



Pimenta racemosa extract ameliorates chemically induced ulcerative colitis in rats by suppressing inflammation and oxidative stress

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Abstract

Pimenta racemosa (*P. racemosa*) tree leaves are used in traditional medicine to alleviate flatulence and gastrointestinal disorders. Ulcerative colitis (UC) is a gastrointestinal inflammatory disease with well-known inflammatory and immunoregulatory pathological features. The complexity of the disease requires multi-targeted treatment. Because of their relative safety and effectiveness, natural products can be utilized as adjunct and alternative agents to conventional UC treatment. So, the present work aimed to isolate phenolic constituents of the plant's 80% aqueous methanol extract (AME) and evaluate its protective effect on acetic acid-induced ulcerative colitis (UC) in rats. The defatted AME was chromatographed, and structures of the isolated compounds were elucidated using UV, NMR spectroscopy and HPLC–ESI–MS analysis. Induction of ulcerative colitis was done using Acetic acid, the protective effect against ulcerative colitis was supported by histopathological and immunohistochemical examinations. The isolated compounds are Kaempferol 3-*O*- β -D-glucopyranoside (kaempferol 3-*O*-glucoside) or (Astragalin) (**1**), quercetin 3-*O*- β -D-glucopyranoside (Isoquercetrin) (**2**), Quercetin (**3**), Catechin (**4**), quercetin 3-*O*- α -L-rhamnospyranoside (Quercetrin) (**5**). The AME of *P. racemosa* significantly alleviated the UC by decreasing the disease activity index and Wallace score. Additionally, GSH, CAT, and SOD were increased, and MDA was decreased, suggesting AME's antioxidant activity. The decreased levels of WBCs, neutrophils, TNF- α , IL-1 β , IL-6, MPO, and *NF- κ B* *p*65 support the AME's anti-inflammatory properties. The highest activity was recorded for the dose of 1000 mg/kg. These findings were in harmony with the histopathological examination and the Alcian Blue staining for mucin content. The current study revealed that the AME of *P. racemosa* has protective activities against UC in rats via its antioxidant and anti-inflammatory properties may be attributed to its phenolic profile.

Keywords *Pimenta racemose* · Phenolic constituents · HPLC-ESI-MS · Ulcerative colitis · Anti-inflammatory · Antioxidant

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Abbreviations

AME	Aqueous methanol extract
UC	Ulcerative colitis
AA	Acetic acid
DAI	Disease active index
CAT	Catalase
MDA	Malondialdehyde
SOD	Superoxide dismutase
ROS	Reactive oxygen species
GSH	Glutathione
MPO	Myeloperoxidase
TNF- α	Tumor necrosis factor alpha
IL-1 β	Interleukin-1 β
IL-6	Interleukin-6
NF- κ B p65	Nuclear factor kappa B p65

Introduction

The two prevalent idiopathic inflammatory bowel diseases that have an impact on people's quality of life are Crohn's disease (CD) and ulcerative colitis (UC) (Kawalec 2016). UC is characterized by a superficial inflammation of both the rectum and colon, while CD is manifested by ulceration, hemorrhage, and edema of the entire intestine (Carty and Rampton 2003).

Regarding the pathophysiology of UC, inflammation and oxidative stress have a well-known role (Alsharif et al. 2022). Neutrophil infiltration and overproduction of inflammatory mediators including tumor necrosis factor-alpha (TNF- α), Interleukin-1 β (IL-1 β), and Interleukin-6 (IL-6) are the main causes of the colonic mucosal ulceration (Guan and Zhang 2017). Chronic inflammation of the colon enhances the overproduction of reactive oxygen species (ROS) that is linked to increased colon cancer risk (Pereira et al. 2015). ROS induce a state of oxidative stress and lipid peroxidation associated with higher levels of malondialdehyde (MDA), which disrupts the oxidant/antioxidant balance. This is seen in UC patients' biopsies as they demonstrate a significant decline in reduced glutathione (GSH) which is a non-enzymatic antioxidant and catalase (CAT) which is an antioxidant enzyme (Rehman et al. 2022).

Acetic acid (AA) -induced UC in rodents is a reproducible UC model that pathologically, morphologically, and symptomatically resembles UC in humans (Fabia et al. 1992). The pathogenesis of AA has been attributed to a massive proton liberation, which causes intracellular acidification, resulting in immense mucosal damage (Randhawa et al. 2014).

The current pharmaceutical agents for treating UC include corticosteroids, thiopurines, aminosaliclates, and anti-TNF- α agents. Drug selection for UC depends on the

degree of symptoms. However, these agents cause many side effects (Farid et al. 2022). By understanding the pathophysiology of UC, researchers expect that agents with anti-inflammatory and antioxidant activities can provide promising treatments for UC. Herbal natural products with abundant flavonoid content have powerful anti-inflammatory and antioxidant effects and they currently are promising alternative conventional therapies for UC (Xue et al. 2023).

Natural products may be utilized as alternative and adjuvant medications to conventional therapies due to their relative safety and efficacy; Recent pharmaceutical industries depend greatly on the detection of plants' secondary metabolites helping in the development of new medicines with valuable biological activity (Soliman et al. 2023).

The order Myrtales consists of nine families of flowering plants. Myrtaceae is one of the well-known plant families belonging to the order Myrtales. It is regarded as the eighth-largest flowering plant family. It comprises about 150 genera and about 5800 species. Myrtaceae plants are distributed worldwide, mainly in South America, Africa, Australia, tropical Asia, and Europe (Guglielmelli et al. 2023).

Genus *Pimenta* contains about 15 species, indigenous to the Caribbean and Central America and widely spread in India, China, and the Middle East. The species of this genus are applied in the manufacturing of condiments, flavoring agents, perfumes, and cosmeceuticals. *Pimenta* species have long been used in traditional medicine to treat several conditions, including fever, rheumatic disorders, toothaches, abdominal pain, pneumonia, colds, diarrhea, and inflammatory disorders. These uses are supported by numerous studies highlighting the diverse pharmacological effects of different *Pimenta* species. Previous research has identified several biological activities, including anticancer, antifungal, anti-inflammatory, antimicrobial, pain-relieving, antioxidant, fever-reducing, blood sugar-lowering, blood pressure-lowering, and insect-repellent properties. These effects contribute to the medicinal value of *Pimenta*, making it a versatile plant in both traditional and modern therapeutic practices (Contreras 2018). It was reported that *Pimenta racemosa* (*P. racemosa*) exerted antiulcerogenic and hepatoprotective activity (Moharram et al. 2018). So, the current work aimed to isolate the polyphenolic compounds and assess the ability of the extract to protect rats from UC caused by AA. Additionally, the aim was extended to study the underlying protective mechanisms.

Methods

Standard material

The standard flavonoids including Luteolin, Apigenin, Quercetin, and Kaempferol were supplied by the Pharmacognosy Department at the Faculty of Pharmacy, Helwan University, Cairo, Egypt. Acetic acid (AA) was bought from CID Pharmaceutical Company (Giza, Egypt). Sulfasalazine was obtained via Mina Pharm Company (Giza, Egypt). Silica gel G60 for column and thin chromatography (70–230 mesh, Merk) and (Merk, Germany) respectively, Silica gel GF254 for pre-coated TLC plates (Merk, Germany), Sephadex LH-20 (Sigma-Aldrich-Steinheim, Germany), Whatman filter paper (1 mm) for paper Chromatography (Whatman Ltd, England), microcrystalline cellulose (Merk-Darmstadt, Germany) and polyamide 6S (Riedel-De-Haen AG, Seelze Haen AG, Seelze Hanver, Germany). Paper chromatography was achieved using solvent systems: (S₁) BAW (n-BuOH – HOAc – H₂O 4: 1: 5, upper layer), (S₂) 15% HOAc (H₂O – HO Ac 85:15). Glucose, rhamnose, xylose, galactose and gentiobiose as standard sugars were brought from Pharmacognosy Department at Faculty of Pharmacy, Helwan University, Cairo, Egypt.

Plant material

The aerial parts of *P. racemosa* were gathered in July 2022, from Giza Zoo, Egypt. Voucher specimen N0. (16Pra3/2022) was stored in the herbarium in the Pharmacognosy Department, at the Faculty of Pharmacy, Helwan University, Egypt. Dr. Trease Labib, a former specialist in plant taxonomy at El Orman Botanical Garden, Egypt was responsible for plant identification.

Animals

After a week of acclimatization to the standard laboratory conditions, which included a 12-h light–dark cycle, a temperature of 23 °C±2 °C, a humidity of 55%, and unrestricted access to a pellet diet and water, healthy adult female Sprague Dawley rats (150–180 g) and albino mice (20–25 g) of both sexes were used in the current study. The Egyptian Organization of Biological Products and Vaccines' breeding unit (Helwan, Egypt) provided the required animals for purchase. Animals were handled according to the guidelines for animal care permitted by the animal care and use committee of the Faculty of Pharmacy, Helwan University (Approval No:12 A2023).

Apparatus and equipment

Rotary evaporator (Buchi, A.G. Switzerland), glass column for chromatography. Ultraviolet lamp for visualization of spots. Nuclear magnetic resonance spectrometer, Bruker 400, MHz for ¹H NMR and 100.40 MHz for ¹³C NMR. The NMR experiments were performed in DMSO, and chemical shifts were given in ppm with tetramethylsilane (TMS) as an internal standard. LC/MS analysis was performed using an LC–MS/MS system (Nexera with LCMS-8045, Shimadzu Corporation, Kyoto, Japan)—HPLC (Nexera LC-30AD) equipped with an autosampler (SIL-30AC), temperature-controlled column oven (CTO-20AC) and photodiode array detector (LC-2030/2040) with detection wavelengths of 235, 254 and 280 nm with λ max absorption at 220–400 nm and coupled to triple quadrupole mass spectrometer (Nexera with LCMS-8045, Shimadzu Corporation, Kyoto, Japan).

