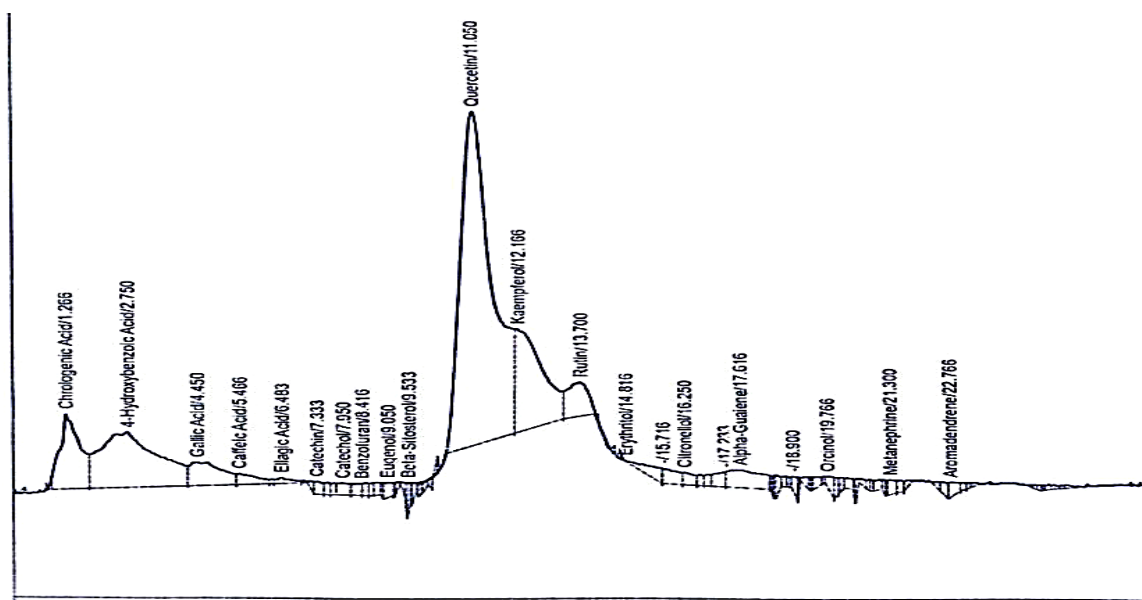


Figure 2: HPLC chromatogram of the ethyl acetate fraction of *C. crepidioides*

The ethyl acetate fraction of *C. crepidioides* showed a range of bioactive compounds, with quercetin having the highest peak area and height, indicating its high relative abundance (Table 1). Other notable flavonoids and phenolic acids include kaempferol, rutin, gallic acid, and chlorogenic acid.

Table 1: HPLC profiles of the ethyl acetate fraction of *C. crepidioides*

Compound	Area (mAU)	Height (mAU)	Retention time (min)
Quercetin	7520.7320	144.107	11.050
4-Hydroxybenzoic Acid	2310.5790	23.544	2.750
Kaempferol	1975.2785	44.327	12.166
Chlorogenic Acid	1060.7840	31.998	1.266
Gallic Acid	552.6750	10.070	4.450
Rutin	524.4750	14.574	13.700
α -Guaiane	435.2880	7.511	17.616
Erythritol	175.3440	0.439	14.816
Caffeic Acid	156.8410	4.769	5.466
Citronellol	103.2780	5.271	16.250
Catechol	102.3055	5.087	7.950
Aromadendrene	95.6350	6.616	22.766
Eugenol	91.7960	6.513	9.050
Benzofuran	82.3060	5.048	8.416
Catechin	81.9950	5.385	7.333
Metanephrene	81.9340	6.247	21.300
Orcinol	77.7860	4.859	19.766
Ellagic Acid	61.0475	2.545	6.483
β -Sitosterol	60.2155	15.439	9.533

Acute oral toxicity study

The test extract was found to be safe, and the mice did not show any lethality or delayed overt toxicity, central and behavioural anomalies following the highest dose (5000 mg/kg) administered in the study.