HPLC/MS

LC-PDA-MS was provided with RP-C18 UPLC column (shim-pack 2 mm×150 mm) owning 2.7 μm particle size using the following grade elution (Acetonitrile (ACN), 0.1% HCOOH in H₂O) 0–2 min (10% ACN); 2–26 min (10% ACN–80% ACN) and 26–33 (100% ACN) with a flow rate of 0.2 mL/min. Positive and negative modes were controlled during LC–MS/MS using electrospray ionization (ESI). LC–MS/MS results were obtained and processed using Lab Solutions software (Shimadzu, Kyoto, Japan). The AME of *P. racemosa* aerial parts were analyzed by LC–MS/MS system (Nexera with LCMS-8045, Shimadzu Corporation, Japan) scanned in positive and negative modes.

Extraction and isolation of polyphenolic compounds

About 1700 g of the air-dried aerial parts of *P. racemosa*, were crushed to a coarse powder and stored at room temperature then extracted with hot aqueous MeOH (80%) under reflux (5×6 L, 60 °C). The total methanolic extracts were concentrated and dried under vacuum at 45 °C to give a dry total extract weighing 400 g. The dried residues were defatted by shaking with Petroleum Ether (6×500 ml). Pet. The ether extract was evaporated under reduced pressure to obtain Pet. Ether fraction. The defatted AME was subjected to desalting by dissolving the residue in the least amount of H₂O and precipitating with excess EtOH (1:10), yielding the EtOH insoluble portion which includes Mainly sugars and traces of phenolic compounds and EtOH soluble portion which was Rich in phenolic compounds. EtOH soluble portion was investigated for phenolic compounds using two dimension-paper chromatography (2D-PC) and solvent

systems S_1 and S_2 , followed by spraying with Naturstoff and FeCl_3 spray reagents.

EthOH soluble portion was concentrated and dried under reduced pressure, 80 gm of the residue was dissolved in the least amount of H_2O then subjected to fractionation on a polyamide column (70×4 cm) eluted with a step gradient by decreasing polarity to 100% MeOH to yield 240 individual fractions, combined into 10 collective fractions based on their resemblance on PC and TLC. The promising fractions were VI (3.2 g, eluted at $\text{H}_2\text{O}/\text{MeOH}$ 80:20%) and VII (1.8 g, eluted at $\text{H}_2\text{O}/\text{MeOH}$ 70:30%). Fraction VI (wt=3.2 g) was re-chromatographed on a silica gel column using ethyl acetate to give 7 individual subfractions (eluted at 100% ethyl acetate); the most promising subfractions were 3 main subfractions (VI a, VI b, VI c). Those three subfractions were purified individually on Sephadex LH-20 with 100% MeOH giving Compound 1, Compound 2, and Compound 3 respectively. Fraction VII (wt=1.8 g) was re-chromatographed on a silica gel column using $\text{CH}_2\text{Cl}_2/\text{MeOH}$ mixtures, giving 5 individual subfractions (eluted at $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 95:5%), the most promising subfractions were 2 main subfractions (VII a—VII d), Fractions VII a and VII b were purified each individually on Sephadex LH-20 C with MeOH (100%), giving Compound 4 and Compound 5 respectively.

Isolated compounds

Compound 1 was obtained as an amorphous powder with a yellow color (18 mg), 2 was obtained as an amorphous powder with a yellow color (15 mg), 3 was purified as an amorphous powder with a yellow color (20 mg), 4 (15 mg) a yellow-colored amorphous powder was obtained and finally, 5 was isolated (25 mg).

In vivo study

Acute toxicity study

In accordance with Moharram et al. (2018) and OECD guideline 425, the acute oral toxicity of the AME of *P. racemosa* aerial parts was assessed in mice. For 14 days, mice were given graded doses up to 5 g/kg, P.O., and their behavior and mortality were monitored. Standard inter-species scaling principles were followed when extrapolating from mice to rats according to OECD guideline 425.

UC induction in rats

UC was induced by intrarectal (IR) administration of AA according to a previously published study by (Dileep et al. 2016) with minor modifications. Rats were fasted for 24 h

with free access to water before induction, then lightly anesthetized with low dose thiopental sodium (30 mg/kg, I.P.) prior to acetic acid administration. A single dose of 4% (v/v) acetic acid solution (prepared in normal saline) was administered intrarectally at a volume of 2 mL per rat using a soft 2 mm pediatric catheter lubricated with Vaseline. The catheter was gently inserted approximately 8 cm into the rectum, and the solution was slowly instilled. After administration, rats were maintained in a head-down position for about 60 s to prevent leakage. Control animals received an equivalent volume (2 mL) of normal saline. This method reliably produces reproducible colonic ulceration and inflammation consistent with ulcerative colitis pathology, as previously reported (Dileep et al. 2016).

Experimental design

Thirty-six rats were randomly allocated into 6 groups ($n=6$):

Group I (Control group): rats received normal saline (2 ml/kg/PO/14 days)+2 ml normal saline single dose, IR at day 15.

Group II (UC group): rats received normal saline (2 ml/kg/PO/14 days)+a single dose of 2 ml of AA IR to induce UC model at day 15.

Group III (Standard group): rats received sulfasalazine (500 mg/kg/PO) for 14 days (Owusu et al. 2020)+AA IR at day 15.

Group IV (AME 250): rats received the AME at a dose of 250 mg/kg/PO, for 14 days+AA IR at day 15.

Group V (AME 500): rats received the AME at a dose of 500 mg/kg/PO, for 14 days+AA IR at day 15.

Group VI (AME 1000): rats received the AME at a dose of 1000 mg/kg/PO, for 14 days+AA IR at day 15.

After the last oral dose of sulfasalazine or the AME as prophylactic treatments, rats were fasted for 24 h before IR administration of either AA or saline at day 15. At day 17 (48 h following the IR instillation), the disease activity index (DAI) was evaluated. After that, the rats were sacrificed under an overdose of thiopental sodium (50 mg/kg) (Ince et al. 2021). The distal colonic sections were separated, opened, cleaned with saline, and examined under a microscope. For histological and immunohistochemical analyses, a portion of the colon was preserved in 10% formalin; for biochemical analysis, the remaining portion was homogenized in phosphate-buffered saline, centrifuged at 10,000 rpm/4 °C/15 min, and then stored at -80 °C. The experimental design is illustrated in Fig. 1.

Estimation of disease activity index (DAI)

DAI is a qualitative index to evaluate UC though using a scoring system to evaluate weight loss percentage, fecal

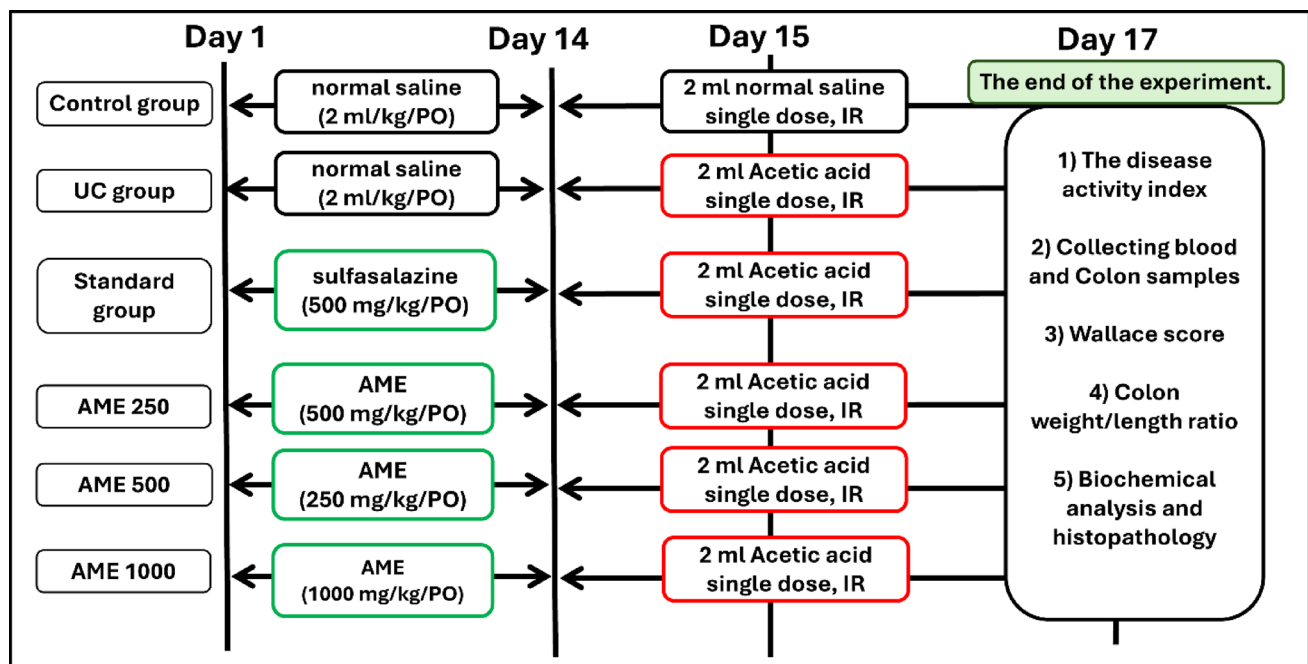


Fig. 1 Experimental design of the study. AME: aqueous methanolic extract, UC: ulcerative colitis; PO: per oral; IR: intrarectally

Table 1 Macroscopic evaluation scale

Score	Criterion
0	No ulceration or inflammation
1	No ulceration with hyperaemia
2	Ulcers without hyperaemia
3	Ulcers and inflammation at one site
4	Two or more sites of ulcer and inflammation
5	Ulcers more than 2 cm

character, and rectal bleeding as follows (Table S1): the percentage weight loss (0=none, 1=1–5%, 2=6–10%, 3=11–20%, 4=>20%), fecal character (0=normal, 1=soft but formed stool, 2=actual soft stool, 3=mild diarrhea, 4=severe diarrhea), and rectal hemorrhage (0=normal; 1=positive hemocult, 2=blood drops in stool visible, 3=mild bleeding, 4=severe hemorrhage). The DAI was estimated using the average values of these items (El-Akabawy and El-Sherif 2019) as follows:

$$\text{DAI calculation} = \frac{(\text{weight loss score} + \text{stool consistency score} + \text{bleeding score})}{3}$$

Haematological parameters

Before scarification, blood samples were obtained from the retro-orbital plexus of the rats into sterile vacutainer tubes coated with EDTA as an anticoagulant. A complete blood count was conducted using the Sysmex KX-21N™ Haematology Analyzer (Sysmex Europe GmbH Co., Germany).

Macroscopic examinations

The macroscopic damage of the colon was assessed using Wallace scores (Table 1) according to the previous study by El-Akabawy and El-Sherif 2019 (El-Akabawy and El-Sherif 2019).

Calculation of colon weight-to-length ratio

To evaluate the degree of edematous colon tissues and the severity of UC in rats, the weight-to-length ratio (mg/cm) was calculated for each rat (Aly et al. 2016).

Estimation of oxidative stress parameters in the colonic tissue homogenate

MDA level in the colonic tissue is an index for lipid peroxidation. MDA was measured using a colorimetric kit (BioVision, Catalog no: K739-100, Milpitas, USA). The non-enzymatic antioxidant, GSH, was quantified calorimetrically using a BioVision kit (Catalog no: K464-100, Milpitas, USA). The level of the antioxidant enzyme SOD was measured by using an ELISA kit (Catalog no: E4584-100, BioVision, Milpitas, USA), while the other antioxidant enzyme, CAT was determined colorimetrically (Catalog no: K773-100, BioVision, Milpitas, USA).

Estimation of TNF- α and IL-1 β in colonic homogenate

Enzyme-linked immunosorbent assay kits were used to quantify the TNF- α (Catalog no: 438205, Bioligand, San Diego, USA), and IL-1 β (Catalog no: E0119 Ra, Bioassay Technology Laboratory, Shanghai, China) in the colonic tissue homogenate according to manufacture instructions.

Estimation of nuclear factor-kappa B p65 (NF- κ B p65) in colonic homogenate

NF- κ B p65 as a transcriptional factor was estimated in the colonic tissue homogenate using an ELISA kit (Catalog no: ABIN2748433, antibodies-online, Pennsylvania, USA).

Estimation of myeloperoxidase (MPO) activity in colonic homogenate

MPO, a marker for neutrophil infiltration, was estimated in the colon tissue homogenate by a BioVision ELISA assay kit (Catalog no: E4581-100, Milpitas, USA).

Determination of colonic tissue protein content

Colonic tissue's protein content was measured, agreeing with the method of Lowry et al. 1951 (Lowry et al. 1951).

Histopathological examination

Samples of colon tissue were cleaned and preserved for 72 h in 10% neutral buffered formalin. After being cut and processed in successive ethanol grades, samples were cleared in xylene before being immersed in the Paraplast tissue embedding medium. Then, 5 μ m thick tissue sections were cut by rotatory microtome to demonstrate the intestinal wall. Sections of tissues were stained and analyzed using the following methods:

1. Hematoxylin and Eosin as the main morphological staining methods.
2. Alcian Blue pH 2.5 to demonstrate goblet cells' acidic mucins.

All standard techniques for sample fixation and staining were done according to Culling et al. 2013 [Culling et al. 2013].

Immunohistochemical staining Deparaffinized 5 μ m thick sections were cut and prepared to be treated with 3% hydrogen peroxide for 20 min, washed with PBS, then incubated with anti-IL6 Antibody (Catalog no: NBP2-16,957, 1:200 – Novus Biologicals, Colorado, USA) overnight at 4 °C,

washed with PBS then incubated with HRP secondary antibody Envision kit (DAKO) 20 min. The sections were washed with PBS, incubated with diaminobenzidine for 10 min, washed with PBS then counterstained with hematoxylin, dehydrated and cleared in xylene, and cover-slipped for microscopic examinations.

Microscopic analysis

Following the method adopted by El Gazzar et al 2023 and Khedr et al 2023 (El Gazzar et al. 2023 and Khedr et al. 2023), at least 6 non-overlapping fields were randomly chosen and scanned to determine the relative mucosal area percentage of reactive goblet cells mucin content in alcian blue stained tissue sections and the mucosal area percentage of relatively mean immunohistochemical expression levels of IL-6 in immune-stained tissue sections. The scan was done using a Full HD microscopic camera worked by a Leica application module for tissue section analysis (Leica Microsystems GmbH, Wetzlar, Germany).

Statistical analysis

Our results were expressed as mean (M) $\pm$ standard error (SE). ANOVA test followed by Tukey's test was used to estimate the significance between groups, and the p -value < 0.05 was considered significant. Version 8 of GraphPad Prism (GraphPad Software Inc., California, USA) was used.

Results

LC-MS/MS analysis results

The AME of *P. racemosa* aerial parts were analyzed, resulting in the identification of 24 metabolites tentatively identified, and results compiled in Table 2. The identification was done by matching their retention time, and MS data to the reported review of the literature. Derivatives of chromones, flavonols, flavones, flavanones, bioflavonoids, and phenolic acids were detected. Chromone derivatives: peak 3, 20 were identified as brevifolin carboxylate, 2-phenoxychromone, 6, 8-di-C-methylcapillarisin as they exhibited the molecular ion peaks [M-H]⁻ 291.10 and 313.12 (m/z), respectively. Bioflavonoids: Peak 24 was identified as Morello flavone as it exhibited the molecular ion peak [M-H]⁻ 555.40 (m/z). Flavonols: Peaks 8, 9, 12, 15 and 17 were identified as quercetin-*O*-galloyl-glucoside, Quercetin 3-*O*- β -D-glucopyranoside, quercetrin, hyperoside, and quercetin as they exhibited the molecular ion peaks [M-H]⁻, 615, 463, 447, 463 and 301(m/z) respectively. Peak 16 was identified

Table 2 Tentative identification of secondary metabolites in AME of *P. racemosa* aerial parts

Peak No	Retention time (min)	Compound name	[M-H] ⁺	Reference
1	0.76	Quinic acid	191.15	(Sebai et al. 2019)
2	1.86	Procyanidin dimer	577.15	(Bystrom et al. 2008; Gujer et al. 1986)
3	2.14	Brevifolin carboxylate	291.10	(Zhu et al. 2015)
4	2.14	Catechin	289.10	(de Souza et al. 2008)
5	3.66	3,5-dimethoxy-4 hydroxy cinnamaldehyde (Sinapaldehyde)	207.15	(Ghareeb et al. 2018)
6	5.36	Hydroxydihydro caffeoylquinic acid	371	(Raslan et al. 2021)
7	5.79	Apigenin 7- <i>O</i> -rutinoside	577.20	(Mitreski et al. 2014)
8	6.49	Quercetin- <i>O</i> -galloyl-glucoside	615.20	(Sobeh et al. 2019)
9	6.64	Quercetin 3- <i>O</i> - β -D-glucopyranoside	463.15	
10	6.99	Ellagic acid pentoside	433.10	(Zhu et al. 2015)
11	7.18	Prunin (Naringenin-7- <i>O</i> -glucoside)	433.15	(Bystrom et al. 2008)
12	7.29	Quercetrin (quercetin 3- <i>O</i> -rhamnoside)	447.15	(Lee et al. 2005)
13	7.47	Salvianolic acid A	493.20	(Marzouk et al. 2007)
14	7.69	1,5-di- <i>O</i> -caffeoylquinic acid	517.15	(Merfort 1992)
15	7.89	Hyperoside (quercetin-3- <i>O</i> -galactoside)	463.20	(Ben Hmed et al. 2020)
16	8.25	Kaempferol 3- <i>O</i> - β -D-glucoside	447.20	(Sebai et al. 2019)
17	8.58	Quercetin	301.10	(de Souza et al. 2008)
18	9.07	Epicatechin gallate	441.20	(Bastos et al. 2007)
19	10.40	Maloyl-hexose	295.20	(Fraternal et al. 2015)
20	10.88	2-phenoxychromone, 6,8-di-C-methylcapillarin	313.15	(Doyle et al. 2018)
21	12.55	Coumaric acid dihexoside	487.45	(Marzouk et al. 2019)
22	14.26	5,7,3',4'-tetrahydroxyflavone-5- <i>O</i> - β -glucosyl-4,8-eriodictyol	721.45	(Gujer et al. 1986)
23	15.98	Maslinic acid (2 α , 3 β -dihydroxyolean-12-en-28-oic acid)	471.40	(Fukumitsu et al. 2016)
24	18.31	Morelloflavone (bioflavonoids)	555.40	(Carrillo-Hormaza et al. 2016)

as Astragalin as it exhibited the molecular ion peak [M-H]⁺ (m/z), 447. Flavone derivatives: Peak 7 was identified as Apigenin 7-*O*-rutinoside as it exhibited the molecular ion peak [M-H]⁺ (m/z) 577. Flavanone: Peak 11 was identified as Prunin (Naringenin-7-*O*-glucoside) as it exhibited the molecular ion peak [M-H]⁺ (m/z) 433. Phenolic acids and natural phenols: Peaks 2, 4, and 18 were identified as Procyanidin dimer, Catechin, and epicatechin gallate as they exhibited the molecular ion peaks [M-H]⁺ (m/z) 577, 289, 441, respectively. Peaks 6, 10, 13, 14, 10, and 21 were identified as hydroxydihydro caffeoylquinic acid, ellagic acid pentoside, salvianolic acid A, 1,5-di-*O*-caffeoylquinic acid, maloyl-hexose, and coumaric acid dihexoside as they exhibited the molecular ion peaks [M-H]⁺ (m/z), 371, 433, 493.20, 517, 295, 487, respectively.