Anti-ulcer activity of extract and fractions of *C. crepidioides*

Anti-ulcer activity of *C. crepidioides* extract in ethanol-induced ulcer models

Crassocephalum crepidioides extract demonstrated significant ($p < 0.05$) dose-dependent gastroprotection. The crude extract (group B) reduced ulcer index from 5.00 ± 1.00 in controls to 2.40 ± 0.46 at 500 mg/kg, with inhibition rising from 6.66% (125 mg/kg) to 52.0% (500 mg/kg), comparable to omeprazole (56.66%; Table 2). The ethyl acetate (group D) fraction showed superior efficacy, lowering the ulcer index to 1.70 ± 0.25 and achieving 66% inhibition at a 500 mg/kg dose, surpassing the standard drug. The methanol fraction (group E) also produced notable reductions ($p < 0.05$), with

the ulcer index decreasing from 4.30 to 2.30 and inhibition increasing from 14 to 54%.

Table 2: Anti-ulcer activity of *C. crepidioides* extract in ethanol-induced ulcer models

Group	Dose (mg/kg)	UI	I (%)
A	0.5 mL	5.00 ± 1.00^b	-
B	125	4.67 ± 1.20^{ab}	6.66
	250	3.60 ± 0.19^{ab}	28.00
	500	2.40 ± 0.46^{ab}	52.00
C	125	n.a	n.a
	250	n.a	n.a
	500	n.a	n.a
D	125	2.80 ± 0.30^{ab}	44.00
	250	2.40 ± 0.19^{ab}	52.00
	500	1.70 ± 0.25^{ab}	66.00
E	125	4.30 ± 0.60^{ab}	14.00
	250	3.40 ± 0.51^{ab}	32.00
	500	2.30 ± 0.20^{ab}	54.00
F	20	2.17 ± 0.17^a	56.66

UI = Ulcer index, I = inhibition, mean $\pm$ SEM; $n = 5$ for each treatment group; treatments compared to negative control^a and standard^b at $p < 0.05$, n.a = no activity (*n*-hexane fraction was not reported as no anti-ulcer activity was detected)

Anti-ulcer activity of *C. crepidioides* extract in indomethacin-induced ulcer model

Crassocephalum crepidioides extract produced significant ($p < 0.05$), dose-dependent protection. The crude extract (group B) reduced ulcer index from 4.80 ± 0.20 in the untreated to 2.00 ± 0.16 at 500 mg/kg, with inhibition rising from 16.67 to 58.33%, comparable to omeprazole (56.25%). The ethyl acetate fraction (group D) was the most potent, lowering ulcer index progressively and achieving 72.92% inhibition at 500 mg/kg, surpassing omeprazole (56.25%). The methanol fraction (group E) also showed dose-dependent activity, reducing ($p < 0.05$) the ulcer index from 4.50 to 2.70 and increasing inhibition from 6.25% to 43.75%. Although less effective than omeprazole, the methanol fraction provided substantial protection. All treated groups demonstrated statistically significant improvements compared to controls ($p < 0.05$; Table 3).

Interaction of *C. crepidioides* constituents with ulcerogenic protein targets

The binding parameters of the ligands (Table 4) showed that among the flavonols (**1**, **2** and **3**) and phenolic acids (**4**, **5** and **6**), **3** and **5**, respectively, elicited significantly stronger binding

(higher negative binding energies) to all the ulcerogenic protein targets than their respective co-crystallised ligands.

Table 3: Anti-ulcer activity of *C. crepidioides* extract on indomethacin-induced rats

Group	Dose (mg/kg)	UI	I (%)
A	0.5 mL	4.80±0.20 ^b	-
B	125	4.00±0.57 ^{ab}	16.67
	250	3.50±0.32 ^{ab}	27.08
	500	2.00±0.16 ^{ab}	58.33
C	125	n.a	n.a
	250	n.a	n.a
	500	n.a	n.a
D	125	2.50±0.16 ^{ab}	47.92
	250	2.00±0.16 ^{ab}	58.33
	500	1.30±0.37 ^{ab}	72.92
E	125	4.50±0.55 ^{ab}	6.25
	250	3.80±0.25 ^{ab}	20.83
	500	2.70±0.17 ^{ab}	43.75
F	20	2.10±0.10 ^{ab}	56.25

UI = Ulcer index, I = inhibition, mean ± SEM; n=5 for each treatment group; treatments compared to negative control^a and standard^b at $p < 0.05$, n.a = no activity (*n*-hexane fraction was not reported as no anti-ulcer activity was detected)

Interaction of rutin and chlorogenic acid with the aggressive factor target

The interactions show that rutin binds to the aggressive ulcerogenic protein via multiple hydrophobic and hydrogen bonds with ASN8A, ASP160A and SER161A (Figure 3 A and B). The chlorogenic acid interacted with the protein through one (1) hydrophobic interaction and eight hydrogen bonds with the GLY76A, THR77A and SER219A amino acids residue (Figure 3 C and D).