Isolation and structure elucidation of phytoconstituents

The chromatographic purification of AME led to the separation of five famous compounds kaempferol 3-*O*- β -D-glucopyranoside (Astragalin) (1), quercetin 3-*O*- β -D-glucopyranoside (Isoquercetrin) (2), Quercetin (3), Catechin (4), quercetin 3-*O*- α -L-rhamnospyranoside (Quercetrin) (5) Fig. 2. The chemical structure of the isolated compounds was established using spectroscopic techniques and comparing obtained data with earlier published ones (Agrawal and Banasal 1989; Liu et al. 2019; Moharram et al. 2018; Marzouk et al. 2007) as well as comparison against authentic samples. Spectra and NMR data are available in detail [Supplementary].

In vivo study

Acute toxicity study

The mice did not exhibit any behavioral changes or mortality at the highest tested dose of AME of *P. racemosa* aerial parts (5 g/kg). Since there was no evidence of toxicity or death, the extract's safety was confirmed by an LD₅₀ > 5000 mg/kg. Accordingly, the highest experimental dose for the study was determined to be one-fifth of the maximum tolerated dose (1000 mg/kg), with intermediate and low doses of 500 mg/kg and 250 mg/kg, respectively, to enable assessment of dose-dependent effects in vivo experiments in rats.

Effect of the AME of *P. racemosa* on the DAI, colon weight-to-length ratio, colon macroscopic score of AA-induced UC rats and Photographic representation of colon

Results are represented by Fig. 3. As presented in Fig. 3A, the DAI score of UC rats was significantly ($p < 0.0001$)

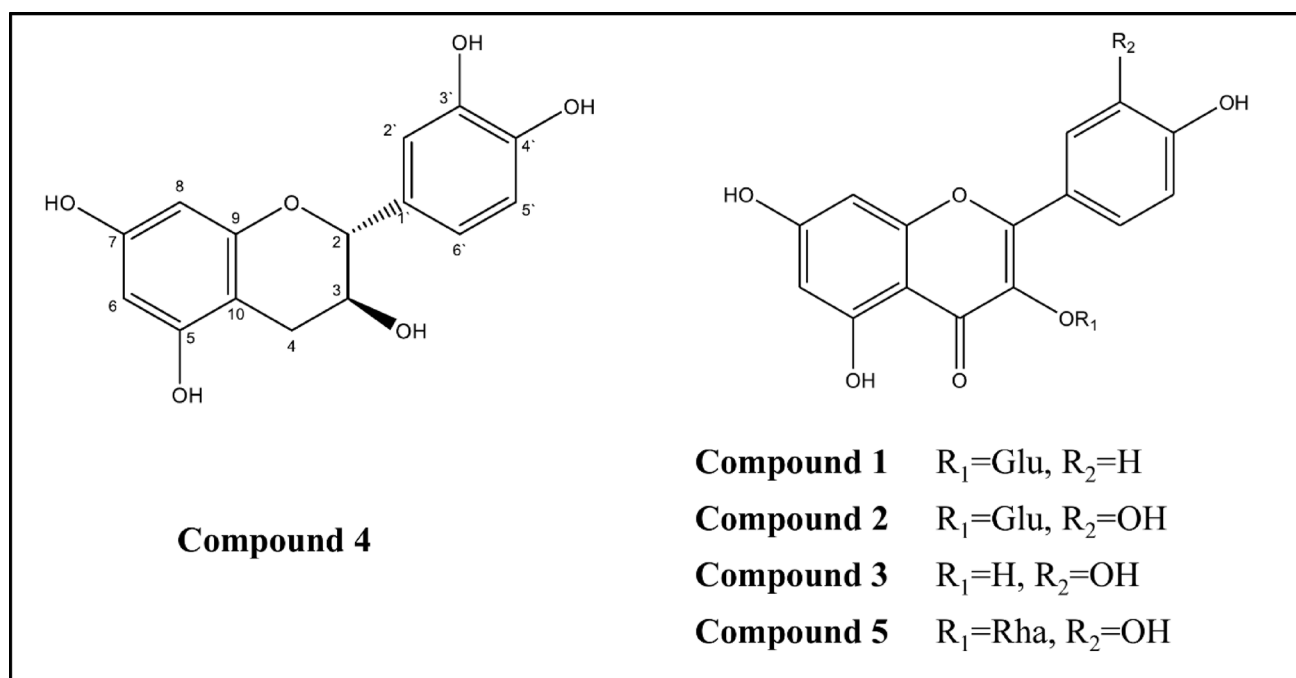


Fig. 2 Structure of isolated compounds from AME of *P. racemosa* aerial parts

increased by 30 folds (5.5 ± 0.4282) compared to the control rats (0.1667 ± 0.0667). The administration of sulfasalazine showed a marked ($p < 0.0001$) decline in DAI score by 60% (0.1667 ± 0.3333), compared to the UC group. In a dose-dependent manner, *P. racemosa* AME in doses of 250, 500, and 1000 mg/kg markedly ($p < 0.0001$) alleviated the severity of the disease and reduced the DAI score by 46.7% (2.333 ± 0.6146), 70% (1.500 ± 0.4282), and 73.3% (1.500 ± 0.4282), respectively, compared to UC group. Interestingly, all doses of the AME showed a non-significant difference compared to the standard sulfasalazine-treated rats. Figure 3B Compared to the control rats (12.20 ± 0.5574), the colon weight/length ratio was markedly ($p < 0.0001$) increased by 1.9 folds in the UC rats (23.13 ± 1.975). Meanwhile, treatment with sulfasalazine and the AME of *P. racemosa* at a dose of 250, 500, and 1000 mg/kg markedly ($p < 0.0001$) declined the mean colon weight/length ratio by 43.8% (13.00 ± 0.7950), 37.1% (14.55 ± 0.6190), 38% (14.35 ± 0.8759), and 43.3% (13.12 ± 0.5394), respectively, compared to the UC rats. Moreover, the AME in the three doses did not reveal any significant change compared to the standard sulfasalazine-treated rats. As demonstrated in Fig. 3C, D the samples from the normal control rats did not show any sign of UC. Administration of AA into the colon evoked edematous inflammatory response and mucosal ulceration with a significantly ($p < 0.0001$) higher macroscopic scoring (4.333 ± 0.2108), compared to the control group. In contrast to the UC rats, sulfasalazine and AME of *P. racemosa* (250, 500, 1000 mg/kg) groups

revealed mild inflammation with less ulceration evidenced by a marked ($p < 0.0001$) inhibition in macroscopic scoring by 73.1% (1.167 ± 0.1667), 65.4% (1.500 ± 0.2236), 69.2% (1.333 ± 0.2108), and 73.1% (1.167 ± 0.3073), respectively, compared to the UC rats.

Effect of the AME of *P. racemosa* on haematological parameters of AA-induced UC rats

As demonstrated in Table 3, all the blood parameters of the control rats fell within the normal range of blood values for healthy rats. Regarding the Hb content, RBCs, and platelets, there was no significant difference among all groups. However, the UC rats showed a marked ($p < 0.0001$) increment in the total WBCs and neutrophil count by 1.6 folds and 2.2 folds (14.75 ± 0.5948 ; 7.850 ± 0.2895), respectively, compared to the control rats (9.067 ± 0.6009 ; 3.500 ± 0.4099). Sulfasalazine and the three doses of the AME of *P. racemosa* markedly ($p < 0.0001$) decreased the level of WBCs by 41.4% (8.650 ± 0.6815), 24.5% (11.13 ± 0.4529), 29.4% (10.42 ± 0.4757), and 34.5% (9.667 ± 0.5840), respectively; and the level of neutrophils by 39.1% (4.783 ± 0.3911), 32.9% (5.267 ± 0.3748), 38.9% (4.800 ± 0.4282), and 39.3% (4.767 ± 0.4780), respectively, compared to the UC rats. Moreover, the AME at doses 500 and 1000 mg/kg did not show a marked change in WBCs and neutrophils levels compared to the sulfasalazine standard group.

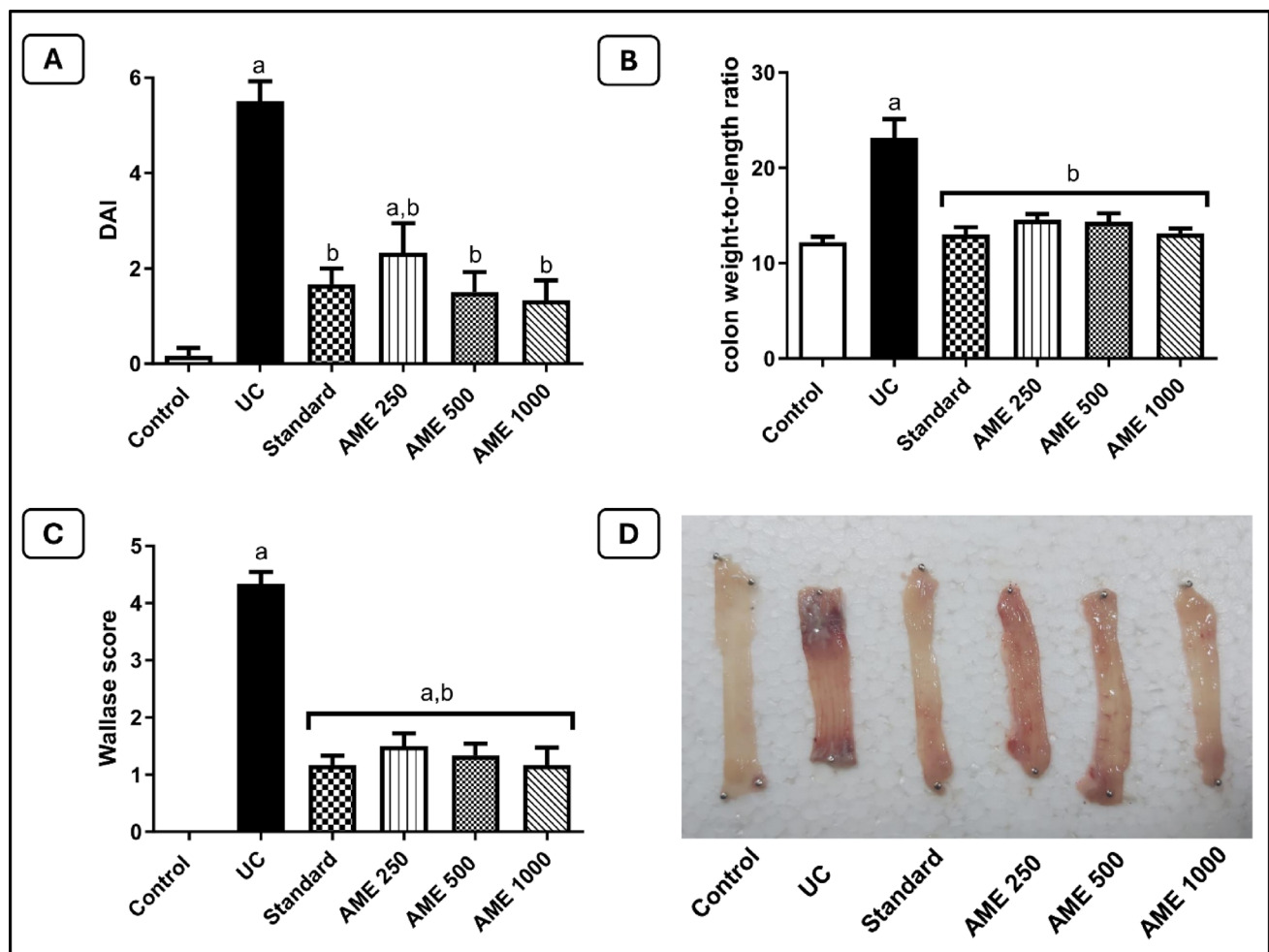


Fig. 3 Effect of the AME of *P. racemosa* on **A:** DAI, **B** Colon weight-to-length ratio, **C:** Colon macroscopic score (Wallase score), **D:** Photographic representation of colon of AA-induced UC rats. Data are

shown as mean±SE. *n*=6. a: significant from the control group at *p*<0.05, b: significant from the UC group at *p*<0.05, c: significant from the standard group at *p*<0.05

Table 3 Effect of the AME of *P. racemosa* on haematological parameters of AA-induced UC rats

	WBCs (× 10 ³ /μL)	NEUT (× 10 ³ /μL)	HB (g/ dL)	RBCs (× 10 ⁶ / μL)	Platelets (× 10 ³ /μL)
Control	9.1±0.6	3.5±0.4	12.8±0.4	6.2±0.2	658±9.1
UC	14.8±0.6 ^a	7.9±0.3 ^a	11.4±0.3	5.5±0.1	630.1±11.0
Standard	8.7±0.7 ^b	4.8±0.4 ^b	12.0±0.2	5.8±0.2	632.2±14.6
AME 250	11.1±0.5 ^{b,c}	5.3±0.4 ^{a,b}	11.1±0.3	5.7±0.2	634.5±5.9
AME 500	10.4±0.5 ^b	4.8±0.4 ^b	11.7±0.3	5.5±0.3	634.5±16.8
AME 1000	9.7±0.6 ^b	4.7±0.5 ^b	12.0±0.5	5.9±0.3	639.5±13.2

Data are shown as mean±SE. *n*=6. ^a significantly different from the control group at *p*<0.05, ^b: significant from the UC group at *p*<0.05, ^c: significant from the standard group at *p*<0.05

Effect of the AME of *P. racemosa* on oxidative stress parameters of AA-induced UC rats

IR injection of AA caused tissue oxidative damage, indicated by the marked (*p*<0.0001) increment in MDA level by 3.7 folds (1.536±0.1270), and decline in GSH, SOD, and CAT levels by 73.8% (1.019±0.09442), 74.2% (0.5532±0.04049), and 75.4% (0.5822±0.05283), respectively, compared to the control rats (0.4143±0.04416, 2.112±0.1028, 3.945±0.1764, 2.368±0.1052; respectively). In comparison with the UC group, treatment with sulfasalazine and AME *P. racemosa* (500, and 1000 mg/kg) markedly (*p*<0.0001) decreased the MDA level by 60.9% (0.5995±0.06245), 46.7% (0.8185±0.04207), and 63.2% (0.5648±0.04476), respectively. AME *P. racemosa* at a dose of 250 mg/kg showed a nonsignificant decrease in MDA level compared to the UC group. Meanwhile, these treatments caused a marked (*p*<0.0001) increase in GSH level by 3 folds (1.682±0.06852), 1.5 folds (0.8228±0.04064),

2.2 folds (1.208 ± 0.07812), and 3.7 folds (2.051 ± 0.04849), respectively; a marked ($p < 0.0001$) increase in SOD level by 2.7 folds (2.796 ± 0.2334), 1.5 folds (1.536 ± 0.06192), 2.1 folds (2.126 ± 0.1016), and 3.2 folds (3.307 ± 0.2788), respectively; and marked ($p < 0.0001$) increase in CAT level by 3 folds (1.771 ± 0.05427), 1.6 folds (0.9530 ± 0.04868), 2.8 folds (1.614 ± 0.06684), and 3.6 folds (2.093 ± 0.06634), respectively, compared to the UC group. These findings support the antioxidant properties of the AME of *P. racemosa*, and the highest effect was recorded for the dose of 1000 mg/kg AME as it showed a nonsignificant change in these measurements compared to the control rats Fig. 4.

Effect of the AME of *P. racemosa* on inflammatory parameters and on the level of MPO of AA-induced UC rats

AA-treated rats showed a marked tissue inflammation evidenced by the marked ($p < 0.0001$) increment in the proinflammatory cytokines TNF- α and IL-1 β levels and

p-NF- κ B p65 levels by 3.6 folds (104.8 ± 6.342), 4.2 folds (4.854 ± 0.3218), and 3.7 folds (3.725 ± 0.1564), respectively compared to the control rats (29.22 ± 2.330 , 1.169 ± 0.07355 , 1.001 ± 0.06411 ; respectively) (Fig. 5. A, B, C). Meanwhile, rats treated with the standard and the AME *P. racemosa* at doses 250, 500, and 1000 mg/kg caused a marked ($p < 0.0001$) decrease in TNF- α levels by 47.6% (54.93 ± 3.576), 19.9% (83.91 ± 3.257), 42.5% (60.22 ± 3.108), and 58.5% (43.53 ± 3.551), respectively; and IL-1 β level by 54.1% (2.226 ± 0.1089), 38.3% (2.997 ± 0.1344), 52.2% (2.320 ± 0.1267), and 59.6% (1.962 ± 0.05079) respectively; and p-NF- κ B p65 level by 55.5% (1.658 ± 0.07257), 21.3% (2.933 ± 0.1010), 45.6% (2.025 ± 0.08662), and 59.1% (1.523 ± 0.09598), respectively, compared to the UC rats. Additionally, the AME at doses 500 and 1000 mg/kg showed a nonsignificant change in these parameters' levels compared to the sulfasalazine standard. Because oxidative stress and inflammatory cell infiltration are linked, the level of MPO as an indicator for