Interaction of rutin and chlorogenic acid with the defensive factor target

Six different hydrophobic interactions and thirteen hydrogen bonds of rutin with THR69A, THR76A, THR79A, SER95A and CYS170A residues were observed (Figure 4 A and B). For C and D (Figure 4), six (6) hydrophobic interactions were detected while the ligand interacted with the protein through eight (8) hydrogen bonds with THR, SER and CYS, showing multiple hydrogen bonds.

Interaction of rutin and chlorogenic acid with microbial factor target

The interaction of rutin with microbial ulcerogenic factor (Figure 5) showed one hydrophobic interaction and four hydrogen bonds with GLN140A. Similarly, chlorogenic acid interacted via four hydrophobic bonds with PHE145A and eight hydrogen bonds with SER143A and HIS144A.

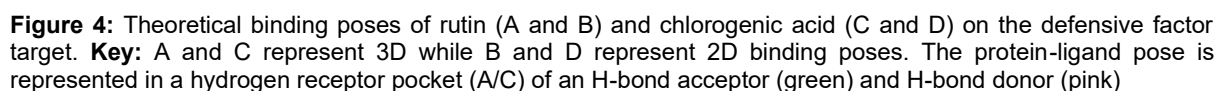
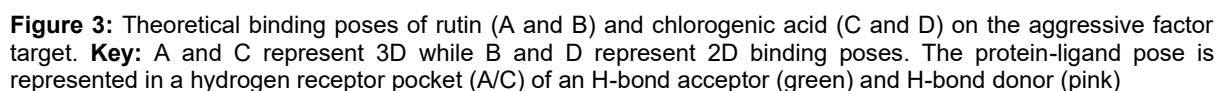
DISCUSSION

Extraction with aqueous methanol recorded 10.4 %w/w yield, which, on CC fractionation using silica gel, furnished three fractions: polar methanol (2.4 %), moderately polar ethyl acetate (2.1%), and non-polar *n*-hexane (1.24 %) fractions. Distribution of the secondary metabolites in the fractions also followed the polarity-dependent fractionation of the crude extract. The HPLC-DAD analysis showed the presence of five principal phenolic markers in both the extract and the most active fraction: quercetin, 4-hydroxybenzoic acid, chlorogenic acid, kaempferol, and rutin. The chemically coherent fingerprint for the bioactive fraction: flavonols (quercetin, kaempferol) dominated the mid-late elution; phenolic acids (chlorogenic, 4-hydroxybenzoic, gallic, caffeic) eluted early; while some terpenoids/volatiles (α -guaiane, aromadendrene, citronellol) and a sterol (β -sitosterol) appeared later. This aligns with recent phytochemical summaries reporting flavonoids and phenolic acids (including quercetin, kaempferol, gallic, caffeic, chlorogenic, rutin) as major constituents of *C. crepidioides* leaves [4].

Table 4: Binding parameters of ligands on ulcerogenic protein targets

Ligand	Aggressive factor		Defensive factor		Microbial factor	
	E (kcal/mol)	Ki (μ M)	E (kcal/mol)	Ki (μ M)	E (kcal/mol)	Ki (μ M)
Native	-3.19	4600	-9.48	0.113	-4.97	228.43
1	-7.44	3.54	-8.54	0.546	-6.41	19.94
2	-7.01	7.30	-8.35	0.757	-6.64	13.59
3	-10.09	0.040	-12.12	0.001	-8.70	0.420
4	-5.25	141.25	-5.52	90.000	-4.48	515.84
5	-8.49	0.597	-10.13	0.037	-7.23	4.99
6	-5.14	171.50	-5.53	88.760	-4.28	730.33