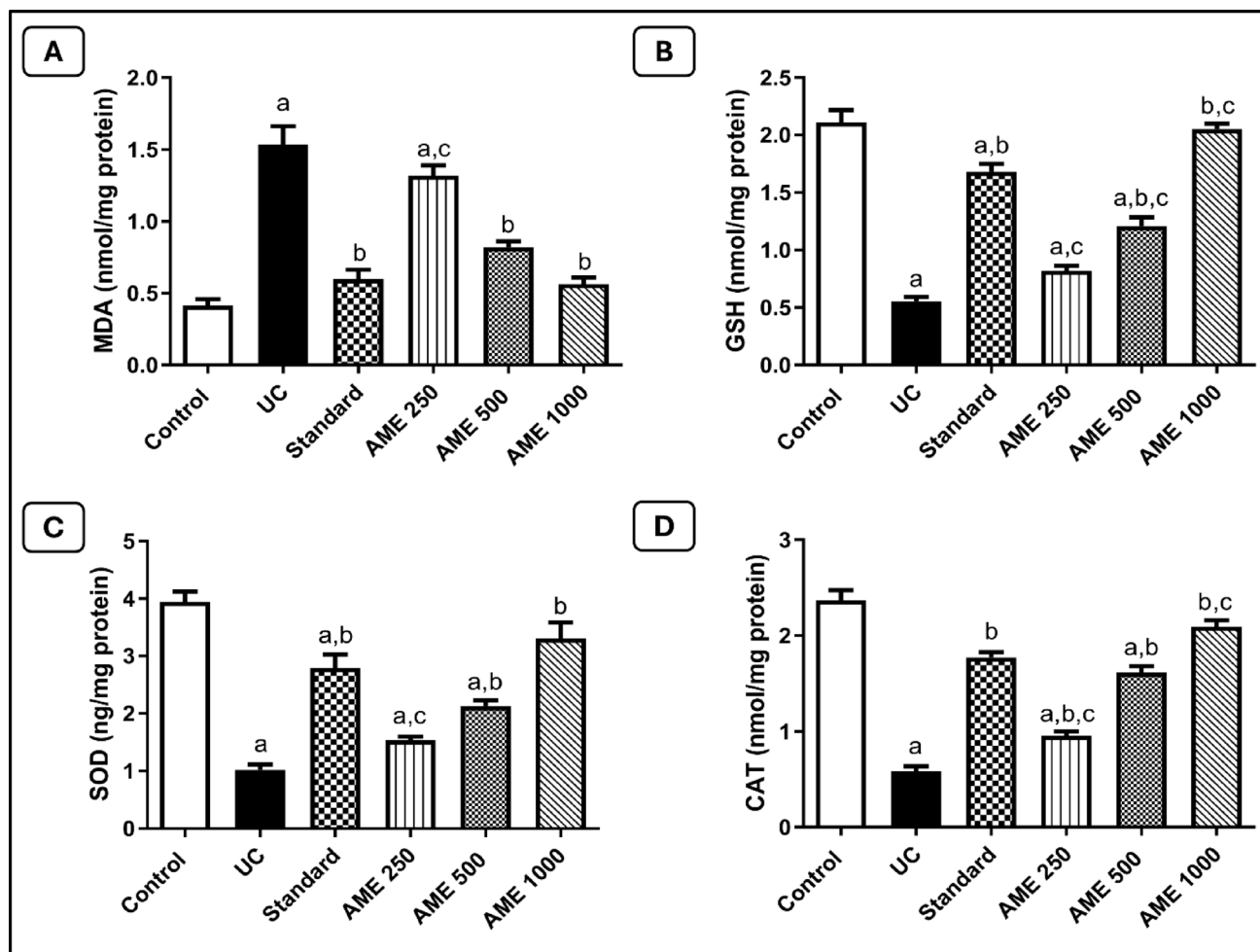


Fig. 4 Effect of the AME of *P. racemosa* on oxidative stress parameters of AA-induced UC rats **A**: MDA, **B**: GSH, **C**: SOD, **D**: CAT. Data are shown as mean $\pm$ SE. $n=6$. a: significantly different from the control

group at $p < 0.05$, b: significant from the UC group at $p < 0.05$, c: significant from the standard group at $p < 0.05$

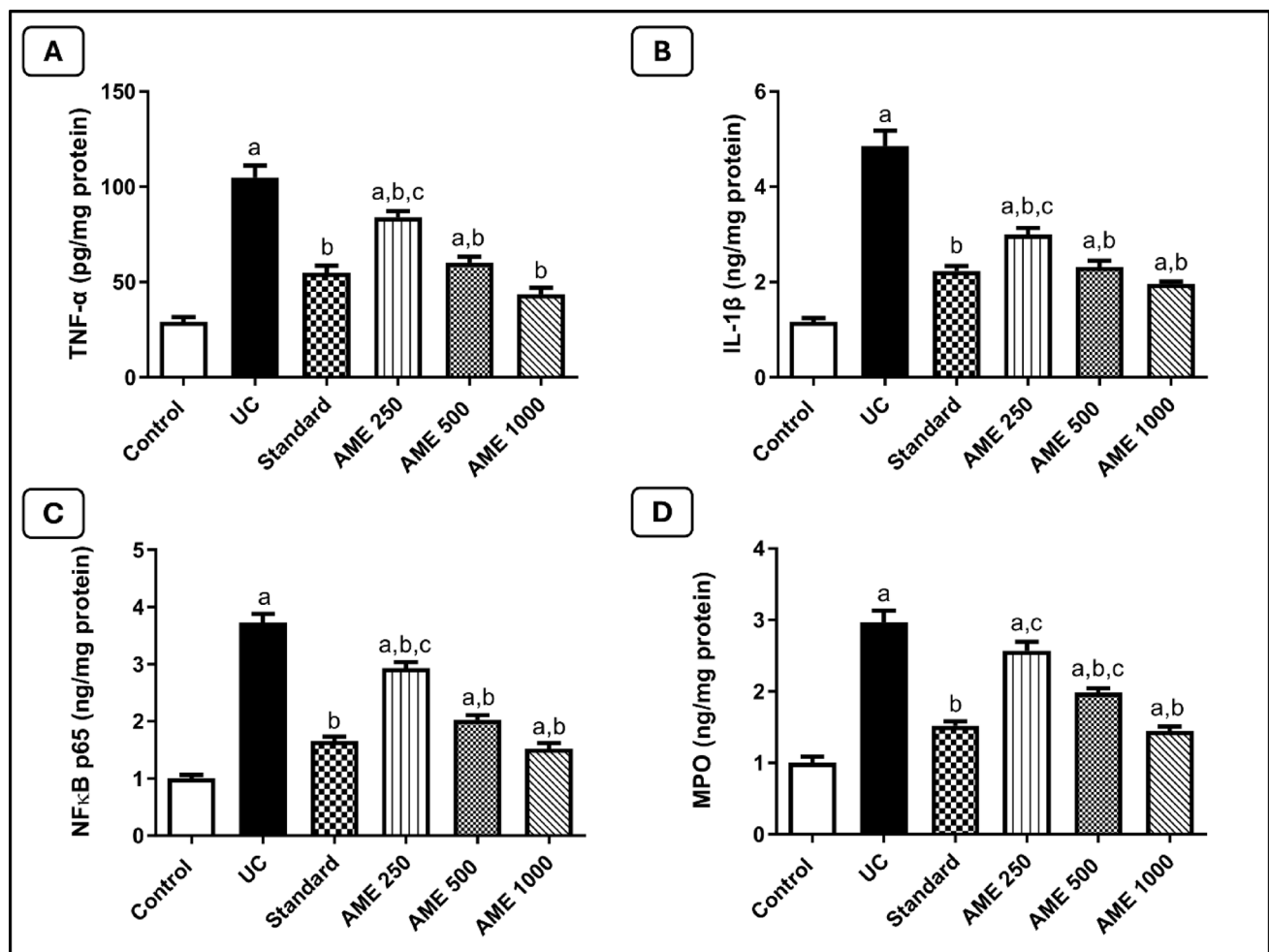


Fig. 5 Effect of the AME of *P. racemosa* on inflammatory parameters of AA-induced UC rats. **A:** TNF- α , **B:** IL-1 β , **C:** p-NF- κ B p65, **D:** MPO. Data are shown as mean $\pm$ SE. $n=6$. a: significant from the con-

trol group at $p<0.05$, b: significant from the UC group at $p<0.05$, c: significant from the standard group at $p<0.05$

this link was measured and found to be markedly ($p<0.0001$) higher in UC rats by 3 folds (2.967 ± 0.1655), compared to the control rats (1.002 ± 0.08497). AME *P. racemosa* at a dose of 250 mg/kg showed a nonsignificant decrease in MPO level (2.568 ± 0.1222), compared to the UC group. Meanwhile, the MPO level was markedly ($p<0.0001$) decreased upon treatment with Sulfasalazine and 500, and 1000 mg/kg AME of *P. racemosa* by 48.9% (1.517 ± 0.06424), 33% (1.987 ± 0.05631), and 51.3% (1.445 ± 0.06211), respectively, compared to the UC rats (Fig. 4 D). The AME at a dose of 1000 mg/kg exhibited a nonsignificant change in the MPO level compared to the standard rats.

Effect of the AME of *P. racemosa* on the histopathological examination of the colonic tissues of AA-induced UC rats

As represented in Fig. 6, normal control samples confirmed normal organized morphological characters of the colon wall, intact colonic crypts showing plentiful goblet

cells (black arrow), and intact covering epithelium with normal submucosa (star) and outer muscular coat. Meanwhile, colitis model samples demonstrated severe ulcerative hemorrhagic colitis with marked mucosal necrotic tissue depress (red arrow) and loss of morphological features of the colonic wall including minimal glandular structures, accompanied by moderate to severe mucosal/submucosal mixed inflammatory cells infiltrates (blue arrow) with hyperaemic vasculatures (red star), as well as marked submucosal and intermuscular edema (black star). 250 mg/kg of AME showed mild protective efficacy with persistent focal records of mucosal necrotic tissue depress and lining epithelium loss (red arrow) with mild glandular structures re-epithelialization (black arrow), inflammatory cells infiltrate (blue arrow) and marked submucosal and intermuscular edema (black star). 500 mg/kg of AME showed higher relative improvement with a higher record of mature goblet cells (black arrow), minimal focal submucosal inflammatory cells (blue arrow) with minor extent to mucosal layer with

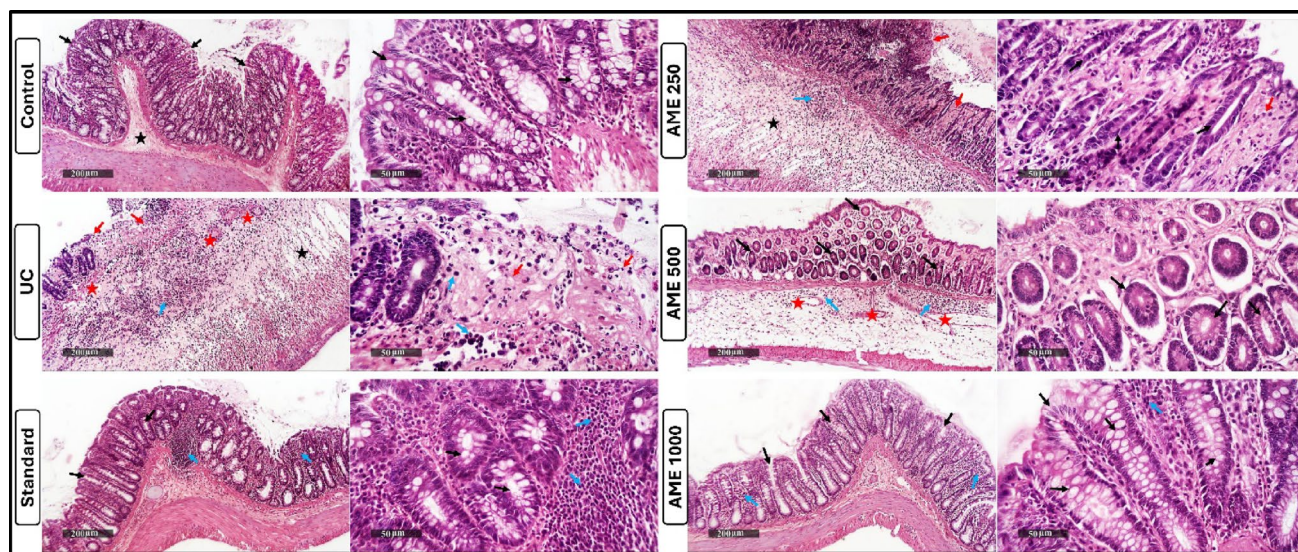


Fig. 6 Effect of the AME of *P. racemosa* on the histopathological examination of the colonic tissues of AA-induced UC rats. The control group showed a normal colon wall with plentiful goblet cells (black arrow) and intact covering epithelium with normal submucosa (star). UC group showed severe ulcerative hemorrhagic colitis with marked mucosal necrotic tissue depress (red arrow), inflammatory cell infiltrates (blue arrow), and marked submucosal and intermuscular edema (black star). 250 mg/kg of AME showed mild protective efficacy with persistent focal records of mucosal necrotic tissue depress and lining epithelium loss (red arrow) with mild glandular structures re-epitheli-

alization (black arrow), inflammatory cells infiltrate (blue arrow) and marked submucosal and intermuscular edema (black star). 500 mg/kg of AME showed higher relative improvement with a higher record of mature goblet cells (black arrow), minimal focal submucosal inflammatory cells (blue arrow), with minor extent to the mucosal layer with many dilated blood vessels (red star). Both standard and 1000 mg/kg of AME showed the highest improvement with a significant increase of mature goblet cells (black arrow), moderate persistence of mucosal/submucosal inflammatory cells (blue arrow), with mild congested blood vessels (red star)

many dilated blood vessels (red star). Interestingly, samples of the AME 1000 mg/kg and standard groups showed a magnificent improvement of colonic wall morphologies with clearly intact epithelium and mucosal glandular elements with an obvious increase of mature goblet cells (black arrow). Significant decreases in mucosal/submucosal inflammatory cell infiltrates were observed in the majority of the examined samples (blue arrow) with mild congestion in the blood vessels (red star). Both standard and 1000 mg/kg of AME showed the highest improvement with a significant increase of mature goblet cells (black arrow), moderate persistence of mucosal/submucosal inflammatory cells (blue arrow) with mild congested blood vessels (red star).

Effect of the AME of *P. racemosa* on the mucins level of AA-induced UC rats

Rats treated with AA showed an altered mucosal barrier evidenced by a marked ($p < 0.0001$) decrease in relative mucin expression by 95.3% (0.6333 ± 0.1944), in comparison to the control rats (13.62 ± 0.6379) Fig. 7. AME *P. racemosa* at a dose of 250 mg/kg showed a nonsignificant decrease in mucin level (0.8500 ± 0.1258). Meanwhile, treatment with standard and the AME *P. racemosa* at doses 500, and 1000 mg/kg preserved the formation of mucosal barrier indicated by the marked ($p < 0.0001$) increment in

mucin expression by 16.7 folds (10.60 ± 0.4789), 14 folds (8.867 ± 0.4372), and 16.5 folds (10.47 ± 0.5402), respectively, compared to the UC rats.

Effect of the AME of *P. racemosa* on the immunohistochemical expression level of IL-6 of AA-induced UC rats

The immunohistochemical expression of IL-6, one of the proinflammatory cytokines involved in UC, was markedly ($p < 0.0001$) increased in UC rats by 3.3 folds (21.07 ± 0.6591), in comparison to the control rats (6.483 ± 0.3188). AME *P. racemosa* at a dose of 250 mg/kg showed a nonsignificant decrease in mucin level (18.87 ± 0.6349). Meanwhile, its expression was markedly ($p < 0.0001$) decreased upon treatment with sulfasalazine standard and 500, and 1000 mg/kg of the AME *P. racemosa* by 54.1% (9.667 ± 0.5725), 38.4% (12.97 ± 0.6328), and 58.6% (8.733 ± 0.4624), respectively, in comparison to the UC rats. These findings support the AME's anti-inflammatory activity Fig. 8.

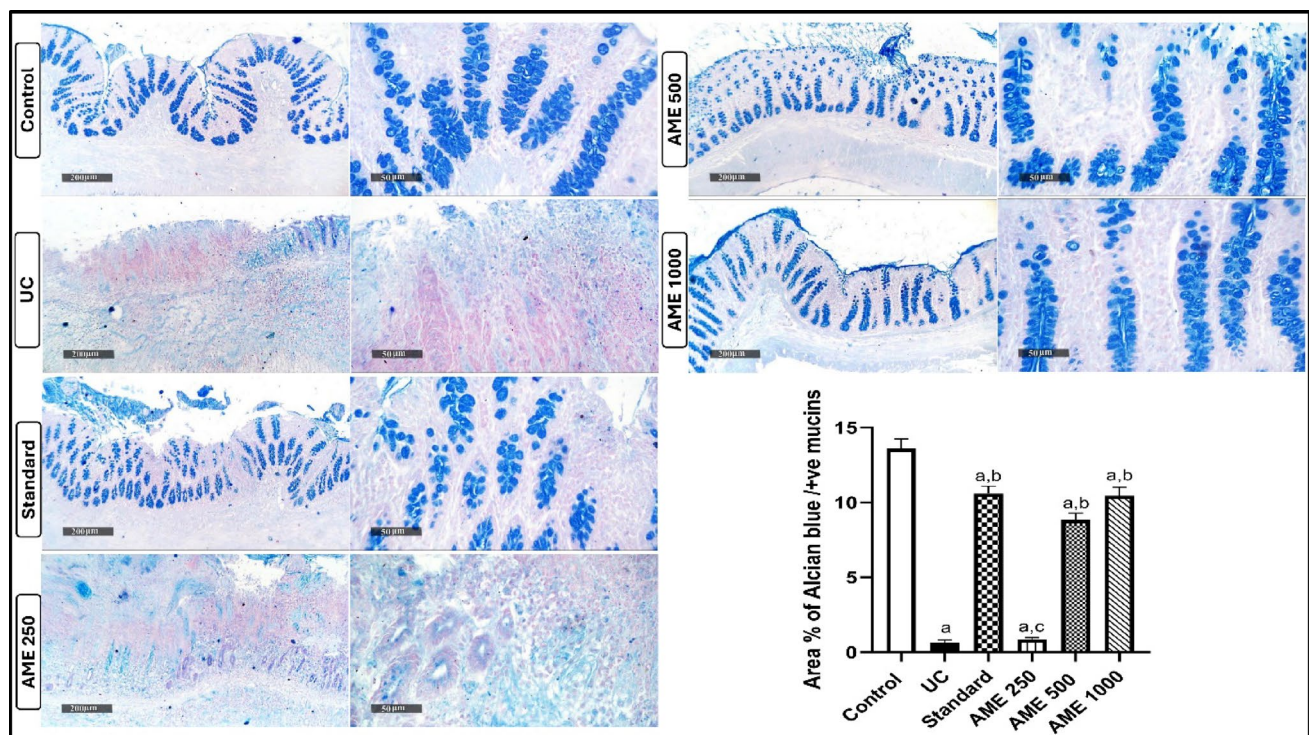


Fig. 7 Effect of the AME of *P. racemosa* on the mucins level of AA-induced UC rats. Data are shown as mean $\pm$ SE. $n=6$. a: significant from the control group at $p<0.05$, b: significant from the UC group at $p<0.05$, c: significant from the standard group at $p<0.05$

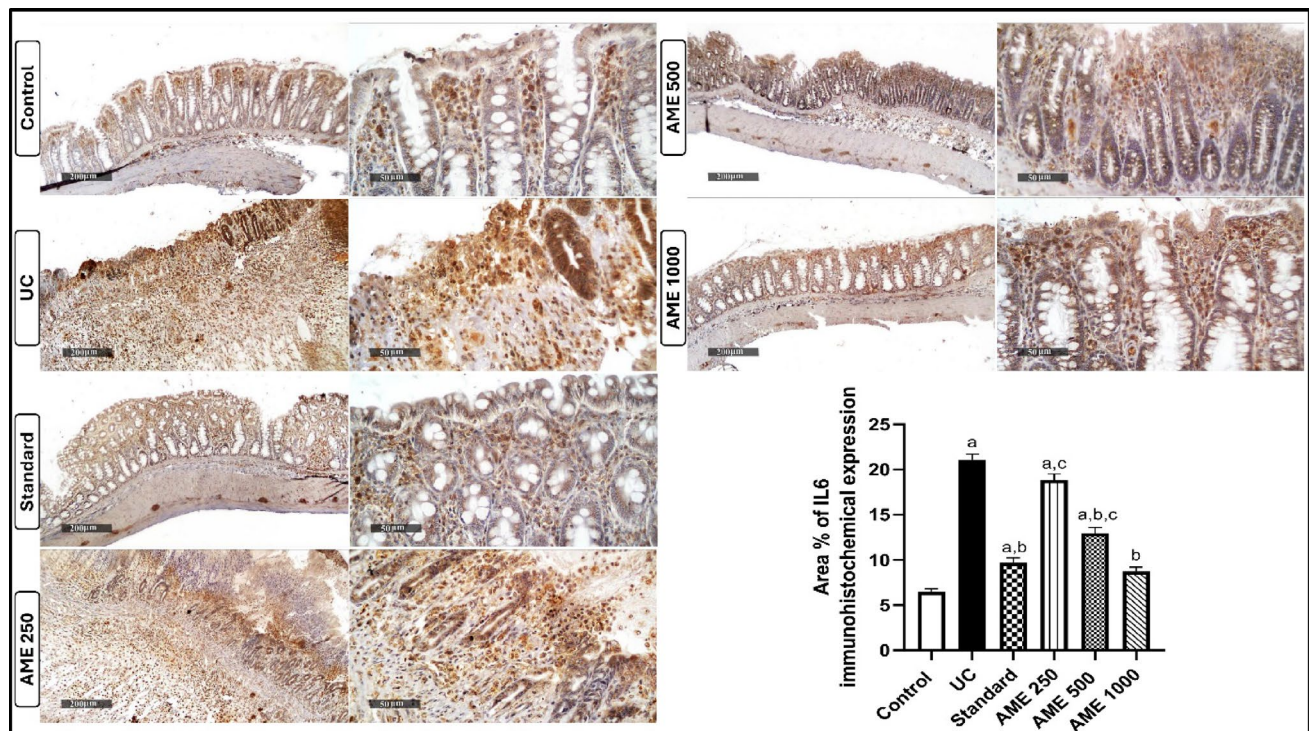


Fig. 8 Effect of the AME of *P. racemosa* on the immunohistochemical expression level of IL-6 of AA-induced UC rats. Data are shown as mean $\pm$ SE. $n=6$. a: significant from the control group at $p<0.05$,

b: significant from the UC group at $p<0.05$, c: significant from the standard group at $p<0.05$

Discussion

Ulcerative colitis (UC) is a known chronic inflammatory multifactorial disorder resulting in inflammation and ulceration of the colonic mucosa (Küpeli et al. 2023). In Egypt and developing countries, the prevalence of UC has markedly increased (Ahmed et al. 2022). Corticosteroids, 5-aminosalicylates, and immunomodulatory are the most widely used agents for treating UC. However, these agents' adverse effects restrict them from being used in the long term (Sales-Campos et al. 2015). That's why; therapeutic options with better compliance and more efficacies are urgently needed.