E = Binding energy, Ki= Inhibition constant



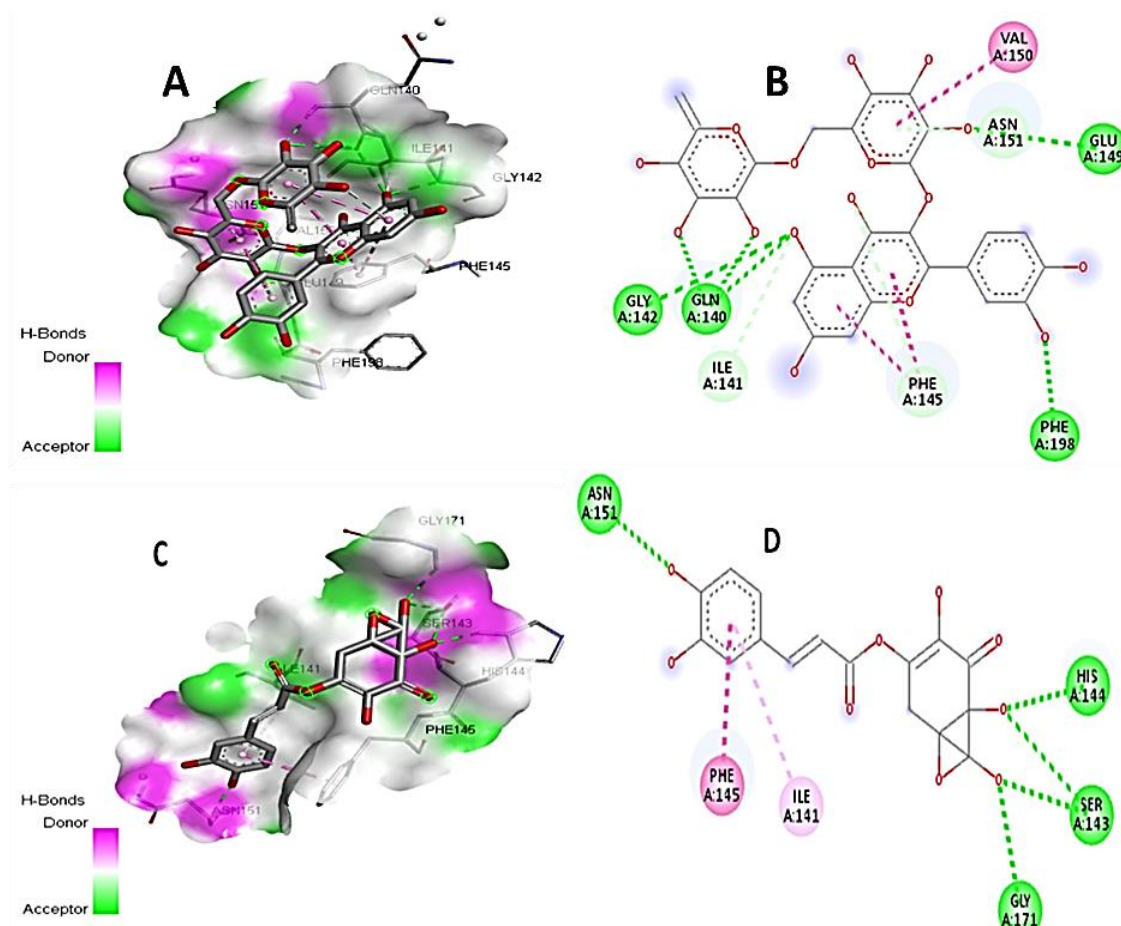


Figure 5: Theoretical binding poses of rutin (A and B) and chlorogenic acid (C and D) on the microbial factor target. **Key:** A and B represent 3D while B and D represent 2D binding poses. The protein-ligand pose is represented in a hydrogen receptor pocket (A/C) of an H-bond acceptor (green) and H-bond donor (pink)

The roles of secondary metabolites in the management of ulcers and the methods of extraction have been elucidated in this study. *Crassocephalum crepidioides* contains flavonoids, tannins, alkaloids, and terpenoids with antioxidant, anti-inflammatory, and antimicrobial properties that aid their cytoprotective effects. Ethanol, methanol and hexane extractions, alongside LC-MS profiling, have been used to isolate these bioactive compounds [5].

Acute oral toxicity (LD_{50}) determination has remained a vital tool in the safety evaluation of substances, and this study is very crucial in the screening of new drugs or compounds before clinical trials [14]. The acute toxicity study indicated that the crude extract showed no signs of any visible manifestations of delayed toxicity throughout the test period in all the phases. As a result, the lack of mortality at 5000 mg/kg demonstrated that the plant leaf extract is relatively non-toxic and its LD_{50} is likely to be higher than the highest tested dose.

The anti-ulcer activity of *C. crepidioides* was evaluated using ethanol- and indomethacin-induced ulcer models, both of which mimic distinct pathogenic mechanisms [3]. Ethanol causes direct gastric mucosal injury through oxidative stress, lipid peroxidation, and depletion of mucus, while indomethacin induces ulcers by inhibiting cyclooxygenase enzymes, thereby reducing prostaglandin synthesis and weakening mucosal defenses [15]. Across both models, the ulcer index (UI) values demonstrated a clear dose-dependent reduction with *C. crepidioides* extract and fractions. Negative control groups (UI, 4.8 – 5.0) consistently showed severe lesions, whereas treatment with the extract and fractions at 500 mg/kg reduced UI significantly, with the ethyl acetate fraction achieving 1.30 ± 0.37 in the indomethacin model. This surpassed the positive control (omeprazole, UI, 2.10 ± 0.10). The inhibition further showed the potency of the ethyl acetate fraction, which at 500 mg/kg achieved 72.92 % inhibition, greater than omeprazole (56.25 %). Crude extract and methanol fraction also showed notable inhibition

(> 43 %) at the highest tested dose, though slightly lower than the standard drug.

Mechanistically, the stronger activity observed in the indomethacin-induced model suggests that *C. crepidioides* enhances prostaglandin levels and bolsters protective factors such as bicarbonate secretion and mucosal blood flow [7]. In contrast, the moderate inhibition in the ethanol-induced model points to cytoprotective mechanisms, including antioxidant defence, free radical scavenging, and stimulation of mucus secretion. Thus, the secondary metabolites such as the flavonoids, tannins, and terpenoids likely act through dual pathways via the restoration of prostaglandin-mediated protection and reinforcement of cytoprotective antioxidant defences [8].

To further understand the *in vivo* anti-ulcer findings, the six most abundant compounds identified from the HPLC-DAD: flavonols (quercetin, kaempferol, rutin) and phenolic acids (gallic, chlorogenic, 4-hydroxybenzoic acids) were docked on defensive (human prostaglandin E receptor EP4), microbial (*H. pylori* urease) and aggressive (monoclinic porcine pepsin) ulcerogenic factor targets. Rutin and chlorogenic acid consistently showed higher binding efficiency on all the ulcerogenic factors simulated in this study with inhibitory constants of rutin (0.04, 0.001 and 0.42 μ M), chlorogenic acid (0.597, 0.037, 4.99 μ M) and binding energies of rutin (-10.09, -12.12, -8.70 kcal/mol), chlorogenic acid (-8.49, -10.13, -7.23 kcal/mol) on monoclinic porcine pepsin, human prostaglandin E receptor EP4 and *H. pylori* urease targets respectively. Compounds of *C. crepidioides* showed variable degrees of interaction with the three ulcerogenic factor targets. Consistent with previous studies, hydrogen bonding interactions shape ligand orientation, stability, and affinity, leading to differences in bioactivities [22]. The rutin (flavonol) and chlorogenic acid (phenolic acid) consistently showed the highest interaction (binding energies & inhibition constants) with the 3 selected targets. Pairwise comparison of **1-3** and **4-6** showed that flavonols recorded stronger interactions than phenolic acids. The interaction showed that the probable mechanism of anti-ulcer activities could be via interaction with the defensive ulcerogenic factors. This further supports the *in vivo* studies, which showed significant activity in the indomethacin-induced ulcer model (enhancing prostaglandin, a defensive or protective factor in ulcer), rather than inhibition of aggressive or microbial factors. The consistently low inhibitory constant of the microbial ulcerogenic factor target by all the compounds suggests less involvement of

inhibition of *H. pylori* as the probable mechanistic insight.

CONCLUSION

The ethyl acetate fraction of *Crassocephalum crepidioides* extract showed a high presence of flavonols and phenolic acids, which could be responsible for the anti-ulcer activity. Higher activity of the crude extract and fractions was observed in the indomethacin-induced model, suggesting prostaglandin level enhancement and increased protective factors as the major probable pathway for anti-ulcer property. Rutin elicited the highest interaction with human prostaglandin E receptor EP4, a defensive factor in the pathogenesis of ulcer, suggesting a protective mechanism of activity. The combination of *in vivo* experimental and *in silico* simulation models supports the anti-ulcer activity of *Crassocephalum crepidioides* extract.

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Ethical approval

Not applicable.

Use of Artificial intelligence/Large language models

The authors confirm that AI-based tools were used solely for language editing and manuscript polishing purposes (e.g., grammar, phrasing, and clarity). No AI tools were used to generate content, interpret data, or influence study design, results, or conclusions. All intellectual contributions, study analyses, and interpretations reported in the manuscript are the original work of the authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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