Natural products have played a pivotal role in preventing and treating UC. They have the advantages of high safety, obvious efficacy, and minimal adverse effects (Xue et al. 2023). *P. racemosa* (Myrtaceae) has been used traditionally for gastrointestinal disorders and osteoarthritis and has shown beneficial effects through its antioxidant and anti-inflammatory activities (Moharram et al. 2018). The present study aimed to inspect the protective effect of the AME of *P. racemosa* against the AA rat model of UC.

The colitis model induced by AA is one of the reasonable models for assessing the efficacy and pharmacological mechanism of agents against UC in rats (Rehman et al. 2022). IR administration of AA causes marked necrosis followed by a massive invasion and infiltration of inflammatory cells into the colonic tissue, vascular dilatation, edema, and ulceration (Randawall et al. 2014).

In the present work, the significant reduction in the DAI was the first indicator of the protective effect of the AME against AA-induced UC. To investigate and understand the pharmacological basis, the colon of the rats was isolated for the macroscopic examination which showed a reduction of the colonic mucosal damage and ulceration. This can be clarified by the ability of *P. racemosa* AME to suppress the infiltration of inflammatory cells as indicated by the significant decline in the number of WBCs and neutrophils. Moreover, the reduction of colonic weight to length ratio which indicates mucosal edema was one of the clues that prove the anti-inflammatory effect of the AME. These findings were further validated through the microscopic histopathological examination using H&E stain that showed a reduction of inflammatory cell infiltration in the colonic tissue samples of *P. racemosa*-administered groups. In addition, Alcian Blue staining showed a significant increase in the relative mucin expression indicating maintaining mucosal barrier integrity. There is an overt link between the inflammatory mediators in the inflamed colonic mucosa and the modulation of ROS (Tian et al. 2017). ROS stimulates the release of a wide range of proinflammatory cytokines and exacerbates tissue damage (Ahmed et al. 2022). The balance between pro-inflammatory and anti-inflammatory cytokines

is urgently required for the homeostasis of the normal colon. Interruption of the cytokines profile is well-known in UC (Alsharif et al. 2022). Elevated levels of NF- κ B and pro-inflammatory cytokines including TNF- α , IL-1 β , and IL-6 were reported in UC (Chen and Sundrud 2016; Müzes 2012).

In the current study, IR administration of AA activates oxidative stress and the overproduction of ROS via intracellular acidification (Hering and Schulzke 2009). As a result of acidification, innate immunity is activated with subsequent phosphorylation and activation of NF- κ B and its-dependent production of inflammatory cytokines TNF- α , IL-1 β , and IL-6. These cytokines stimulate cytotoxic oxidant release and finally disrupt oxidant/antioxidant balance (Elhefnawy et al. 2023; Rehman et al. 2022). MPO is one of these cytotoxic oxidants released from the neutrophils during tissue inflammation. Its level is directly proportionate to the number of neutrophils in the inflamed colonic tissues (El Akabawy and El-Sherif 2019). It has oxidation potential for chloride ions resulting in neutrophil-dependent oxidative damage and destruction. Therefore, its level is linked with MDA as an indicator of lipid peroxidation (Out-Boakye et al. 2023).

Treatment with the AME demonstrated antioxidant and anti-inflammatory effects against AA-induced UC. The antioxidant effect was evidenced by the lowered level of MDA, elevated GSH level (a non-enzymatic antioxidant), and elevated SOD and CAT levels which are classified as enzymatic antioxidant enzymes. Further, the NF- κ B p65, TNF- α , IL-1 β , IL-6, and MPO levels were found to be significantly decreased, explaining the anti-inflammatory activity of the AME which was in harmony with low counts of neutrophils and WBCs. These ameliorative effects of the AME may be accredited to its flavonoid fracture as several previous studies documented the valuable potential of flavonoids on various rodent colitis experimental models including dextran sulfate sodium, acetic acid, or trinitrobenzene sulfonic acid model. These investigations have demonstrated the anti-inflammatory properties of different flavonoids, including glycosides and aglycones as well as those from various chemical classes, including flavanols (catechins), flavones (apigenin7-O-rutinoside), and flavonols (kaempferol, quercetin, and quercetrin).

Astragalin belonging to Kaempferol glycosides was identified in our study. It is well documented that kaempferol has strong antioxidants and anti-inflammatory activity against various diseases and studies have demonstrated that kaempferol may help protect against experimental colitis in mice. It was shown that Kaempferol downregulated inflammatory mediators such as IL-6, IL-1 β , COX-2, TNF- α , iNOS, MPO, prostaglandin E2, and nitric oxide levels in colonic mucosa, which in turn alleviated the symptoms of UC. Furthermore,

it was shown that kaempferol can significantly increase the healing of the intestinal epithelium. Accordingly, the content of astragalin in the extract can count for its observed antioxidant and anti-inflammatory effect (Xue et al. 2023).

Quercetin's antioxidant qualities can be crucial for the extract's effective free radical scavenging properties as previously showed strong antioxidant properties (Veza et al. 2016). By inhibiting oxidative stress, regulating macrophage activity, and lowering apoptosis, quercetin can reduce intestinal inflammation (Veza et al. 2016). Moreover, Quercetrin can prevent colitis in several ways. It exhibits anti-inflammatory properties, mostly through suppressing inflammatory factors including iNOS, COX2, NOX1, TNF- α , and IL-1 β in rodents, decreasing neutrophil activity, and preventing bacterial translocation, resulting in limited mucosal barrier rupture (Veza et al. 2016).

Flavanols are another factor in the protective effect of the extract. Flavanols can be either polymers (proanthocyanins) or monomers (catechins). A polyphenolic ring and six oxygenated heterocycles that carry another polyphenolic ring coalesce to form the chemical structure of catechins. The polyphenol ring's 3', 4' catechol structure effectively scavenges peroxide, superoxide, and peroxyxynitrite radicals. The preventive effect of flavanols in experimental models of UC in rats and mice has been validated by numerous studies (Veza et al. 2016).

Besides having antibacterial properties, catechins also have anti-inflammatory, anti-tumor, antioxidant, and anti-aging properties. It improves intestinal mucosal inflammation by increasing the production of GSH, reducing NO and MDA, increasing SOD, and inhibiting the production of NF- κ B and proinflammatory cytokines. Through lowering the generation of inflammatory factors and enhancing oxidative stress in intestinal tissues, catechins protect against intestinal inflammation and decrease the severity of ulcerative colitis.

Collectively, the major components found in AME of *P. racemosa* aerial parts are characterized by antioxidant, anti-inflammatory, and immunomodulatory properties which can explain the protective effect of the extract against the AA model of UC in rats.

Our preventative strategy shows that AME lowers histological damage, inflammatory mediators, and oxidative stress markers when administered before AA. These findings imply that AME functions, at least partially, by preserving the integrity of the mucosal barrier and regulating early inflammatory responses. Future research is necessary to ascertain whether AME can reverse established colitis, to identify the best therapeutic windows, and to describe the dose-response relationships, even though our findings support AME's potential as a preventive or adjunctive agent.

Conclusion

The result of this study proved the isolation and identification of phenolic constituents of AME of *P. racemosa* aerial parts. The AME exhibited antioxidant, anti-inflammatory, and immunomodulatory effects against AA-induced ulcerative colitis (UC) in rats, which may be attributed to its phenolic profiling. Because of the urgent need to find new adjuvant and complementary herbal medications for many diseases, our study recommends more clinical studies for *P. racemosa* as a palliative natural therapy against UC.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10787-025-02060-x>.

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Authors' contribution Fathia S. Elshaarawy, Mohamed I. Abd-elhady, and Haitham A. Ibrahim: Conceptualization, Supervision, Investigation, Formal analysis, Resources, Methodology, Writing—original draft; and Writing—review & editing. Wafaa Hamdy: Methodology, Data curation, Writing—original draft. Merhan E. Ali, Asmaa A. Ahmed, and Elsayed K. El-Sayed: Investigation; Methodology, Data curation, Writing—review & editing.

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Data availability The data and figures supporting our findings can be found in the main text. NMR spectra of the isolated compounds are available as supplementary files.

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval Animal care and experimental were approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Pharmacy, Helwan University, Egypt (Protocol No:12 A2023), and conducted according to the European Community Directive (68/609/EEC), a national rule on animal care that is consistent with the NIH Guidelines for the Care and Use of Laboratory Animals (8th edition). The authors confirm that the study is reported in accordance with ARRIVE guidelines.

Consent for publication Not applicable.

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